PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

MATED THAT 30 MILLION PEOPLE TAN INDOORS IN THE UNITED STATES ANNUALLY, INCLUDING >2 MILLION ADOLESCENTS. THE RELATIONSHIP OF SUN EXPOSURE TO MELANOMA DEVELOPMENT IS LESS CLEAR-CUT, BUT SUGGESTIVE EVIDENCE SUPPORTS AN ASSOCIATION. THE STRON-GEST RISK FACTORS FOR MELANOMA INCLUDE POSITIVE FAMILY HISTORY FOR MEL-ANOMA, MULTIPLE DYSPLASTIC NEVI, AND PRIOR MELANOMA. MELANOMAS OCCASIONALLY DEVELOP BY THE TEENAGE YEARS, INDICATING THAT THE LATENT PE-RIOD FOR TUMOR GROWTH IS LESS THAN THAT OF NONMELANOMA SKIN CANCER. MELANOMAS ARE AMONG THE MOST RAPIDLY INCREASING OF ALL HUMAN MA-LIGNANCIES (CHAP. 83). EPIDEMIOLOGIC STUDIES OF IMMIGRANT POPULA-TIONS OF SIMILAR ETHNIC STOCK INDICATE THAT INDIVIDUALS BORN IN ONE AREA OR WHO MIGRATE TO THE SAME LOCALE BEFORE AGE 10 HAVE HIGHER AGE-SPE-CIFIC MELANOMA RATES THAN INDIVIDUALS ARRIVING LATER. IT IS THUS REASON-ABLE TO CONCLUDE THAT LIFE IN A SUNNY CLIMATE FROM BIRTH OR EARLY CHILDHOOD INCREASES THE RISK OF MELANOMA. IN GENERAL, RISK DOES NOT CORRELATE WITH CUMULATIVE SUN EXPOSURE BUT MAY RELATE TO THE DURATION AND EXTENT OF EXPOSURE IN CHILDHOOD. EPIDEMIOLOGIC STUDIES HAVE SHOWN THAT INDOOR TANNING IS A RISK FACTOR FOR MELANOMA. META-ANALYSIS OF 17 CASE-CONTROL STUDIES IN PATIENTS WITH MELANOMA CONCLUDED THAT THE PROTECTIVE EFFECT OF SUNSCREENS AGAINST THIS TYPE OF TUMOR COULD NOT BE SUBSTANTIATED, BUT THIS IS LIKELY DUE TO FAILURE TO CONTROL FOR CONFOUNDING FACTORS SUCH AS SUNSCREEN STABILITY AND FRE-QUENCY OF APPLICATION. SINCE NO PROSPECTIVE STUDIES ARE AVAILABLE TO AD-DRESS THIS ISSUE, IT SEEMS REASONABLE TO RECOMMEND THAT PATIENTS AT RISK FOR MELANOMA UTILIZE PHOTOPROTECTION SUCH AS SUN AVOIDANCE, HIGH SUN PROTECTIVE FACTOR (SPF) SUNSCREENS, AND PROTECTIVE CLOTHING.

IMMUNOLOGIC EFFECTS EXPOSURE TO SOLAR RADIATION CAUSES LOCAL (INHIBI-TION OF IMMUNE RESPONSES TO ANTIGENS APPLIED AT THE IRRADIATED SITE) AND SYSTEMIC (INHIBITION OF IMMUNE RESPONSES TO ANTIGENS APPLIED AT REMOTE UNIRRADIATED SITES) IMMUNOSUPPRESSION. THE ACTION SPECTRUM FOR UV-INDUCED IMMUNOSUPPRESSION CLOSELY MIMICS THE ABSORPTION SPECTRUM OF DNA. PYRIMIDINE DIMERS IN LCS MAY INHIBIT ANTIGEN PRE-SENTATION. THE ABSORPTION SPECTRUM OF EPIDERMAL UROCANIC ACID
CLOSELY MIMICS THE ACTION SPECTRUM FOR UV-B-INDUCED IMMUNOSUPPRESSION. TRANS-CIS ISOMERIZATION OF UROCANIC ACID IN THE STRATUM CORNEUM LEADS TO ITS SYSTEMIC ABSORPTION AND CONSEQUENT IMMUNOSUPPRESSIVE EFFECTS. FURTHERMORE ADMINISTRATION OF MODEST DOSES OF UV-B TO HUMAN SKIN REDUCES THE DEGREE OF ALLERGIC SENSITIZATION TO THE POTENT CONTACT ALLERGEN, DINITROCHLOROBENZENE. THIS IS ASSOCIATED WITH ROS-INDUCED DEPLETION OF EPIDERMAL LCS. HIGHER DOSES OF UV-RADIATION EVOKE DIMINISHED IMMUNOLOGIC RESPONSES TO ANTIGENS INTRODUCED EITHER EPICUTANEOUSLY OR INTRACUTANEOUSLY AT SITES DISTANT FROM THE IRRADIATED SITE. THESE SUPPRESSED RESPONSES ARE ALSO ASSOCIATED WITH THE INDUCTION OF ANTIGEN-SPECIFIC SUPPRESSOR T LYMPHOCYTES AND MAY BE MEDIATED BY AS YET UNDEFINED FACTORS THAT ARE RELEASED FROM EPIDERMAL CELLS AT THE IRRADIATED SITE. ONE IMPORTANT CONSEQUENCE OF CHRONIC SUN EXPOSURE AND THE CONCOMITANT IMMUNOSUPPRESSION IS ENHANCED RISK OF SKIN CANCER. PERHAPS THE MOST GRAPHIC DEMONSTRATION OF THE ROLE OF IMMUNOSUPPRESSION IN ENHANCING THE RISK OF NONMELANOMA SKIN CANCER HAS COME FROM STUDIES OF PATIENTS RECEIVING ORGAN TRANSPLANTATION WHO ARE ON CHRONIC IMMUNOSUPPRESSIVE ANTIREJECTION DRUG REGIMENS. MORE THAN 50% OF TRANSPLANT PATIENTS DEVELOP BCCS AND SCCS, AND THESE Cancers ARE THE MOST COMMON MALIGNANCY ARISING IN IMMUNOSUPPRESSED SOLID-ORGAN TRANSPLANT RECIPIENTS. HUMAN PAPILLOMA VIRUSES (HPVs) MAY ALSO PLAY A ROLE IN THE INCREASED RISK OF SCCS IN THESE PATIENTS SINCE TUMORS DISPLAY AN HPV DNA CARRIAGE RATE OF ALMOST 80%. THESE PATIENTS REQUIRE CLOSE PERIODIC MONITORING AND RIGOROUS PHOTOPROTECTION USING SUNSCREENS, PROTECTIVE CLOTHING, AND SUN AVOIDANCE.

PHOTOSENSITIVITY DISEASES

TABLE 57-2 CLASSIFICATION OF PHOTOSENSITIVITY DISEASES

<table>
<thead>
<tr>
<th>TYPE</th>
</tr>
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<tbody>
<tr>
<td>GENETIC</td>
</tr>
<tr>
<td>METABOLIC</td>
</tr>
<tr>
<td>PHOTOTOXIC</td>
</tr>
<tr>
<td>INTERNAL</td>
</tr>
</tbody>
</table>
EXTERNAL
PHOTOALLERGIC
IMMEDIATE
DELAYED

NEOPLASTIC

IDIOPATHIC

PHOTOAGGRAVATED

DISEASE

ERYTHROPOIETIC PORPHYRIA
ERYTHROPOIETIC PROTOPORPHYRIA
PORPHYRIA CUTANEA TARDA-FAMILIAL
VARIEGATE PORPHYRIA
HEPATOERYTHROPOIETIC PORPHYRIA
ALBINISM
XERODERMA PIGMENTOSUM
ROTHMUND-THOMPSON DISEASE
BLOOM SYNDROME
COCKAYNE'S DISEASE
KINDLER SYNDROME
PHENYLKETONURIA
PORPHYRIA CUTANEA TARDA-SPORADIC
HARTNUP DISEASE
KWASHIORKOR
PELLAGRA
CARCINOID SYNDROME

DRUGS
DRUGS, PLANTS, FOOD

SOLAR URTICARIA
DRUG PHOTOALLERGY
PERSISTENT LIGHT REACTION/CHRONIC ACTINIC
DERMATITIS
PHOTOAGING
ACTINIC KERATOSIS
MELANOMA AND NONMELANOMA SKIN
CANCER
POLYMORPHOUS LIGHT ERUPTION
HYDROA AESTIVALE
ACTINIC PRURIGO
LUPUS ERYTHEMATOSUS
SYSTEMIC
SUBACUTE CUTANEOUS
DISCOID
DERMATOMYOSITIS
HERPES SIMPLEX
LICHEN PLANUS ACTINICUS
ACNE VULGARIS (AESTIVALE)

OF PORPHYRIA CUTANEA TARDA (PCT) TYPICALLY BEGINS IN THE FOURTH AND
FIFTH DECADES. A HISTORY OF EXPOSURE TO TOPICAL AND SYSTEMIC DRUGS AND CHEMICALS MAY PROVIDE IMPORTANT CLUES. MANY CLASSES OF DRUGS CAN CAUSE PHOTOSENSITIVITY ON THE BASIS OF EITHER PHOTOTOXICITY OR PHOTOALLERGY. FRAGRANCES SUCH AS MUSK AMBRETTE THAT WERE PREVIOUSLY PRESENT IN NUMEROUS COSMETIC PRODUCTS ARE ALSO POTENT PHOTOSENSITIZERS.

EXAMINATION OF THE SKIN MAY ALSO OFFER IMPORTANT CLUES. ANATOMIC AREAS THAT ARE NATURALLY PROTECTED FROM DIRECT SUNLIGHT SUCH AS THE HAIRY SCALP, THE UPPER EYELIDS, THE RETROAURICULAR AREAS, AND THE INFRANASAL AND SUBMENTAL REGIONS MAY BE SPARED, WHEREAS EXPOSED AREAS SHOW CHARACTERISTIC FEATURES OF THE PATHOLOGIC PROCESS. THESE ANATOMIC LOCALIZATION PATTERNS ARE OFTEN HELPFUL, BUT NOT INFALLIBLE, IN MAKING THE DIAGNOSIS. FOR EXAMPLE, AIRBORNE CONTACT SENSITIZERS THAT ARE BLOWN ONTO THE SKIN MAY PRODUCE DERMATITIS THAT CAN BE DIFFICULT TO DISTINGUISH FROM PHOTOSENSITIVITY, DESPITE THE FACT THAT SUCH MATERIAL MAY TRIGGER SKIN REACTIVITY IN AREAS SHIELDED FROM DIRECT SUNLIGHT.

MANY DERMATOLOGIC CONDITIONS MAY BE CAUSED OR AGGRAVATED BY SUNLIGHT (TABLE 57-2). THE ROLE OF LIGHT IN EVOKING THESE RESPONSES MAY BE DEPENDENT ON GENETIC ABNORMALITIES RANGING FROM WELL-DESCRIBED DEFECTS IN DNA REPAIR THAT OCCUR IN XP TO THE INHERITED ABNORMALITIES IN HEME SYNTHESIS THAT CHARACTERIZE THE PORPHYRIAS. IN CERTAIN PHOTOSENSITIVITY DISEASES, THE CHROMOPHORE HAS BEEN IDENTIFIED, WHEREAS IN THE MAJORITY, THE ENERGY-ABSORBING AGENT IS UNKNOWN.

POLYMORPHOUS LIGHT ERUPTION AFTER SUNBURN, THE MOST COMMON TYPE OF PHOTOSENSITIVITY DISEASE IS POLYMORPHOUS LIGHT ERUPTION (PLE), THE MECHANISM OF WHICH IS UNKNOWN. MANY AFFECTED INDIVIDUALS NEVER SEEK MEDICAL ATTENTION BECAUSE THE CONDITION IS OFTEN TRANSIENT, BECOMING MANIFEST EACH SPRING WITH INITIAL SUN EXPOSURE BUT THEN SUB-03 MISTAKES

353 CHAPTER 57 PHOTOSENSITIVITY AND OTHER REACTIONS TO LIGHT

SIDING SPONTANEOUSLY WITH CONTINUING EXPOSURE, A PHENOMENON KNOWN AS “HARDENING.” THE MAJOR MANIFESTATIONS OF PLE INCLUDE PRURITIC (OFTEN INTENSELY SO) ERYTHEMATOUS PAPULES THAT MAY COALESCE INTO PLAQUES IN A PATCHY DISTRIBUTION ON EXPOSED AREAS OF THE TRUNK AND FOREARMS. THE FACE IS USUALLY LESS SERIOUSLY INVOLVED. THE DIAGNOSIS CAN BE CONFIRMED BY SKIN BIOPSY AND BY PERFORMING PHOTOTEST PROCEDURES IN WHICH SKIN IS EXPOSED TO MULTIPLE ERYTHEMA DOSES OF UV-A AND UV-B. THE ACTION SPECTRUM FOR PLE IS USUALLY WITHIN THESE PORTIONS OF THE SOLAR SPECTRUM.

TREATMENT OF THIS PLE INCLUDES THE USE OF SUNSCREENS AND THE INDUCTION OF HARDENING BY THE CAUTIOUS ADMINISTRATION OF ARTIFICIAL UV-B (BROAD-BAND OR NARROW-BAND) AND/OR UV-A RADIATION FOR 2-3 WEEKS.
PRIOR TO INITIAL SUN EXPOSURE.

PHOTOSENSITIVITY DISORDERS

PHOTOSENSITIVITY DISORDERS ARE RELATED TO THE TOPICAL OR SYSTEMIC ADMINISTRATION OF DRUGS AND OTHER CHEMICALS. BOTH REACTIONS REQUIRE THE ABSORPTION OF ENERGY BY A DRUG OR CHEMICAL RESULTING IN THE PRODUCTION OF AN EXCITED-STATE PHOTOSENSITIZER THAT CAN TRANSFER ITS ABSORBED ENERGY TO A Bystander MOLECULE OR TO MOLECULAR OXYGEN, THEREBY GENERATING TISSUE-DESTRUCTIVE CHEMICAL SPECIES, INCLUDING ROS.

PHOTOSENSITIVITY IS A NONIMMUNOLOGIC REACTION CAUSED BY DRUGS AND CHEMICALS, A FEW OF WHICH ARE LISTED IN TABLE 57-3. THE USUAL CLINICAL MANIFESTATIONS INCLUDE ERYTHEMA RESEMBLING A SUNBURN REACTION THAT QUICKLY DESQUAMATES, OR “PEELS,” WITHIN SEVERAL DAYS. IN ADDITION, EDEMA, VESICLES, AND BULLAE MAY OCCUR.

PHOTOALLERGY IS MUCH LESS COMMON AND IS DISTINCT IN THAT THIS IS AN IMMUNOPATHOLOGIC PROCESS. THE EXCITED-STATE PHOTOSENSITIZER MAY CREATE HIGHLY UNSTABLE HAPTENIC FREE RADICALS THAT BIND COVALENTLY TO MACROMOLECULES TO FORM A FUNCTIONAL ANTIGEN CAPABLE OF EVOKING A DELAYED HYPERSENSITIVITY RESPONSE. SOME OF THE DRUGS AND CHEMICALS THAT PRODUCE PHOTOALLERGY ARE LISTED IN TABLE 57-4. THE CLINICAL MANIFESTATIONS TYPICALLY DIFFER FROM THOSE OF PHOTOTOXICITY IN THAT AN INTENSELY PRURITIC ECZEMATOUS DERMATITIS TENDS TO PREDOMINATE AND EVOLVES INTO LIKENIFIED, THICKENED, “LEATHERY” CHANGES IN SUN-EXPOSED AREAS. A SMALL SUBSET (PERHAPS 5-10%) OF PATIENTS WITH PHOTOALLERGY MAY DEVELOP A PERSISTENT EXQUISITE HYPERSENSITIVITY TO LIGHT EVEN WHEN THE OFFENDING DRUG OR CHEMICAL IS IDENTIFIED AND ELIMINATED, A CONDITION KNOWN AS PERSISTENT LIGHT REACTION.

A VERY UNCOMMON TYPE OF PERSISTENT PHOTOSENSITIVITY IS KNOWN AS CHRONIC ACTINIC DERMATITIS. THESE PATIENTS ARE TYPICALLY ELDERLY MEN WITH A LONG HISTORY OF PREEXISTING ALLERGIC CONTACT DERMATITIS OR PHOTOSENSITIVITY. THEY ARE USUALLY EXQUISITELY SENSITIVE TO UV-B, UV-A, AND VISIBLE WAVELENGTHS.

DIAGNOSTIC CONFIRMATION OF PHOTOTOXICITY AND PHOTOALLERGY CAN OFTEN BE OBTAINED USING PHOTOTEST PROCEDURES. IN PATIENTS WITH SUSPECTED PHOTOTOXICITY, DETERMINING THE MINIMAL ERYTHEMA DOSE (MED) WHILE THE PATIENT IS EXPOSED TO A SUSPECTED AGENT AND THEN REPEATING THE MED AFTER DISCONTINUATION OF THE AGENT MAY PROVIDE A CLUE TO THE CAUSATIVE DRUG OR CHEMICAL. PHOTOPATCH TESTING CAN BE PERFORMED TO CONFIRM THE DIAGNOSIS OF PHOTOALLERGY. THIS IS A SIMPLER VARIATION OF ORDI-

**TABLE 57-3 PHOTOTOXIC DRUGS**

AMIODARONE  
DACARBazine  
FLUOROQUINOLONES  
5-FLUORO URACIL  
FUROSEMIDE  
NALIDIXIC ACID  
PHENOTHIAZINES  
PSORALENS  
RETINOIDS
SULFONAMIDES
SULFONYLUREAS
TETRACYCLINES
THIAZIDES
VINBLASTINE

TOPICAL

+

+/-

SYSTEMIC

+
+
+
+
+
+
+
+
+
+
+
+
+
+

TABLE 57-4 PHOTOALLERGIC DRUGS

6-METHYLCOUMARIN
AMINOBENZOIC ACID AND ESTERS
BITHIONOL
CHLORPROMAZINE
DICLOFENAC
FLUOROQUINOLONES
HALOGENATED SALICYLANILIDES
HYPERICIN (ST JOHN'S WORT)
MUSK AMBRETTE
PIROXICAM
PROMETHAZINE
SULFONAMIDES
SULFONYLUREAS

TOPICAL

+
+


SYSTEMIC PATCH TESTING IN WHICH A SERIES OF KNOWN PHOTOALLERGENS IS APPLIED TO THE SKIN IN DUPLICATE AND ONE SET IS IRRADIATED WITH A SUBERYTHEMA DOSE OF UV-A. DEVELOPMENT OF ECZEMATOUS CHANGES AT SITES EXPOSED TO SENSITIZER AND LIGHT IS A POSITIVE RESULT. THE CHARACTERISTIC ABNORMALITY IN PATIENTS WITH PERSISTENT LIGHT REACTION IS A DIMINISHED THRESHOLD TO ERYTHEMA EVOLED BY UV-B. PATIENTS WITH CHRONIC ACTINIC DERMATITIS USUALLY MANIFEST A BROAD SPECTRUM OF UV HYPERRESPONSIVENESS AND REQUIRE METICULOUS PHOTOPROTECTION INCLUDING AVOIDING SUN EXPOSURE, HIGH (>30) SPF SUNSCREENS, AND IN SEVERE CASES SYSTEMIC IMMUNOSUPPRESSION, PREFERABLY WITH AZATHIOPRINE (1-2 MG/KG PER DAY). THE MANAGEMENT OF DRUG PHOTOSENSITIVITY INVOLVES FIRST AND FOREMOST THE ELIMINATION OF EXPOSURE TO THE CHEMICAL AGENTS RESPONSIBLE FOR THE REACTION AND MINIMIZATION OF SUN EXPOSURE. THE ACUTE SYMPTOMS OF PHOTOTOXICITY MAY BE AMELIORATED BY COOL, MOIST COMPRESSES, TOPICAL GLUCOCORTICOIDS, AND SYSTEMICALLY ADMINISTERED NSAIDS. IN SEVERELY AFFECTED INDIVIDUALS, A RAPIDLY TAPERED COURSE OF SYSTEMIC GLUCOCORTICOIDs MAY BE USEFUL. JUDICIOUS USE OF ANALGESICS MAY BE NECESSARY. PHOTOALLERGIC REACTIONS REQUIRE A SIMILAR MANAGEMENT APPROACH. FURTHERMORE, PATIENTS WITH PERSISTENT LIGHT REACTION AND CHRONIC ACTINIC DERMATITIS MUST BE METICULOUSLY PROTECTED AGAINST LIGHT EXPOSURE. IN SELECTED PATIENTS IN WHOM CHRONIC SYSTEMIC HIGH-DOSE GLUCOCORTICOIDS POSE UNACCEPTABLE RISKS, IT MAY BE NECESSARY TO EMPLOY IMMUNOSUPPRESSIVE DRUGS SUCH AS AZATHIOPRINE, CYCLOPHOSPHAMIDE, CYCLOSPORINE, OR MYCOPHENOLATE MOFETIL.

PORPHYRIA THE PORPHYRIAS (CHAP. 352) ARE A GROUP OF DISEASES THAT
GENE LINKED TO HEMOCHROMATOSIS. THIS COULD CONTRIBUTE TO THE IRON OVERLOAD SEEN IN PCT, ALTHOUGH IRON STATUS AS MEASURED BY SERUM FERRITIN, IRON LEVELS, AND TRANSFERRIN SATURATION IS NO DIFFERENT FROM THAT IN PCT PATIENTS WITHOUT HFE MUTATIONS. PRIOR HEPATITIS C VIRUS INFECTION APPEARS TO BE AN INDEPENDENT RISK FACTOR FOR PCT.

TREATMENT OF PCT CONSISTS OF REPEATED PHLEBOTOMIES TO DIMINISH THE EXCESSIVE HEPATIC IRON STORES AND/OR INTERMITTENT LOW DOSES OF THE ANTIMALARIAL DRUGS CHLOROQUINE AND HYDROXYCHLOROQUINE. LONG-TERM REMISSION OF THE DISEASE CAN BE ACHIEVED IF THE PATIENT ELIMINATES EXPOSURE TO PORPHYRINOPTERGENIC AGENTS.

ERYTHROPOIETIC PROTOPORPHYRIA ORIGINES IN THE BONE MARROW AND IS DUE TO A DECREASE IN THE MITOCHONDRIAL ENZYME FERROCHELATASE SECONDARY TO NUMEROUS GENE MUTATIONS. THE MAJOR CLINICAL FEATURES INCLUDE AN ACUTE PHOTOSENSITIVITY CHARACTERIZED BY SUBJECTIVE BURNING AND STINGING OF EXPOSED SKIN THAT OFTEN DEVELOPS DURING OR JUST AFTER EXPOSURE. THERE MAY BE ASSOCIATED SKIN SWELLING AND, AFTER REPEATED EPISODES, A WAXLIKE SCARRING.

THE DIAGNOSIS IS CONFIRMED BY DEMONSTRATION OF ELEVATED LEVELS OF FREE ERYTHROCYTE PROTOPORPHYRIN. DETECTION OF INCREASED PLASMA PROTOPORPHYRIN HELPS TO DIFFERENTIATE LEAD POISONING AND IRON-DEFICIENCY ANEMIA, IN BOTH OF WHICH ELEVATED ERYTHROCYTE PROTOPORPHYRIN LEVELS OCCUR IN THE ABSENCE OF CUTANEOUS PHOTOSENSITIVITY AND OF ELEVATED PLASMA PROTOPORPHYRIN LEVELS.

TREATMENT CONSISTS OF REDUCING SUN EXPOSURE AND THE ORAL ADMINISTRATION OF THE CAROTENOID P-CAROTENE, WHICH IS AN EFFECTIVE SCAVENGER OF FREE RADICALS. THIS DRUG INCREASES TOLERANCE TO SUN EXPOSURE IN MANY AFFECTED INDIVIDUALS, ALTHOUGH IT HAS NO EFFECT ON DEFICIENT FERROCHELATASE.

AN ALGORITHM FOR MANAGING PATIENTS WITH PHOTOSENSITIVITY IS ILLUSTRATED IN FIG. 57-1.

PHOTOPROTECTION

SINCE PHOTOSENSITIVITY OF THE SKIN RESULTS FROM EXPOSURE TO SUNLIGHT, IT Follows THAT ABSOLUTE AVOIDANCE OF THE SUN WOULD ELIMINATE THESE DISORDERS. UNFORTUNATELY, CONTEMPORARY LIFE-PROTECt makes THIS AN IMPRACTICAL ALTERNATIVE FOR MOST INDIVIDUALS, AND THIS HAS LED TO A SEARCH FOR BETTER APPROACHES TO PHOTOPROTECTION.

NATURAL PHOTOPROTECTION IS PROVIDED BY STRUCTURAL PROTEINS IN THE EPIDERMIS, PARTICULARLY KERATINS AND MELANIN. THE AMOUNT OF MELANIN AND ITS DISTRIBUTION IN CELLS IS GENETICALLY REGULATED, AND INDIVIDUALS OF DARKER COMPLEXION (SKIN TYPES IV-VI) ARE AT DECREASED RISK FOR THE DEVELOPMENT OF ACUTE SUNBURN AND CUTANEOUS MALIGNANCY.

OTHER FORMS OF PHOTOPROTECTION INCLUDE CLOTHING AND SUNSCREENS. CLOTHING CONSTRUCTED OF TIGHTLY WOVEN SUN-PROTECTIVE FABRICS, IRRESPECTIVE OF COLOR, AFFORDS SUBSTANTIAL PROTECTION. WIDE-BRIMMED HATS, LONG
SLEEVES, AND TROUSERS ALL REDUCE DIRECT EXPOSURE. SUNSCREENS ARE NOW CONSIDERED TO BE OVER-THE-COUNTER DRUGS AND CATEGORY I INGREDIENTS ARE RECOGNIZED BY THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) AS MONOGRAPHED AND SAFE AND EFFECTIVE. THESE ARE LISTED IN TABLE 57-5. SUNSCREENS ARE RATED FOR THEIR PHOTOPROTECTIVE EFFECT BY THEIR SPF. THE SPF IS SIMPLY A RATIO OF THE TIME REQUIRED TO PRODUCE SUNBURN ERYTHEMA WITH AND WITHOUT SUNSCREEN APPLICATION. THE MONOGRAPH STIPULATES THAT SUNSCREENS MUST BE RATED ON A SCALE RANGING FROM MINIMAL (SPF *2 AND *12) TO MODERATE (SPF *12 AND *30) TO HIGH (SPF *30, LABELED AS 30+). NO SPF NUMBER >30 CAN BE PLACED ON THE LABEL. IN ADDITION TO LIGHT ABSORPTION, A CRITICAL DETERMINANT OF THE SUSTAINED PHOTOPROTECTIVE EFFECT OF SUNSCREENS IS THEIR WATER-RESISTANCE. FIGURE 57-1 AN ALGORITHM FOR THE DIAGNOSIS OF A PATIENT WITH PHOTO SENSITIVITY.

TABLE 57-5 FDA CATEGORY 1 MONOGRAPHED SUNSCREEN INGREDIENTS###A

INGREDIENTS

P-AMINOBENZOIC ACID (PABA)
AVOBENZONE
CINOXATE
DIOXYBENZONE (BENZOPHENONE-8)
ECAMSULE###B
HOMOSALATE
MENTHYL ANTHRANILATE
OCTOCRYLENE
OCTYL METHOXYCINNAMATE
OCTYL SALICYLATE
OXYPHENAZONE (BENZOPHENONE-3)
PADIMATEO (OCTYL DIMETHYL PABA)
PHENYLBENZIMIDAZOLE SULFONIC ACID
SULISOBENZONE (BENZOPHENONE-4)
TITANIUM DIOXIDE
TROLAMINE SALICYLATE
ZINC OXIDE

MAXIMUM CONCENTRATION, %

15
3
3
3
15
15
5
10
7.5
5
6
8
### CHAPTER 58 ANEMIA AND POLYCYTHEMIA

THE FDA MONOGRAPH HAS ALSO DEFINED STRICT TESTING CRITERIA FOR SUNSCREENS MAKING THIS CLAIM. SOME DEGREE OF PHOTOPROTECTION CAN ALSO BE ACHIEVED BY LIMITING THE TIME OF EXPOSURE DURING THE DAY. SINCE THE MAJORITY OF AN INDIVIDUAL’S TOTAL LIFETIME SUN EXPOSURE MAY OCCUR BY THE AGE OF 18, IT IS IMPORTANT TO EDUCATE PARENTS AND YOUNG CHILDREN ABOUT THE HAZARDS OF SUNLIGHT. SIMPLY ELIMINATING EXPOSURE AT MIDDAY WILL SUBSTANTIALLY REDUCE LIFETIME UV-B EXPOSURE.

**PHOTOTHERAPY AND PHOTOCHEMOTHERAPY**

UV CAN ALSO BE USED THERAPEUTICALLY. THE ADMINISTRATION OF UV-B ALONE OR IN COMBINATION WITH TOPICALLY APPLIED AGENTS CAN INDUCE REMISSIONS OF PSORIASIS AND ATOPIC DERMATITIS. PHOTOCHEMOTHERAPY IN WHICH TOPICALLY APPLIED OR SYSTEMICALLY ADMINISTERED PSORALENS ARE COMBINED WITH UV-A (PUVA) IS ALSO EFFECTIVE IN TREATING PSORIASIS AND IN THE EARLY STAGES OF CUTANEOUS T CELL LYMPHOMA AND VITILIGO. PSORALENS ARE TRICYCLIC FUROCOUMARINS THAT, WHEN INTERCALATED INTO DNA AND EXPOSED TO UV-A, FORM ADDUCTS WITH PYRIMIDINE BASES AND EVENTUALLY FORM DNA CROSS-LINKS. THESE STRUCTURAL CHANGES ARE THOUGHT TO DECREASE DNA SYNTHESIS AND RELATE TO THE IMPROVEMENT THAT OCCURS IN PSORIASIS. THE REASON THAT PUVA PHOTOCHEMOTHERAPY IS EFFECTIVE IN CUTANEOUS T CELL LYMPHOMA IS NOT CLEAR.

**SECTION 10 HEMATOLOGIC ALTERATIONS**

58 ANEMIA AND POLYCYTHEMIA

JOHN W. ADAMSON, DAN L. LONGO

**HEMATOPOIESIS AND THE PHYSIOLOGIC BASIS OF RED CELL PRODUCTION**

*HEMATOPOIESIS* IS THE PROCESS BY WHICH THE FORMED ELEMENTS OF THE BLOOD ARE PRODUCED. THE PROCESS IS REGULATED THROUGH A SERIES OF STEPS BEGINNING WITH THE PLURIPOTENT HEMATOPOIETIC STEM CELL. STEM CELLS ARE CAPABLE OF PRODUCING RED CELLS, ALL CLASSES OF GRANULOCYTES, MONOCYTES, PLATELETS, AND THE CELLS OF THE IMMUNE SYSTEM. THE PRECISE MOLECULAR MECHANISM—EITHER INTRINSIC TO THE STEM CELL ITSELF, OR THROUGH THE AC-
TION OF EXTRINSIC FACTORS BY WHICH THE STEM CELL BECOMES COMMITTED TO A GIVEN LINEAGE IS NOT FULLY DEFINED. HOWEVER, EXPERIMENTS IN MICE SUGGEST THAT ERYTHROID CELLS COME FROM A COMMON ERYTHROID/MEGA-KARYOCYTE PROGENITOR THAT DOES NOT DEVELOP IN THE ABSENCE OF EXPRESSION OF THE GATA-1 AND FOG-1 (FRIEND OF GATA-1) TRANSCRIPTION FACTORS (CHAP. 68). FOLLOWING LINEAGE COMMITMENT, HEMATOPOIETIC PROGENITOR AND PRECURSOR CELLS COME INCREASINGLY UNDER THE REGULATORY INFLUENCE OF GROWTH FACTORS AND HORMONES. FOR RED CELL PRODUCTION, ERYTHROPOIETIN (EPO) IS THE REGULATORY HORMONE. EPO IS REQUIRED FOR THE MAINTENANCE OF COMMITTED ERYTHROID PROGENITOR CELLS THAT, IN THE ABSENCE OF THE HORMONE, UNDERGO PROGRAMMED CELL DEATH (APOPTOSIS). THE REGULATED PROCESS OF RED CELL PRODUCTION IS ERYTHROPOIESIS, AND ITS KEY ELEMENTS ARE ILLUSTRATED IN FIG. 58-1.

In the bone marrow, the first morphologically recognizable erythroid precursor is the pronormoblast. This cell can undergo 4-5 cell divisions that result in the production of 16-32 mature red cells. With increased EPO production, or the administration of EPO as a drug, early progenitor cell numbers are amplified and, in turn, give rise to increased numbers of erythrocytes. The regulation of EPO production itself is linked to O2 availability. In mammals, O2 is transported to tissues bound to the hemoglobin contained within circulating red cells. The mature red cell is 8 *m in diameter, anucleate, discoid in shape, and extremely pliable in order to

In addition to its effects on DNA, PUVA photochemotherapy also stimulates epidermal thickening and melanin synthesis; the latter provides the rationale for its use in the depigmenting disease vitiligo. Oral 8-METHOXypsoralen and UV-A appear to be most effective in this regard, but as many as 100 treatments extending over 12-18 months may be required to promote satisfactory repigmentation. Not surprisingly the major side effects of long-term UV-B phototherapy and PUVA photochemotherapy mimic those seen in individuals with chronic sun exposure and include skin dryness, actinic keratoses, and an increased risk of skin cancer. Despite these risks, the therapeutic index of these modalities continues to be excellent.

FURTHER READINGS
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FIGURE 58-1 THE PHYSIOLOGIC REGULATION OF RED CELL PRODUCTION BY
TISSUE OXYGEN TENSION. HB. HEMOGLOBIN.

TRAVERSE THE MICROCIRCULATION SUCCESSFULLY; ITS MEMBRANE INTEGRITY IS MAINTAINED BY THE INTRACELLULAR GENERATION OF ATP. NORMAL RED CELL PRODUCTION RESULTS IN THE DAILY REPLACEMENT OF 0.8-1% OF ALL CIRCULATING RED CELLS IN THE BODY, SINCE THE AVERAGE RED CELL LIVES 100-120 DAYS. THE ORGAN RESPONSIBLE FOR RED CELL PRODUCTION IS CALLED THE ERYTHRON. THE ERYTHRON IS A DYNAMIC ORGAN MADE UP OF A RAPIDLY PROLIFERATING POOL OF MARROW ERYTHROID PRECURSOR CELLS AND A LARGE MASS OF MATURE CIRCULATING RED BLOOD CELLS. THE SIZE OF THE RED CELL MASS REFLECTS THE BALANCE OF RED CELL PRODUCTION AND DESTRUCTION. THE PHYSIOLOGIC BASIS OF RED CELL PRODUCTION AND DESTRUCTION PROVIDES AN UNDERSTANDING OF THE MECHANISMS THAT CAN LEAD TO ANEMIA. THE PHYSIOLOGIC REGULATOR OF RED CELL PRODUCTION, THE GLYCOPROTEIN HORMONE EPO, IS PRODUCED AND RELEASED BY PERITUBULAR CAPILLARY LINING CELLS WITHIN THE KIDNEY. THESE CELLS ARE HIGHLY SPECIALIZED EPITHELIAL-LIKE CELLS. A SMALL AMOUNT OF EPO IS PRODUCED BY HEPATOCYTES. THE

PAGE NO. 5

356 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

FUNDAMENTAL STIMULUS FOR EPO PRODUCTION IS THE AVAILABILITY OF O\textsubscript{2} FOR TISSUE METABOLIC NEEDS. IMPAIRED O\textsubscript{2} DELIVERY TO THE KIDNEY CAN RESULT FROM A DECREASED RED CELL MASS (ANEMIA), IMPAIRED O\textsubscript{2} LOADING OF THE HEMOGLOBIN MOLECULE OR A HIGH O\textsubscript{2} AFFINITY MUTANT HEMOGLOBIN (HYPOXEMIA), OR, RARELY, IMPAIRED BLOOD FLOW TO THE KIDNEY (RENAL ARTERY STENOSIS). EPO GOVERNS THE DAY-TO-DAY PRODUCTION OF RED CELLS, AND AMBIENT LEVELS OF THE HORMONE CAN BE MEASURED IN THE PLASMA BY SENSITIVE IMMUNOASSAYS—THE NORMAL LEVEL BEING 10-25 U/L. WHEN THE HEMOGLOBIN CONCENTRATION FALLS BELOW 100-120 G/L (10-12 G/DL), PLASMA EPO LEVELS INCREASE IN PROPORTION TO THE SEVERITY OF THE ANEMIA (FIG. 58-2). IN CIRCULATION, EPO HAS A HALF-CLEARANCE TIME OF 6-9 H. EPO ACTS BY BINDING TO SPECIFIC RECEPTORS ON THE SURFACE OF MARROW ERYTHROID PRECURSORS, INDUCING THEM TO PROLIFERATE AND TO MATURE. WITH EPO STIMULATION, RED CELL PRODUCTION CAN INCREASE FOUR- TO FIVEFOLD WITHIN A 1- TO 2-WEEK PERIOD BUT ONLY IN THE PRESENCE OF ADEQUATE NUTRIENTS, ESPECIALLY IRON. THE FUNCTIONAL CAPACITY OF THE ERYTHRON, THEREFORE, REQUIRES NORMAL RENAL PRODUCTION OF EPO, A FUNCTIONING ERYTHROID MARROW, AND AN ADEQUATE SUPPLY OF SUBSTRATES FOR HEMOGLOBIN SYNTHESIS. A DEFECT IN ANY OF THESE KEY COMPONENTS CAN LEAD TO ANEMIA. GENERALLY, ANEMIA IS RECOGNIZED IN THE LABORATORY WHEN A PATIENT’S HEMOGLOBIN LEVEL OR HEMATOCRIT IS REDUCED BELOW AN EXPECTED VALUE
(THE NORMAL RANGE). THE LIKELIHOOD AND SEVERITY OF ANEMIA ARE DEFINED BASED ON THE DEVIATION OF THE PATIENT’S HEMOGLOBIN/HEMATOCRIT FROM VALUES EXPECTED FOR AGE- AND SEX-MATCHED NORMAL SUBJECTS. THE HEMOGLOBIN CONCENTRATION IN ADULTS HAS A GAUSSIAN DISTRIBUTION. THE MEAN HEMATOCRIT VALUE FOR ADULT MALES IS 47% (* SD 7) AND THAT FOR ADULT FEMALES IS 42% (* 5). ANY SINGLE HEMATOCRIT OR HEMOGLOBIN VALUE CARRIES WITH IT A LIKELIHOOD OF ASSOCIATED ANEMIA. THUS, A HEMATOCRIT OF *39% IN AN ADULT MALE OR <35% IN AN ADULT FEMALE HAS ONLY ABOUT A 25% CHANCE OF BEING NORMAL. SUSPECTED LOW HEMOGLOBIN OR HEMATOCRIT VALUES ARE MORE EASILY INTERPRETED IF PREVIOUS VALUES FOR THE SAME PATIENT ARE KNOWN FOR COMPARISON. THE WORLD HEALTH ORGANIZATION (WHO) DEFINES ANEMIA AS A HEMOGLOBIN LEVEL < 130 G/L (13 G/DL) IN MEN AND <120 G/L (12 G/DL) IN WOMEN.


ANEMIA

CLINICAL PRESENTATION OF ANEMIA

SIGNS AND SYMPTOMS ANEMIA IS MOST OFTEN RECOGNIZED BY ABNORMAL SCREENING LABORATORY TESTS. PATIENTS LESS COMMONLY PRESENT WITH ADVANCED ANEMIA AND ITS ATTENDANT SIGNS AND SYMPTOMS. ACUTE ANEMIA IS NEARLY ALWAYS DUE TO BLOOD LOSS OR HEMOLYSIS. IF BLOOD LOSS IS MILD, ENHANCED O2 DELIVERY IS ACHIEVED THROUGH CHANGES IN THE O2-HEMOGLOBIN DISSOCIATION CURVE MEDIATED BY A DECREASED PH OR INCREASED CO2 (BOHR EFFECT). WITH ACUTE BLOOD LOSS, HYPOVolemIA DOMINATES THE CLINICAL PICTURE AND THE HEMATOCRIT AND HEMOGLOBIN LEVELS DO NOT REFLECT THE VOLUME OF BLOOD LOST. SIGNS OF VASCULAR INSTABILITY APPEAR WITH ACUTE LOSSES OF 10-15% OF THE TOTAL BLOOD VOLUME. IN SUCH PATIENTS, THE ISSUE IS NOT ANEMIA BUT HYPOTENSION AND DECREASED ORGAN PERFUSION. WHEN >30% OF THE BLOOD VOLUME IS LOST SUDDENLY, PATIENTS ARE UNABLE TO COMPENSATE WITH THE USUAL MECHANISMS OF VASCULAR CONTRACTION AND CHANGES IN REGIONAL BLOOD FLOW. THE PATIENT PREFERS TO REMAIN SUPINE AND WILL SHOW POSTURAL HYPOTENSION AND TACHYCARDIA. IF THE VOLUME OF BLOOD LOST IS >40% (I.E., >2 L IN THE AVERAGE-SIZED ADULT), SIGNS OF HYPOVOLEMIC SHOCK INCLUDING CONFUSION, DYSPNEA, DIAPHORESIS, HYPOTENSION, AND TACHYCARDIA APPEAR (CHAP. 101). SUCH PATIENTS HAVE

FIGURE 58-2 ERYTHROPOIETIN LEVELS IN RESPONSE TO ANEMIA. WHEN THE HEMOGLOBIN LEVEL FALLS TO 120 G/L (12 G/DL), PLASMA ERYTHROPOIETIN LEVELS INCREASE LOGARITHMICALLY. IN THE PRESENCE OF RENAL DISEASE OR CHRONIC INFLAMMATION, EPO LEVELS ARE TYPICALLY LOWER THAN EXPECTED FOR A PARTICULAR LEVEL OF ANEMIA. AS INDIVIDUALS AGE, THE LEVEL OF EPO NEEDED TO SUSTAIN NORMAL HEMOGLOBIN LEVELS APPEARS TO INCREASE. (FROM HILLMAN ET AL.)
SIGNIFICANT DEFICITS IN VITAL ORGAN PERFUSION AND REQUIRE IMMEDIATE VOLUME REPLACEMENT. WITH ACUTE HEMOLYTIC DISEASE, THE SIGNS AND SYMPTOMS DEPEND ON THE MECHANISM THAT LEADS TO RED CELL DESTRUCTION. INTRAVASCULAR HEMOLYSIS WITH RELEASE OF FREE HEMOGLOBIN MAY BE ASSOCIATED WITH ACUTE BACK PAIN, FREE HEMOGLOBIN IN THE PLASMA AND URINE, AND RENAL FAILURE. SYMPTOMS ASSOCIATED WITH MORE CHRONIC OR PROGRESSIVE ANEMIA DEPEND ON THE AGE OF THE PATIENT AND THE ADEQUACY OF BLOOD SUPPLY TO CRITICAL ORGANS. SYMPTOMS ASSOCIATED WITH MODERATE ANEMIA INCLUDE FATIGUE, LOSS OF STAMINA, BREATHLESSNESS, AND TACHYCARDIA (PARTICULARLY WITH PHYSICAL EXERTION). HOWEVER, BECAUSE OF THE INTRINSIC COMPENSATORY MECHANISMS THAT GOVERN THE O\textsuperscript{2}-HEMOGLOBIN DISSOCIATION CURVE, THE GRADUAL ONSET OF ANEMIA-PARTICULARLY IN YOUNG PATIENTS-MAY NOT BE ASSOCIATED WITH SIGNS OR SYMPTOMS UNTIL THE ANEMIA IS SEVERE [HEMOGLOBIN <70-80 G/L (7-8 G/DL)]. WHEN ANEMIA DEVELOPS OVER A PERIOD OF DAYS OR WEEKS, THE TOTAL BLOOD VOLUME IS NORMAL TO SLIGHTLY INCREASED AND CHANGES IN CARDIAC OUTPUT AND REGIONAL BLOOD FLOW HELP COMPENSATE FOR THE OVERALL LOSS IN O\textsuperscript{2}-CARRYING CAPACITY. CHANGES IN THE POSITION OF THE O\textsuperscript{2}-HEMOGLOBIN DISSOCIATION CURVE ACCOUNT FOR SOME OF THE COMPENSATORY RESPONSE TO ANEMIA. WITH CHRONIC ANEMIA, INTRACELLULAR LEVELS OF 2, 3-BISPHOSPHOGLYCERATE RISE, SHIFTING THE DISSOCIATION CURVE TO THE RIGHT AND FACILITATING O\textsuperscript{2} UNLOADING. THIS COMPENSATORY MECHANISM CAN ONLY MAINTAIN NORMAL TISSUE O\textsuperscript{2} DELIVERY IN THE FACE OF A 20-30 G/L (2-3 G/DL) DEFICIT IN HEMOGLOBIN CONCENTRATION. FINALLY, FURTHER PROTECTION OF O\textsuperscript{2} DELIVERY TO VITAL ORGANS IS ACHIEVED BY THE SHUNTING OF BLOOD AWAY FROM ORGANS THAT ARE RELATIVELY RICH IN BLOOD SUPPLY, PARTICULARLY THE KIDNEY, GUT, AND SKIN.

CERTAIN DISORDERS ARE COMMONLY ASSOCIATED WITH ANEMIA. CHRONIC INFLAMMATORY STATES (E.G., INFECTION, RHEUMATOID ARTHRITIS) ARE ASSOCIATED WITH MILD TO MODERATE ANEMIA, WHEREAS LYMPHOPROLIFERATIVE DISORDERS, SUCH AS CHRONIC LYMPHOCYTIC LEUKEMIA AND CERTAIN OTHER B CELL NEOPLASMS, MAY BE ASSOCIATED WITH AUTOIMMUNE HEMOLYSIS.

APPROACH TO THE PATIENT:

ANEMIA

THE EVALUATION OF THE PATIENT WITH ANEMIA REQUIRES A CAREFUL HISTORY AND PHYSICAL EXAMINATION. NUTRITIONAL HISTORY RELATED TO DRUGS OR ALCOHOL INTAKE AND FAMILY HISTORY OF ANEMIA SHOULD ALWAYS BE ASSESSED. CERTAIN GEOGRAPHIC BACKGROUNDS AND ETHNIC ORIGINS ARE ASSOCIATED WITH AN INCREASED LIKELIHOOD OF AN INHERITED DISORDER OF THE HEMOGLOBIN MOLECULE OR INTERMEDIARY METABOLISM. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY AND CERTAIN HEMOGLOBINOPathIES ARE SEEN MORE COMMONLY IN THOSE OF MIDDLE EASTERN OR AFRICAN ORIGIN, INCLUDING AFRICAN AMERICANS WHO HAVE A HIGH FREQUENCY OF G6PD DEFICIENCY. OTHER INFORMATION THAT MAY BE USEFUL INCLUDES EXPOSURE TO CERTAIN TOXIC AGENTS OR DRUGS AND SYMPTOMS RELATED TO OTHER DISORDERS COMMONLY ASSOCIATED WITH ANEMIA. THESE
INCLUDE SYMPTOMS AND SIGNS SUCH AS BLEEDING, FATIGUE, MALAISE, FEVER, WEIGHT LOSS, NIGHT SWEATS, AND OTHER SYSTEMIC SYMPTOMS. CLUES TO THE MECHANISMS OF ANEMIA MAY BE PROVIDED ON PHYSICAL EXAMINATION BY FINDINGS OF INFECTION, BLOOD IN THE STOOL, LYMPHADENOPATHY, SPLENOMEGALY, OR PETECHiae. SPLENOMEGALY AND LYMPHADENOPATHY, SPLENOMEGALY, OR PETECHiae.

PAGE NO. 6

357 CHAPTER 58 ANEMIA AND POLYCYTHEMIA

TABLE 58-1 LABORATORY TESTS IN ANEMIA DIAGNOSIS

I. COMPLETE BLOOD COUNT (CBC)
A. RED BLOOD CELL COUNT
   1. HEMOGLOBIN
   2. HEMATOCRIT
   3. RETICULOCYTE COUNT
B. RED BLOOD CELL INDICES
   1. MEAN CELL VOLUME (MCV)
   2. MEAN CELL HEMOGLOBIN (MCH)
   3. MEAN CELL HEMOGLOBIN CONCENTRATION (MCHC)
   4. RED CELL DISTRIBUTION WIDTH (RDW)
C. WHITE BLOOD CELL COUNT
   1. CELL DIFFERENTIAL
   2. NUCLEAR SEGMENTATION OF NEUTROPHILS
D. PLATELET COUNT
E. CELL MORPHOLOGY
   1. CELL SIZE
   2. HEMOGLOBIN CONTENT
   3. ANISOCYTOSIS
   4. POIKILOCYTOSIS
   5. POLYCHROMASIA

II. IRON SUPPLY STUDIES
A. SERUM IRON
B. TOTAL IRON-BINDING CAPACITY
C. SERUM FERRITIN

III. MARROW EXAMINATION
A. ASPIRATE
   1. M/E RATIO###A
   2. CELL MORPHOLOGY
   3. IRON STAIN
B. BIOPSY
   1. CELULARITY
   2. MORPHOLOGY

###AM/E RATIO, RATIO OF MYELOID TO ERYTHROID PRECURSORS.
NOPATHY SUGGEST AN UNDERLYING LYMPHOPROLIFERATIVE DISEASE, WHILE
PETECHIAE SUGGEST PLATELET DYSFUNCTION. PAST LABORATORY MEASURE-
MENTS MAY BE HELPFUL TO DETERMINE A TIME OF ONSET.
IN THE ANEMIC PATIENT, PHYSICAL EXAMINATION MAY DEMONSTRATE A
FORCEFUL HEARTBEAT, STRONG PERIPHERAL PULSES, AND A SYSTOLIC “FLOW”
MURMUR. THE SKIN AND MUCOUS MEMBRANES MAY BE PALE IF THE HE-
MOGLOBIN IS <80-100 G/L (8-10 G/DL). THIS PART OF THE PHYSICAL EX-
AMINATION SHOULD FOCUS ON AREAS WHERE VESSELS ARE CLOSE TO THE
SURFACE SUCH AS THE MUCOUS MEMBRANES, NAIL BEDS, AND PALMAR
CREASES. IF THE PALMAR CREASES ARE LIGHTER IN COLOR THAN THE SURROUND-
ING SKIN WHEN THE HAND IS HYPEREXTENDED, THE HEMOGLOBIN LEVEL IS
USUALLY <80 G/L (8 G/DL).

LABORATORY EVALUATION

TABLE 58-1 LISTS THE TESTS USED IN THE INI-
TIAL WORKUP OF ANEMIA. A ROUTINE COMPLETE BLOOD COUNT (CBC) IS
REQUIRED AS PART OF THE EVALUATION AND INCLUDES THE HEMOGLOBIN, HE-
MATOCRIT, AND RED CELL INDICES: THE MEAN CELL VOLUME (MCV) IN FEM-
TOLITERS, MEAN CELL HEMOGLOBIN (MCH) IN PICOGRAMS PER CELL, AND
MEAN CONCENTRATION OF HEMOGLOBIN PER VOLUME OF RED CELLS
(MCHC) IN GRAMS PER LITER (NON-SL: GRAMS PER DECILITER). THE RED
CELL INDICES ARE CALCULATED AS SHOWN IN TABLE 58-2, AND THE NORMAL
VARIATIONS IN THE HEMOGLOBIN AND HEMATOCRIT WITH AGE ARE SHOWN IN
TABLE 58-3. A NUMBER OF PHYSIOLOGIC FACTORS AFFECT THE CBC INCLUDING AGE, SEX, PREGNANCY, SMOKING, AND ALTITUDE. HIGH-NORMAL HE-
MOGLOBIN VALUES MAY BE SEEN IN MEN AND WOMEN WHO LIVE AT
ALTITUDE OR SMOKE HEAVILY. HEMOGLOBIN ELEVATIONS DUE TO SMOKING
REFLECT NORMAL COMPENSATION DUE TO THE DISPLACEMENT OF O\textsubscript{2} BY CO
IN HEMOGLOBIN BINDING. OTHER IMPORTANT INFORMATION IS PROVIDED
BY THE RETICULOCYTE COUNT AND MEASUREMENTS OF IRON SUPPLY INCLUDE-
ing SERUM IRON, TOTAL IRON-BINDING CAPACITY (TIBC: AN INDIRECT MEAS-
URE OF THE TRANSFERRIN LEVEL), AND SERUM FERRITIN. MARKED ALTERATIONS
IN THE RED CELL INDICES USUALLY REFLECT DISORDERS OF MATURATION OR
IRON DEFICIENCY. A CAREFUL EVALUATION OF THE PERIPHERAL BLOOD SMEAR

TABLE 58-2 RED BLOOD CELL INDICES

INDEX

\[
\text{MEAN CELL VOLUME (MCV)} = \frac{(\text{HEMATOCRIT X 10})}{(\text{RED \text{CELL COUNT X 10}}^*)} \\
\text{MEAN CELL HEMOGLOBIN (MCH)} = \frac{(\text{HEMOGLOBIN X 10})}{(\text{RED \text{CELL COUNT X 10}}^*)} \\
\text{MEAN CELL HEMOGLOBIN CONCENTRATION} = \frac{(\text{HEMOGLOBIN X 10})}{\text{HEMATOCRIT, OR MCH/MCV}}
\]

NORMAL VALUE

90 * 8 FL
30 * 3 PG
33 *2\%
TABLE 58-3 CHANGES IN NORMAL HEMOGLOBIN/HEMATOCRIT VALUES WITH AGE AND PREGNANCY

AGE/SEX
AT BIRTH
CHILDHOOD
ADOLESCENCE
ADULT MAN
ADULT WOMAN (MENSTRUATING)
ADULT WOMAN (POSTMENOPAUSAL)
DURING PREGNANCY

<table>
<thead>
<tr>
<th></th>
<th>AT BIRTH</th>
<th>CHILDHOOD</th>
<th>ADOLESCENCE</th>
<th>ADULT MAN</th>
<th>ADULT WOMAN (MENSTRUATING)</th>
<th>ADULT WOMAN (POSTMENOPAUSAL)</th>
<th>DURING PREGNANCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMOGLOBIN G/DL</td>
<td>17</td>
<td>12</td>
<td>13</td>
<td>16 (*2)</td>
<td>13 (*2)</td>
<td>14 (*2)</td>
<td>12 (*2)</td>
</tr>
<tr>
<td>HEMATOCRIT%</td>
<td>52</td>
<td>36</td>
<td>40</td>
<td>47 (*6)</td>
<td>40 (*6)</td>
<td>42 (*6)</td>
<td>37 (*6)</td>
</tr>
</tbody>
</table>

SOURCE: FROM HILLMAN ET AL.

IS IMPORTANT, AND CLINICAL LABORATORIES OFTEN PROVIDE A DESCRIPTION OF BOTH THE RED AND WHITE CELLS, A WHITE CELL DIFFERENTIAL COUNT, AND THE PLATELET COUNT. IN PATIENTS WITH SEVERE ANEMIA AND ABNORMALITIES IN RED BLOOD CELL MORPHOLOGY AND/OR LOW RETICULOCYTE COUNTS, A BONE MARROW ASPIRATE OR BIOPSY MAY BE IMPORTANT TO ASSIST IN THE DIAGNOSIS. OTHER TESTS OF VALUE IN THE DIAGNOSIS OF SPECIFIC ANEMIAS ARE DISCUSSED IN CHAPTERS ON SPECIFIC DISEASE STATES.

THE COMPONENTS OF THE CBC ALSO HELP IN THE CLASSIFICATION OF ANEMIA. MICROCYTOSIS IS REFLECTED BY A LOWER THAN NORMAL MCV (<80), WHEREAS HIGH VALUES (>100) REFLECT MACROCYTOSIS. THE MCH AND MCHC REFLECT DEFECTS IN HEMOGLOBIN SYNTHESIS (HYPOCHROMIA). AUTOMATED CELL COUNTERS DESCRIBE THE RED CELL VOLUME DISTRIBUTION WIDTH (RDW). THE MCV (REPRESENTING THE PEAK OF THE DISTRIBUTION CURVE) IS INSSENSITIVE TO THE APPEARANCE OF SMALL POPULATIONS OF MACROCYTES OR MICROCYTES. AN EXPERIENCED LABORATORY TECHNICIAN WILL BE ABLE TO IDENTIFY MINOR POPULATIONS OF LARGE OR SMALL CELLS OR HYPOCHROMIC CELLS BEFORE THE RED CELL INDICES CHANGE.

PERIPHERAL BLOOD SMEAR THE PERIPHERAL BLOOD SMEAR PROVIDES IMPORTANT INFORMATION ABOUT DEFECTS IN RED CELL PRODUCTION. AS A COMPLEMENT TO THE RED CELL INDICES, THE BLOOD SMEAR ALSO REVEALS VARIATIONS IN CELL SIZE (ANISOCYTOSIS) AND SHAPE (POIKILOCYTOSIS). THE DEGREE OF ANISOCYTOSIS USUALLY CORRELATES WITH INCREASES IN THE RDW.
OR THE RANGE OF CELL SIZES. POIKILOCYTOSIS SUGGESTS A DEFECT IN THE MATURATION OF RED CELL PRECURSORS IN THE BONE MARROW OR FRAGMENTATION OF CIRCULATING RED CELLS. THE BLOOD SMEAR MAY ALSO REVEAL POLYCHROMASIA-RED CELLS THAT ARE SLIGHTLY LARGER THAN NORMAL AND GRAYISH BLUE IN COLOR ON THE WRIGHT-GIEMSA STAIN. THESE CELLS ARE RETICULOCYTES THAT HAVE BEEN PREMATURELY RELEASED FROM THE BONE MARROW, AND THEIR COLOR REPRESENTS RESIDUAL AMOUNTS OF RIBOSOMAL RNA. THESE CELLS APPEAR IN CIRCULATION IN RESPONSE TO EPO STIMULATION OR TO ARCHITECTURAL DAMAGE OF THE BONE MARROW (FIBROSIS, INFILTRATION OF THE MARROW BY MALIGNANT CELLS, ETC.) THAT RESULTS IN THEIR DISORDERED RELEASE FROM THE MARROW. THE APPEARANCE OF NUCLEATED RED CELLS, HOWELL-JOLLY BODIES, TARGET CELLS, SICKLE CELLS, AND OTHERS MAY PROVIDE CLUES TO SPECIFIC DISORDERS (FIGS. 58-3 TO 58-11).

FIGURE 58-3 NORMAL BLOOD SMEAR (WRIGHT'S STAIN). HIGH-POWER FIELD SHOWING NORMAL RED CELLS, A NEUTROPHIL, AND A FEW PLATELETS. (FROM HILLMAN ET AL.)

361 CHAPTER 58 ANEMIA AND POLYCYTHEMIA

FIGURE 58-17 THE PHYSIOLOGIC CLASSIFICATION OF ANEMIA. CBC, COMPLETE BLOOD COUNT.

BE DISTINGUISHED BY THE RED CELL INDICES, BY EXAMINATION OF THE PERIPHERAL BLOOD SMEAR, OR BY A MARROW EXAMINATION. IF THE RED CELL INDICES ARE NORMAL, THE ANEMIA IS ALMOST CERTAINLY HYPOPROLIFERATIVE IN NATURE. MATURATION DISORDERS ARE CHARACTERIZED BY INEFFECTIVE RED CELL PRODUCTION AND A LOW RETICULOCYTE PRODUCTION INDEX. BIZARRE RED CELL SHAPES-MACROCYTES OR HYPOCHROMIC MICROCYTES-ARE SEEN ON THE PERIPHERAL BLOOD SMEAR. WITH A HYPOPROLIFERATIVE ANEMIA, NO ERYTHROID HYPERPLASIA IS NOTED IN THE MARROW, WHEREAS PATIENTS WITH INEFFECTIVE RED CELL PRODUCTION HAVE ERYTHROID HYPERPLASIA AND AN M/E RATIO < 1:1.

HYPOPROLIFERATIVE ANEMIAS AT LEAST 75% OF ALL CASES OF ANEMIA ARE HYPOPROLIFERATIVE IN NATURE. A HYPOPROLIFERATIVE ANEMIA REFLECTS ABSOLUTE OR RELATIVE MARROW FAILURE IN WHICH THE ERYTHROID MARROW HAS NOT PROLIFERATED APPROPRIATELY FOR THE DEGREE OF ANEMIA. THE MAJORITY OF HYPOPROLIFERATIVE ANEMIAS ARE DUE TO MILD TO MODERATE IRON DEFICIENCY OR INFLAMMATION. A HYPOPROLIFERATIVE ANEMIA CAN RESULT FROM MARROW DAMAGE, IRON DEFICIENCY, OR INADEQUATE EPO STIMULATION. THE LAST MAY REFLECT IMPAIRED RENAL FUNCTION, SUPPRESSION OF EPO PRODUCTION BY IN-
FLAMMATORY CYTOKINES SUCH AS INTERLEUKIN 1, OR REDUCED TISSUE NEEDS FOR O2 FROM METABOLIC DISEASE SUCH AS HYPOTHYROIDISM. ONLY OCCASSIONALLY IS THE MARROW UNABLE TO PRODUCE RED CELLS AT A NORMAL RATE, AND THIS IS MOST PREVALENT IN PATIENTS WITH RENAL FAILURE. WITH DIABETES MELLITUS OR MYELOMA, THE EPO DEFICIENCY MAY BE MORE MARKED THAN WOULD BE PREDICTED BY THE DEGREE OF RENAL INSUFFICIENCY. IN GENERAL, HYPOPROLIFERATIVE ANEMIAS ARE CHARACTERIZED BY NORMOCYTIC, NORMOCHROMIC RED CELLS, ALTHOUGH MICROCYTIC, HYPOCHROMIC CELLS MAY BE OBSERVED WITH MILD IRON DEFICIENCY OR LONG-STANDING CHRONIC INFLAMMATORY DISEASE. THE KEY LABORATORY TESTS IN DISTINGUISHING BETWEEN THE VARIOUS FORMS OF HYPOPROLIFERATIVE ANEMIA INCLUDE THE SERUM IRON AND IRON-BINDING CAPACITY, EVALUATION OF RENAL AND THYROID FUNCTION, A MARROW BIOPSY OR ASPIRATE TO DETECT MARROW DAMAGE OR INFILTRATIVE DISEASE, AND SERUM FERRITIN TO ASSESS IRON STORES. OCCASIONALLY, AN IRON STAIN OF THE MARROW WILL BE NEEDED TO DETERMINE THE PATTERN OF IRON DISTRIBUTION. PATIENTS WITH THE ANEMIA OF ACUTE OR CHRONIC INFLAMMATION SHOW A DISTINCTIVE PATTERN OF SERUM IRON (LOW), TIBC (NORMAL OR LOW), PERCENT TRANSFERRIN SATURATION (LOW), AND SERUM FERRITIN (NORMAL OR HIGH). THESE CHANGES IN IRON VALUES ARE BROUGHT ABOUT BY HEPcidin, THE IRON REGULATORY HORMONE THAT IS INCREASED IN INFLAMMATION (CHAP. 98). A DISTINCT PATTERN OF RESULTS IS NOTED IN MILD TO MODERATE IRON DEFICIENCY (LOW SERUM IRON, HIGH TIBC, LOW PERCENT TRANSFERRIN SATURATION, LOW SERUM FERRITIN) (CHAP. 98). MARROW DAMAGE BY DRUGS, SUCH AS THE ANTI-RETROVIRALS USED TO TREAT HIV INFECTION, INFILTRATIVE DISEASE SUCH AS LEUKEMIA OR LYMPHOMA, OR MARROW APLASIA CAN USUALLY BE DIAGNOSED FROM THE PERIPHERAL BLOOD AND BONE MARROW MORPHOLOGY. WITH INFILTRATIVE DISEASE OR FIBROSIS, A MARROW BIOPSY IS REQUIRED.

MATURATION DISORDERS THE PRESENCE OF ANEMIA WITH AN INAPPROPRIATELY LOW RETICULOCYTE PRODUCTION INDEX, MACRO- OR MICROCYTOSIS ON SMEAR, AND ABNORMAL RED CELL INDICES SUGGESTS A MATURATION DISORDER. MATURATION DISORDERS ARE DIVIDED INTO TWO CATEGORIES: NUCLEAR MATURATION DEFECTS, ASSOCIATED WITH MACROCYTOSIS AND ABNORMAL MARROW DEVELOPMENT, AND CYTOPLASMIC MATURATION DEFECTS, ASSOCIATED WITH MICROCYTOSIS AND HYPOCHROMIA USUALLY FROM DEFECTS IN HEMOGLOBIN SYNTHESIS. THE INAPPROPRIATELY LOW RETICULOCYTE PRODUCTION INDEX IS A REFLECTION OF THE INEFFECTIVE ERYTHROPOIESIS THAT RESULTS FROM THE DESTRUCTION WITHIN THE MARROW OF DEVELOPING ERYTHROBLASTS. BONE MARROW EXAMINATION SHOWS ERYTHROID HYPERPLASIA. NUCLEAR MATURATION DEFECTS RESULT FROM VITAMIN B12 OR FOLIC ACID DEFICIENCY, DRUG DAMAGE, OR MYELODYSPLASIA. DRUGS THAT INTERFERE WITH CELLULAR DNA METABOLISM, SUCH AS METHOTREXATE OR ALKYLATING AGENTS, CAN PRODUCE A NUCLEAR MATURATION DEFECT. ALCOHOL, ALONE, IS ALSO CAPABLE OF PRODUCING MACROCYTOSIS AND A VARIABLE DEGREE OF ANEMIA, BUT THIS IS USUALLY ASSOCIATED WITH FOLIC ACID DEFICIENCY. MEASUREMENTS OF FOLIC ACID AND VITAMIN B12 ARE KEY NOT ONLY IN IDENTIFYING THE SPECIFIC

BLOOD LOSS/HEMOLYTIC ANEMIA IN CONTRAST TO ANEMIAS ASSOCIATED WITH AN INAPPROPRIATELY LOW RETICULOCYTE PRODUCTION INDEX, HEMOLYSIS IS ASSOCIATED WITH RED CELL PRODUCTION INDICES *2.5 TIMES NORMAL. THE STIMULATED ERYTHROPOIESIS IS REFLECTED IN THE BLOOD SMEAR BY THE APPEARANCE OF INCREASED NUMBERS OF POLYCHROMATOPHILIC MACROCYTES. A MARROW EXAMINATION IS RARELY INDICATED IF THE RETICULOCYTE PRODUCTION INDEX IS INCREASED APPROPRIATELY. THE RED CELL INDICES ARE TYPICALLY NORMOCYTIC OR SLIGHTLY MACROCYTIC, REFLECTING THE INCREASED NUMBER OF RETICULOCYTES. ACUTE BLOOD LOSS IS NOT ASSOCIATED WITH AN INCREASED RETICULOCYTE PRODUCTION INDEX BECAUSE OF THE TIME REQUIRED TO INCREASE EPO PRODUCTION AND, SUBSEQUENTLY, MARROW PROLIFERATION. SUBACUTE BLOOD LOSS MAY BE ASSOCIATED WITH MODEST RETICULOCYTOSIS. ANEMIA FROM CHRONIC
BLOOD LOSS PRESENTS MORE OFTEN AS IRON DEFICIENCY THAN WITH THE
PICTURE
OF INCREASED RED CELL PRODUCTION.
THE EVALUATION OF BLOOD LOSS ANEMIA IS USUALLY NOT DIFFICULT. MOST
PROBLEMS ARISE WHEN A PATIENT PRESENTS WITH AN INCREASED RED CELL PRO-
DUCTION INDEX FROM AN EPISODE OF ACUTE BLOOD LOSS THAT WENT UNRECOG-
IZED. THE CAUSE OF THE ANEMIA AND INCREASED RED CELL PRODUCTION MAY
NOT BE OBVIOUS. THE CONFIRMATION OF A RECOVERING STATE MAY REQUIRE
OBSERVATIONS OVER A PERIOD OF 2-3 WEEKS, DURING WHICH THE HEMOGLO-
BIN CONCENTRATION WILL BE SEEN TO RISE AND THE RETICULOCYTE PRODUCTION
INDEX FALL.
HEMOLYTIC DISEASE, WHILE DRAMATIC, IS AMONG THE LEAST COMMON
FORMS OF ANEMIA. THE ABILITY TO SUSTAIN A HIGH RETICULOCYTE PRODUCTION
INDEX REFLECTS THE ABILITY OF THE ERYTHROID MARROW TO COMPENSATE FOR
HEMOLYSIS AND, IN THE CASE OF EXTRAVASCULAR HEMOLYSIS, THE EFFICIENT RE-
CYCLING OF IRON FROM THE DESTROYED RED CELLS TO SUPPORT RED CELL PRODUC-
TION. WITH INTRAVASCULAR HEMOLYSIS, SUCH AS PAROXYSMAL NOCTURNAL
HEMOGLOBINURIA, THE LOSS OF IRON MAY LIMIT THE MARROW RESPONSE. THE
LEVEL OF RESPONSE DEPENDS ON THE SEVERITY OF THE ANEMIA AND THE NATURE
OF THE UNDERLYING DISEASE PROCESS.
HEMOGLOBINOPATHIES, SUCH AS SICKLE CELL DISEASE AND THE THALAS-
SEMIAS, PRESENT A MIXED
PICTURE. THE RETICULOCYTE INDEX MAY BE HIGH
BUT IS INAPPROPRIATELY LOW FOR THE DEGREE OF MARROW ERYTHROID HYPER-
PLASIA (CHAP. 99).
HEMOLYTIC ANEMIAS PRESENT IN DIFFERENT WAYS. SOME APPEAR SUDDEN-
LY AS AN ACUTE, SELF-LIMITED EPISODE OF INTRAVASCULAR OR EXTRAVASCULAR
HEMOLYSIS, A PRESENTATION PATTERN OFTEN SEEN IN PATIENTS WITH AUTOIM-
MUNE HEMOLYSIS OR WITH INHERITED DEFECTS OF THE EMBDEN-MEYERHOF
PATHWAY OR THE GLUTATHIONE REDUCTASE PATHWAY. PATIENTS WITH
INHERITED
DISORDERS OF THE HEMOGLOBIN MOLECULE OR RED CELL MEMBRANE
GENERALLY
HAVE A LIFELONG CLINICAL HISTORY TYPICAL OF THE DISEASE PROCESS. THOSE
WITH CHRONIC HEMOLYTIC DISEASE, SUCH AS HEREDITARY SPHEROCYTOSIS, MAY
ACTUALLY PRESENT NOT WITH ANEMIA BUT WITH A COMPLICATION STEMMING
FROM THE PROLONGED INCREASE IN RED CELL DESTRUCTION SUCH AS SYMPTO-
OMATIC BILIRUBIN GALLSTONES OR SPLENOMEGALY. PATIENTS WITH CHRONIC
HEMOLYSIS ARE ALSO SUSCEPTIBLE TO APLASTIC CRISES IF AN INFECTIOUS
PROCESS
INTERRUPTS RED CELL PRODUCTION.
THE DIFFERENTIAL DIAGNOSIS OF AN ACUTE OR CHRONIC HEMOLYTIC EVENT RE-
QUIRES THE CAREFUL INTEGRATION OF FAMILY HISTORY, THE PATTERN OF
CLINICAL
PRESENTATION AND- WHETHER THE DISEASE IS CONGENITAL OR ACQUIRED-BY A
CAREFUL EXAMINATION OF THE PERIPHERAL BLOOD SMEAR. PRECISE DIAGNOSIS
MAY REQUIRE MORE SPECIALIZED LABORATORY TESTS, SUCH AS HEMOGLOBIN
ELECTROPHORESIS OR A SCREEN FOR RED CELL ENZYMES. ACQUIRED DEFECTS IN
RED CELL SURVIVAL ARE OFTEN IMMUNOLOGICALLY MEDIATED AND REQUIRE A DI-
RECT OR INDIRECT ANTIGLOBULIN TEST OR A COLD AGGLUTININ TITER TO DETECT THE PRESENCE OF HEMOLYTIC ANTIBODIES OR COMPLEMENT-MEDIATED RED CELL DESTRUCTION.

ANEMIA

AN OVERRIDING PRINCIPLE IS TO INITIATE TREATMENT OF MILD TO MODERATE ANEMIA ONLY WHEN A SPECIFIC DIAGNOSIS IS MADE RARELY, IN THE ACUTE SETTING, ANEMIA MAY BE SO SEVERE THAT RED CELL TRANSFUSIONS ARE REQUIRED BEFORE A SPECIFIC DIAGNOSIS IS MADE. WHETHER THE ANEMIA IS OF ACUTE OR GRADUAL ONSET, THE SELECTION OF THE APPROPRIATE TREATMENT IS DETERMINED BY THE DOCUMENTED CAUSE(S) OF THE ANEMIA. OFTEN, THE CAUSE OF THE ANEMIA MAY BE MULTIFACTORIAL. FOR EXAMPLE, A PATIENT WITH SEVERE RHEUMATOID ARTHRITIS WHO HAS BEEN TAKING ANTI-INFLAMMATORY DRUGS MAY HAVE A HYPO PROLIFERATIVE ANEMIA ASSOCIATED WITH CHRONIC INFLAMMATION AS WELL AS CHRONIC BLOOD LOSS ASSOCIATED WITH INTERMITTENT GASTROINTESTINAL BLEEDING. IN EVERY CIRCUMSTANCE, IT IS IMPORTANT TO EVALUATE THE PATIENT'S IRON STATUS FULLY BEFORE AND DURING THE TREATMENT OF ANY ANEMIA. TRANSFUSION IS DISCUSSED IN CHAP. 107; IRON THERAPY IS DISCUSSED IN CHAP. 98; TREATMENT OF MEGAOBLASTIC ANEMIA IS DISCUSSED IN CHAP. 100; TREATMENT OF OTHER ENTITIES IS DISCUSSED IN THEIR RESPECTIVE CHAPTERS (SICKLE CELL ANEMIA. CHAP. 99; HEMOLYTIC ANEMIAS, CHAP. 101; APLASTIC ANEMIA AND MYELODYSPLASIA, CHAP. 102).

THERAPEUTIC OPTIONS FOR THE TREATMENT OF ANEMIAS HAVE EXPANDED DRAMATICALLY DURING THE PAST 25 YEARS. BLOOD COMPONENT THERAPY IS AVAILABLE AND SAFE. RECOMBINANT EPO AS AN ADJUNCT TO ANEMIA MANAGEMENT HAS TRANSFORMED THE LIVES OF PATIENTS WITH CHRONIC RENAL FAILURE ON DIALYSIS AND MADE SOME IMPROVEMENTS IN THE QUALITY OF LIFE OF ANEMIC CANCER PATIENTS RECEIVING CHEMOTHERAPY. IMPROVEMENTS IN THE MANAGEMENT OF SICKLE CELL CRISSES AND SICKLE CELL ANEMIA HAVE ALSO TAKEN PLACE. EVENTUALLY, PATIENTS WITH INHERITED DISORDERS OF GLOBIN SYNTHESIS OR MUTATIONS IN THE GLOBIN GENE, SUCH AS SICKLE CELL DISEASE, MAY BENEFIT FROM THE SUCCESSFUL INTRODUCTION OF TARGETED GENETIC THERAPY (CHAP 65).

POLYCYTHEMIA

POLYCYTHEMIA IS DEFINED AS AN INCREASE IN CIRCULATING RED BLOOD CELLS
ABOVE NORMAL. THIS INCREASE MAY BE REAL OR ONLY APPARENT BECAUSE OF A DECREASE IN PLASMA VOLUME (SPURIOUS OR RELATIVE POLYCYTHEMIA). THE TERM ERYTHROCYTOSIS MAY BE USED INTERCHANGEABLY WITH POLYCYTHEMIA, BUT SOME DRAW A DISTINCTION BETWEEN THEM: ERYTHROCYTOSIS IMPLIES DOCUMENTATION OF INCREASED RED CELL MASS, WHEREAS POLYCYTHEMIA REFERS TO ANY INCREASE IN RED CELLS. OFTEN PATIENTS WITH POLYCYTHEMIA ARE DETECTED THROUGH AN INCIDENTAL FINDING OF ELEVATED HEMOGLOBIN OR HEMATOCRIT LEVELS. CONCERN THAT THE HEMOGLOBIN LEVEL MAY BE ABNORMALLY HIGH IS USUALLY TRIGGERED AT 170 G/L (17 G/DL) FOR MEN AND 150 G/L (15 G/DL) FOR WOMEN. HEMATOCRIT LEVELS >50% IN MEN OR >45% IN WOMEN MAY BE ABNORMAL. HEMATOCRITS >60% IN MEN AND >55% IN WOMEN ARE ALMOST INVARiABLY ASSOCIATED WITH AN INCREASED RED CELL MASS.

HISTORIC FEATURES USEFUL IN THE DIFFERENTIAL DIAGNOSIS INCLUDE SMOKING HISTORY; LIVING AT HIGH ALTITUDE; OR A HISTORY OF CONGENITAL HEART DISEASE, PEPTIC ULCER DISEASE, SLEEP APNEA, CHRONIC LUNG DISEASE, OR RENAL DISEASE.

PATIENTS WITH POLYCYTHEMIA MAY BE ASYMPTOMATIC OR EXPERIENCE SYMPTOMS RELATED TO THE INCREASED RED CELL MASS OR AN UNDERLYING DISEASE PROCESS THAT LEADS TO INCREASED RED CELL PRODUCTION. THE DOMINANT SYMPTOMS FROM INCREASED RED CELL MASS ARE RELATED TO HYPERVISCOSITY AND THROMBOSIS (BOTH VENOUS AND ARTERIAL), BECAUSE THE BLOOD VISCOSITY INCREASES LOGARITHMICALLY AT HEMATOCRIT >55%. MANIFESTATIONS RANGE FROM DIGITAL ISCHEMIA TO BUDD-CHIARI SYNDROME WITH HEPATIC VEIN THROMBOSIS. ABDOMINAL THROMBOSES ARE PARTICULARLY COMMON. NEUROLOGIC SYMPTOMS SUCH AS VERTIGO, TINNITUS, HEADACHE, AND VISUAL DISTURBANCES MAY OCCUR. HYPERTENSION IS OFTEN PRESENT. PATIENTS WITH POLYCYTHEMIA VERA MAY HAVE AQUAGENIC PRURITUS AND SYMPTOMS RELATED TO HEPATOSPLENOMEGALY. PATIENTS MAY HAVE EASY BRUISING, EPISTAXIS, OR BLEEDING FROM THE GASTROINTESTINAL TRACT. PATIENTS WITH HYPOXEMIA MAY DEVELOP CYANOSIS ON MINIMAL EXERTION OR HAVE HEADACHE, IMPAIRED MENTAL ACUITY, AND FATIGUE.

THE PHYSICAL EXAMINATION USUALLY REVEALS A RUDDY COMPLEXION. SPLEENOMEGALY FAVORS POLYCYTHEMIA VERA AS THE DIAGNOSIS (CHAP. 103). THE PRESENCE OF CYANOSIS OR EVIDENCE OF A RIGHT-TO-LEFT SHUNT SUGGESTS CONGENITAL HEART DISEASE PRESENTING IN THE ADULT, PARTICULARLY TETRALOGY OF FALLOT OR EISENMENGER SYNDROME (CHAP. 229). INCREASED BLOOD VISCOSITY RAISES PULMONARY ARTERY PRESSURE; HYPOXEMIA CAN LEAD TO INCREASED PULMONARY VASCULAR RESISTANCE. TOGETHER THESE FACTORS CAN PRODUCE COR PULMONALE.

POLYCYTHEMIA CAN BE SPURIOUS (RELATED TO A DECREASE IN PLASMA VOLUME: Gaisbock’s Syndrome), PRIMARY, OR SECONDARY IN ORIGIN. THE SECONDARY CAUSES ARE ALL ASSOCIATED WITH INCREASES IN EPO LEVELS: EITHER A PHYSIOLOGICALLY ADAPTED APPROPRIATE ELEVATION BASED ON TISSUE HYPOXIA (LUNG DISEASE, HIGH ALTITUDE, CO POISONING, HIGH-AFFINITY HEMOGLOBIN-OPTHY) OR AN ABNORMAL OVERPRODUCTION (RENAL CYSTS, RENAL ARTERY STE- NOSIS, TUMORS WITH ECTOPIC EPO PRODUCTION). A RARE FAMILIAL FORM OF
Polycythemia is associated with normal EPO levels but hyperresponsive EPO receptors due to mutations.

**Approach to the Patient:**

**Polycythemia**

As shown in Fig. 58-18, the first step is to document the presence of an increased red cell mass using the principle of isotope dilution by administering $^{15}$Cr-labeled autologous red blood cells to the patient and sampling blood radioactivity over a 2-h period. If the red cell mass is normal (<36 ml/kg in men, <32 ml/kg in women), the patient has spurious polycythemia. If the red cell mass is increased (>36 ml/kg in men, >32 ml/kg in women), serum EPO levels should be measured. If EPO levels are low or unmeasurable, the...

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**363 Chapter 59 Bleeding and Thrombosis**

**Figure 58-18 An Approach to Diagnosing Patients With Polycythemia.** RBC, red blood cell; EPO, erythropoietin; COPD, chronic obstructive pulmonary disease; AV, atrioventricular; IVP, intravenous pyelogram; HCT, hematocrit.

Cell mass is normal (<36 ml/kg in men, <32 ml/kg in women), the patient has spurious polycythemia. If the red cell mass is increased (>36 ml/kg in men, >32 ml/kg in women), serum EPO levels should be measured. If EPO levels are low or unmeasurable, the...

**59 Bleeding and Thrombosis**

Barbara A. Konkle

The human hemostatic system provides a natural balance between procoagulant and anticoagulant forces. The procoagulant forces include platelet adhesion and aggregation and fibrin clot formation; anticoagulant forces include the natural inhibitors of coagulation and fibrinolysis. Under normal circumstances, hemostasis is regulated to promote blood flow; however, it is also prepared to clot blood rapidly to arrest blood flow and prevent exsanguination. After bleeding is successfully halted, the system remodels the damaged vessel to restore normal blood flow.

The major components of the hemostatic system, which function in concert, are (1) platelets and other formed elements of blood, such as monocytes and red cells; (2) plasma proteins (the coagulation and fibrinolytic factors and inhibitors); and (3) the vessel wall itself.

**Steps of Normal Hemostasis**

**Platelet Plug Formation**
ON VASCULAR INJURY, PLATELETS ADHERE TO THE SITE OF INJURY, USUALLY THE DE-
NUDED VASCULAR INTIMAL SURFACE. PLATELET ADHESION IS MEDIATED PRIMARY BY VON WILLEBRAND FACTOR (VWF), A LARGE MULTIMERIC PROTEIN PRESENT IN BOTH PLASMA AND IN THE EXTRACELLULAR MATRIX OF THE SUBENDOTHELIAL VESSEL WALL, WHICH SERVES AS THE PRIMARY “MOLECULAR GLUE,” PROVIDING SUFFICIENT STRENGTH TO WITHSTAND THE HIGH LEVELS OF SHEAR STRESS THAT WOULD TEND TO DETACH THEM WITH THE FLOW OF BLOOD. PLATELET ADHESION IS

PATIENT MOST LIKELY HAS POLYCYTHEMIA VERA. ANCILLARY TESTS THAT SUPPORT THIS DIAGNOSIS INCLUDE ELEVATED WHITE BLOOD CELL COUNT, INCREASED ABSOLUTE BASOPHIL COUNT, AND THROMBOCYTOSIS. A MUTATION IN JAK-2 (VAL617PHE), A KEY MEMBER OF THE CYTOKINE INTRACELLULAR SIGNALING PATHWAY, CAN BE FOUND IN 70-95% OF PATIENTS WITH POLYCYTHEMIA VERA.

IF SERUM EPO LEVELS ARE ELEVATED, ONE ATTEMPTS TO DISTINGUISH WHETHER THE ELEVATION IS A PHYSIOLOGIC RESPONSE TO HYPOXIA OR IS RELATED TO AUTONOMOUS PRODUCTION. PATIENTS WITH LOW ARTERIAL O2 SATURATION (<92%) SHOULD BE FURTHER EVALUATED FOR THE PRESENCE OF HEART OR LUNG DISEASE, IF THEY ARE NOT LIVING AT HIGH ALTITUDE. PATIENTS WITH NORMAL O2 SATURATION WHO ARE SMOKERS MAY HAVE ELEVATED EPO LEVELS BECAUSE OF CO DISPLACEMENT OF O2. IF CARBOXYHEMOGLOBIN (COHB) LEVELS ARE HIGH, THE DIAGNOSIS IS SMOKER’S POLYCYTHEMIA. SUCH PATIENTS SHOULD BE URGED TO STOP SMOKING. THOSE WHO CANNOT STOP SMOKING REQUIRE PHLEBOTOMY TO CONTROL THEIR POLYCYTHEMIA. PATIENTS WITH NORMAL O2 SATURATION WHO DO NOT SMOKE EITHER HAVE AN ABNORMAL HEMOGLOBIN THAT DOES NOT DELIVER O2 TO THE TISSUES (EVALUATED BY FINDING ELEVATED O2-HEMOGLO-

BIN AFFINITY) OR HAVE A SOURCE OF EPO PRODUCTION THAT IS NOT RESPONDING TO THE NORMAL FEEDBACK INHIBITION. FURTHER WORKUP IS DICTATED BY THE DIFFERENTIAL DIAGNOSIS OF EPO-PRODUCING NEOPLASMS. HEPATOMA, UTERINE LEIOMYOMA, AND RENAL CANCER OR CYSTS ARE ALL DETECTABLE WITH ABDOMINOPELVIC CT SCANS. CEREBELLAR HEMANGIOMAS MAY PRODUCE EPO, BUT THEY NEARLY ALWAYS PRESENT WITH LOCALIZING NEUROLOGIC SIGNS AND SYMPTOMS RATHER THAN POLYCYTHEMIA-RELATED SYMPTOMS.

ACKNOWLEDGMENT

DR. ROBERT S. HILLMAN WROTE THIS CHAPTER IN THE 14TH EDITION, AND ELEMENTS OF HIS CHAPTER WERE RETAINED HERE.

FURTHER READINGS

HILLMAN RS ET AL: HEMATOLOGY IN CLINICAL PRACTICE, 4TH ED. NEW YORK, MCGRAW-HILL, 2005

ALSO FACILITATED BY DIRECT BINDING TO SUBENDOTHELIAL COLLAGEN THROUGH SPECIFIC PLATELET MEMBRANE COLLAGEN RECEPTORS. PLATELET ADHESION RESULTS IN SUBSEQUENT PLATELET ACTIVATION AND
AGGREGATION. THIS PROCESS IS ENHANCED AND AMPLIFIED BY HUMORAL MEDIATORS IN
PLASMA (E.G., EPINEPHRINE, THROMBIN); MEDIATORS RELEASED FROM ACTIVATED
PLATELETS (E.G., ADENOSINE DIPHOSPHATE, SEROTONIN); AND VESSEL WALL
EXTRA-
CELLULAR MATRIX CONSTITUENTS THAT COME IN CONTACT WITH ADHERENT
PLATELETS
(E.G., COLLAGEN, VWF). ACTIVATED PLATELETS UNDERGO THE RELEASE
REACTION,
DURING WHICH THEY SECRETE CONTENTS THAT FURTHER PROMOTE AGGREGATION
AND INHIBIT THE NATURALLY ANTICOAGULANT ENDOTHELIAL CELL FACTORS.
DURING
PLATELET AGGREGATION (PLATELET-PLATELET INTERACTION), ADDITIONAL
PLATELETS
ARE RECRUITED FROM THE CIRCULATION TO THE SITE OF VASCULAR
INJURY, LEADING TO
THE FORMATION OF AN OCCLUSIVE PLATELET THROMBUS. THE PLATELET PLUG IS AN-
CHORED AND STABILIZED BY THE DEVELOPING FIBRIN MESH.
THE PLATELET GLYCO PROTEIN (GP) IIB/IIIA (*###IIB*###3) COMPLEX IS THE MOST
ABUNDANT RECEPTOR ON THE PLATELET SURFACE. PLATELET ACTIVATION
CONVERTS
THE NORMALLY INACTIVE GPIIB/IIIA RECEPTOR INTO AN ACTIVE RECEPTOR, EN-
ABLING BINDING TO FIBRINOGEN AND VWF. BECAUSE THE SURFACE OF EACH
PLATE-
LET HAS ABOUT 50,000 GPIIB/IIIA FIBRINOGEN BINDING SITES, NUMEROUS
ACTIVATED PLATELETS RECRUITED TO THE SITE OF VASCULAR INJURY CAN
RAPIDLY
FORM AN OCCLUSIVE AGGREGATE BY MEANS OF A DENSE NETWORK OF
INTERCELLULAR
FIBRINOGEN BRIDGES. SINCE THIS RECEPTOR IS THE KEY MEDIATOR OF PLATELET
AG-
GREGATION, IT HAS BECOME AN EFFECTIVE TARGET FOR ANTIPLATELET THERAPY.

FIBRIN CLOT FORMATION

PLASMA COAGULATION PROTEINS (CLOTTING FACTORS) NORMALLY CIRCULATE IN
PLASMA IN THEIR INACTIVE FORMS. THE SEQUENCE OF COAGULATION PROTEIN
RE-
ACTIONS THAT CULMINATE IN THE FORMATION OF FIBRIN WAS ORIGINALLY DE-

PAGE NO. 10

364 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF
DISEASES

SCRIBED AS A WATERFALL OR A CASCADE. TWO PATHWAYS
OF BLOOD COAGULATION HAVE BEEN DESCRIBED IN THE
PAST: THE SO-CALLED EXTRINSIC, OR TISSUE FACTOR, PATH-
WAY AND THE SO-CALLED INTRINSIC, OR CONTACT ACTIVA-
TION, PATHWAY. WE NOW KNOW THAT COAGULATION IS
NORMAL INITIATED THROUGH TISSUE FACTOR (TF) EX-
POSURE AND ACTIVATION THROUGH THE CLASSIC EXTRINSIC
PATHWAY, BUT WITH CRITICALLY IMPORTANT AMPLIFICA-
TION THROUGH ELEMENTS OF THE CLASSIC INTRINSIC
PATHWAY, AS ILLUSTRATED IN FIG. 59-1. THESE REAC-
TIONS TAKE PLACE ON PHOSPHOLIPID SURFACES, USUALLY
THE ACTIVATED PLATELET SURFACE. COAGULATION TESTING
IN THE LABORATORY CAN REFLECT OTHER INFLUENCES DUE
to THE ARTIFICIAL NATURE OF THE IN VITRO SYSTEMS USED
(SEE BELOW).
THE IMMEDIATE TRIGGER FOR COAGULATION IS VAS-
CULAR DAMAGE THAT EXPOSES BLOOD TO TF THAT IS
CONSTITUTIVELY EXPRESSED ON THE SURFACES OF SUB-
ENDOTHELIAL CELLULAR COMPONENTS OF THE VESSEL
WALL, SUCH AS SMOOTH-MUSCLE CELLS AND FIBRO-
BLASTS. TF IS ALSO PRESENT IN CIRCULATING MICROPAR-
TICLES, PRESUMABLY SHED FROM CELLS INCLUDING
MONOCYTES AND PLATELETS. TF BINDS THE SERINE
PROTEASE FACTOR VILA; THE COMPLEX ACTIVATES FACTOR
X TO FACTOR XA. ALTERNATIVELY, THE COMPLEX CAN IN-
DIRECTLY ACTIVATE FACTOR X BY INITIALLY CONVERTING
FACTOR IX TO FACTOR IXA, WHICH THEN ACTIVATES FAC-
TOR X. THE PARTICIPATION OF FACTOR XI IN HEMOSTASIS IS NOT DEPENDENT ON
ITS ACTIVATION BY FACTOR XIIA BUT RATHER ON ITS POSITIVE FEEDBACK
ACTIVA-
TION BY THROMBIN. THUS, FACTOR XIA FUNCTIONS IN THE PROPAGATION AND
AMPLIFICATION, RATHER THAN IN THE INITIATION, OF THE COAGULATION
CASCADE.
FACTOR XA, WHICH CAN BE FORMED THROUGH THE ACTIONS OF EITHER THE
TISSUE FACTOR/FACTOR VIIA COMPLEX OR FACTOR IXA (WITH FACTOR VIIIA AS A
COFACTOR), CONVERTS PROTHROMBIN TO THROMBIN, THE PIVOTAL PROTEASE OF
THE COAGULATION SYSTEM. THE ESSENTIAL COFACTOR FOR THIS REACTION IS
FACTOR VA. LIKE THE HOMOLOGOUS FACTOR VIIIA, FACTOR VA IS PRODUCED BY THROM-
BİN-INDUCED LIMITED PROTEOLYSIS OF FACTOR V. THROMBIN IS A MULTIFUNC-
TIONAL ENZYME THAT CONVERTS SOLUBLE PLASMA FIBRINOGEN TO AN
INSOLUBLE
FIBRIN MATRIX. FIBRIN POLYMERIZATION INVOLVES AN ORDERLY PROCESS OF IN-
TERMOL EcULAR ASSOC IATIONS (FIG. 59-2). THROMBIN ALSO ACTIVATES FACTOR
XIII (FIBRIN-STABILIZING FACTOR) TO FACTOR XIIIIA, WHICH COVALENTLY CROSS-
LINKS AND THEREBY STABILIZES THE FIBRIN CLOT.
THE ASSEMBLY OF THE CLOTTING FACTORS ON ACTIVATED CELL MEMBRANE
SURFACES GREATLY ACCELERATES THEIR REACTION RATES AND ALSO SERVES TO LOCAL-
IZE BLOOD DOTTING TO SITES OF VASCULAR INJURY. THE CRITICAL CELL
MEMBRANE
COMPONENTS, ACIDIC PHOSPHOLIPIDS, ARE NOT NORMALLY EXPOSED ON REST-
ING CELL MEMBRANE SURFACES. HOWEVER, WHEN PLATELETS, MONOCYTES, AND
ENDOTHELIAL CELLS ARE ACTIVATED BY VASCULAR INJURY OR INFLAMMATORY
STIM-
ULI, THE PROCOAGULANT HEAD GROUPS OF THE MEMBRANE ANIONIC PHOSPHO-
LIPIDS BECOME TRANSLOCATED TO THE SURFACES OF THESE CELLS OR RELEASED AS
PART OF MICROPARTICLES, MAKING THEM AVAILABLE TO SUPPORT AND PROMOTE
ANTITHROMBOTIC MECHANISMS

SEVERAL PHYSIOLOGIC ANTITHROMBOTIC MECHANISMS ACT IN CONCERT TO PREVENT CLOTTING UNDER NORMAL CIRCUMSTANCES. THESE MECHANISMS OPERATE TO PRESERVE BLOOD FLUIDITY AND LIMIT BLOOD CLOTTING TO SPECIFIC FOCAL SITES OF VASCULAR INJURY. ENDOTHELIAL CELLS HAVE MANY ANTITHROMBOTIC EFFECTS. THEY PRODUCE PROSTAGLANDIN, NITRIC OXIDE, AND ECTOADPASE/CD39, WHICH ACT TO INHIBIT PLATELET BINDING, SECRETION, AND AGGREGATION. ENDOTHELIAL CELLS PRODUCE ANTICOAGULANT FACTORS INCLUDING HEPARAN PROTEOGLYCANS, ANTITHROMBIN, TF PATHWAY INHIBITOR, AND THROMBOMODULIN. THEY ALSO ACTIVATE FIBRINOLYTIC MECHANISMS THROUGH THE PRODUCTION OF TISSUE PLASMINOGEN ACTIVATOR 1, UROKINASE, PLASMINOGEN ACTIVATOR INHIBITOR, AND ANNEXIN-2. THE SITES OF ACTION OF THE MAJOR PHYSIOLOGIC ANTITHROMBOTIC PATHWAYS ARE SHOWN IN FIG. 59-3. ANTITHROMBIN (OR ANTITHROMBIN III) IS THE MAJOR PLASMA PROTEASE INHIBITOR OF THROMBIN AND THE OTHER CLOTTING FACTORS IN COAGULATION.

FIGURE 59-1 COAGULATION IS INITIATED BY TISSUE FACTOR (TF) EXPOSURE, WHICH, WITH FACTOR (F) VIIA, ACTIVATES FIX AND FX, WHICH IN TURN, WITH FVIII AND FV AS COFACTORs, RESPECTIVELY, RESULTS IN THROMBIN FORMATION AND SUBSEQUENT CONVERSION OF FIBRINOGEN TO FIBRIN. THROMBIN ACTIVATES FXI, FVIII, AND FV, AMPLIFYING THE COAGULATION SIGNAL. ONCE THE TF/FVIIA/FXA COMPLEX IS FORMED, TISSUE FACTOR PATHWAY INHIBITOR (TFPI) INHIBITS THE TF/FVIIA PATHWAY, MAKING COAGULATION DEPENDENT ON THE AMPLIFICATION LOOP THROUGH FIX/FVIII. COAGULATION REQUIRES CALCIUM (NOT SHOWN) AND TAKES PLACE ON PHOSPHOLIPID SURFACES, USUALLY THE ACTIVATED PLATELET MEMBRANE.

ANTITHROMBIN NEUTRALIZES THROMBIN AND OTHER ACTIVATED COAGULATION FACTORS BY FORMING A COMPLEX BETWEEN THE ACTIVE SITE OF THE ENZYME AND THE REACTIVE CENTER OF ANTITHROMBIN. THE RATE OF FORMATION OF THESE INACTIVATING COMPLEXES INCREASES BY A FACTOR OF SEVERAL THOUSAND IN THE PRESENCE OF HEPARIN. ANTITHROMBIN INACTIVATION OF THROMBIN AND OTHER ACTIVATED CLOTTING FACTORS OCCURS PHYSIOLOGICALLY ON VASCULAR SURFACES, WHERE GLYCOSAMINOGLYCANS, INCLUDING HEPARAN SULFATES, ARE PRESENT TO CATALYZE THESE REACTIONS. INHERITED QUANTITATIVE OR QUALITATIVE DEFICIENCIES OF ANTITHROMBIN LEAD TO A LIFELONG PREDISPOSITION TO VENOUS THROMBOEMBOLISM.

FIGURE 59-2 FIBRIN FORMATION AND DISSOLUTION. A. FIBRINOGEN IS A TRINODULAR STRUCTURE CONSISTING OF 2 D DOMAINS AND 1 E DOMAIN. THROM-
BIN ACTIVATION RESULTS IN AN ORDERED LATERAL ASSEMBLY OF PROTOFIBRILS (B) WITH NONCOVALENT ASSOCIATIONS. FXIIIA CROSS-LINKS THE D DOMAINS ON ADJACENT MOLECULES (C). FIBRIN AND FIBRINOGEN (NOT SHOWN) LYSIS BY PLASMIN OCCURS AT DISCRETE SITES AND RESULTS IN INTERMEDIATE FIBRIN(OGEN) DEGRADATION PRODUCTS (NOT SHOWN) D-DIMERS ARE THE PRODUCT OF COMPLETE LYSIS OF FIBRIN, MAINTAINING THE CROSS-LINKED D DOMAINS.

365 CHAPTER 59 BLEEDING AND THROMBOSIS

FIGURE 59-3 SITES OF ACTION OF THE FOUR MAJOR PHYSIOLOGIC ANTI-THROMBOTIC PATHWAYS: ANTITHROMBIN (AT); PROTEIN C/S (PC/PS); TISSUE FACTOR PATHWAY INHIBITOR (TFPI), AND THE FIBRINOLYTIC SYSTEM, CONSISTING OF PLASMINOGEN, PLASMINOGEN ACTIVATOR (PA), AND PLASMIN. PT, PROTHROMBIN; TH, THROMBIN. FDP, FIBRIN (OGEN) DEGRADATION PRODUCTS. [MODIFIED FROM BA KONKLE, AL SCHAFER, IN DP ZIPES ET AL (EDS): BRAUNWALD’S HEART DISEASE, 7TH ED.PHILADELPHIA, SAUNDERS, 2005.]

PROTEIN C IS A PLASMA GLYCOPROTEIN THAT BECOMES AN ANTICOAGULANT WHEN IT IS ACTIVATED BY THROMBIN. THE THROMBIN-INDUCED ACTIVATION OF PROTEIN C OCCURS PHYSIOLOGICALLY ON THROMBOMODULIN, A TRANSMEMBRANE PROTEOGLYCAN BINDING SITE FOR THROMBIN ON ENDOTHELIAL CELL SURFACES. THE BINDING OF PROTEIN C TO ITS RECEPTOR ON ENDOTHELIAL CELLS PLACES IT IN PROXIMITY TO THE THROMBIN-THROMBOMODULIN COMPLEX, THEREFORE ENHANCING ITS ACTIVATION EFFICIENCY. ACTIVATED PROTEIN C ACTS AS AN ANTICOAGULANT BY CLEAVING AND INACTIVATING ACTIVATED FACTORS V AND VIII. THIS REACTION IS ACCELERATED BY A COFACTOR, PROTEIN S, WHICH, LIKE PROTEIN C, IS A GLYCOPROTEIN THAT UNDERGOES VITAMIN K-DEPENDENT POSTTRANSLATIONAL MODIFICATION. QUANTITATIVE OR QUALITATIVE DEFICIENCIES OF PROTEIN C OR PROTEIN S, OR RESISTANCE TO THE ACTION OF Activated PROTEIN C BY A SPECIFIC MUTATION AT ITS TARGET CLEAVAGE SITE IN FACTOR VA (FAC-TOR V LEIDEN), LEAD TO HYPERCOAGULABLE STATES.

TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IS A PLASMA PROTEASE INHIBITOR THAT REGULATES THE TF-INDUCED EXTRINSIC PATHWAY OF COAGULATION. TFPI INHIBITS THE TF/FVIIA/FXA COMPLEX, ESSENTIALLY TURNING OFF THE TF/FVIIA INITIATION OF COAGULATION, WHICH THEN BECOMES DEPENDENT ON THE “AMPLIFICATION LOOP” VIA FXI AND FVIII ACTIVATION BY THROMBIN. TFPI IS BOUND TO LIPOPROTEIN AND CAN ALSO BE RELEASED BY HEPARIN FROM ENDOTHELIAL CELLS, WHERE IT IS BOUND TO GLYCOSAMINOGLYCANS, AND FROM PLATELETS. THE HEPARIN-MEDIATED RELEASE OF TFPI MAY PLAY A ROLE IN THE ANTICOAGULANT EFFECTS OF UNFRACTIONATED AND LOW-MOLECULAR-WEIGHT HEPARINS.

THE FIBRINOLYTIC SYSTEM
ANY THROMBIN THAT ESCAPES THE INHIBITORY EFFECTS OF THE PHYSIOLOGIC ANTI-COAGULANT SYSTEMS IS AVAILABLE TO CONVERT FIBRINOGEN TO FIBRIN. IN RESPONSE, THE ENDOGENOUS FIBRINOLYTIC SYSTEM IS THEN ACTIVATED TO DISPOSE OF INTRAVASCULAR FIBRIN AND THEREBY MAINTAIN OR REESTABLISH THE PATENCY OF THE CIRCULATION. JUST AS THROMBIN IS THE KEY PROTEASE ENZYME OF THE COAGULATION SYSTEM, PLASMIN IS THE MAJOR PROTEASE ENZYME OF THE FIBRINOLYTIC SYSTEM, ACTING TO DIGEST FIBRIN TO FIBRIN DEGRADATION PRODUCTS. THE GENERAL SCHEME OF FIBRINOLYSIS IS SHOWN IN FIG. 59-4.

FIGURE 59-4 A SCHEMATIC DIAGRAM OF THE FIBRINOLYTIC SYSTEM. TISSUE PLASMINOGEN ACTIVATOR (TPA) IS RELEASED FROM ENDOTHELIAL CELLS, BINDS THE FIBRIN CLOT, AND ACTIVATES PLASMINOGEN TO PLASMIN EXCESS FIBRIN IS DEGRATED BY PLASMIN TO DISTINCT DEGRADATION PRODUCTS (FDPS). ANY FREE PLASMIN ISCOMPLEXED WITH **2-ANTIPLASMIN (**2PI).

THE PLASMINOGEN ACTIVATORS, TISSUE TYPE PLASMINOGEN ACTIVATOR (TPA) AND THE UROKINASE TYPE PLASMINOGEN ACTIVATOR (UPA), CLEAVE THE ARG560-VAL561 BOND OF PLASMINOGEN TO GENERATE THE ACTIVE ENZYME PLASMIN. THE LYSINE-BINDING SITES OF PLASMIN (AND PLASMINOGEN) PERMIT IT TO BIND TO FIBRIN, SO THAT PHYSIOLOGIC FIBRINOLYSIS IS “FIBRIN SPECIFIC.” BOTH PLASMINOGEN (THROUGH ITS LYSINE-BINDING SITES) AND TPA POSSESS SPECIFIC AFFINITY FOR FIBRIN AND THEREBY BIND SELECTIVELY TO CLOTS. THE ASSEMBLY OF A TERNARY COMPLEX, CONSISTING OF FIBRIN, PLASMINOGEN, AND TPA, PROMOTES THE LOCALIZED INTERACTION BETWEEN PLASMINOGEN AND TPA AND GREATLY ACCELERATES THE RATE OF PLASMINOGEN ACTIVATION TO PLASMIN. MOREOVER, PARTIAL DEGRADATION OF FIBRIN BY PLASMIN EXPOSES NEW PLASMINOGEN AND TPA BINDING SITES IN CARBOXY-TERMINUS LYSINE RESIDUES OF FIBRIN FRAGMENTS TO ENHANCE THESE REACTIONS FURTHER. THIS CREATES A HIGHLY EFFICIENT MECHANISM TO GENERATE PLASMIN FOCALLY ON THE FIBRIN CLOT, WHICH THEN BECOMES PLASMIN’S SUBSTRATE FOR DIGESTION TO FIBRIN DEGRADATION PRODUCTS. PLASMIN CLEAVES FIBRIN AT DISTINCT SITES OF THE FIBRIN MOLECULE LEADING TO THE GENERATION OF CHARACTERISTIC FIBRIN FRAGMENTS DURING THE PROCESS OF FIBRINOLYSIS (FIG. 59-2). THE SITES OF PLASMIN CLEAVAGE OF FIBRIN ARE THE SAME AS THOSE IN FIBRINOGEN. HOWEVER, WHEN PLASMIN ADS ON COVALENTLY CROSS-LINKED FIBRIN, D-DIMERS ARE RELEASED; HENCE, D-DIMERS CAN BE MEASURED IN PLASMA AS A RELATIVELY SPECIFIC TEST OF FIBRIN (RATHER THAN FIBRINOGEN) DEGRADATION. D-DIMER ASSAYS CAN BE USED AS SENSITIVE MARKERS OF BLOOD CLOT FORMATION, AND SOME HAVE BEEN VALIDATED FOR CLINICAL USE TO EXCLUDE THE DIAGNOSIS OF DEEP VENOUS THROMBOSIS (DVT) AND PULMONARY EMBOLISM IN SELECTED POPULATIONS. PHYSIOLOGIC REGULATION OF FIBRINOLYSIS OCCURS PRIMARILY AT TWO LEVELS: (1) PLASMINOGEN ACTIVATOR INHIBITORS (PAIS), SPECIFICALLY PAI1 AND PAI2, INHIBIT THE PHYSIOLOGIC PLASMINOGEN ACTIVATORS; AND (2) **2-ANTIPLASMIN (**2PI).
ANTI-PLASMIN INHIBITS PLASMIN. PAI1 IS THE PRIMARY INHIBITOR OF TPA AND UPA IN PLASMA. ANTIPLASMIN IS THE MAIN INHIBITOR OF PLASMIN IN HUMAN PLASMA, INACTIVATING ANY NONFIBRIN CLOT-ASSOCIATED PLASMIN.

**APPROACH TO THE PATIENT:**

**BLEEDING AND THROMBOSIS**

**CLINICAL PRESENTATION** DISORDERS OF HEMOSTASIS MAY BE EITHER INHERITED OR ACQUIRED. A DETAILED PERSONAL AND FAMILY HISTORY IS KEY IN DETERMINING THE CHRONICITY OF SYMPTOMS AND THE LIKELIHOOD OF THE DISORDER BEING INHERITED AND IT PROVIDES CLUES TO UNDERLYING CONDITIONS THAT HAVE CONTRIBUTED TO THE BLEEDING OR THROMBOTIC STATE. IN ADDITION, THE HISTORY CAN GIVE CLUES AS TO THE ETIOLOGY BY DETERMINING (1) THE BLEEDING (MUCOSAL AND/OR JOINT) OR THROMBOSIS (ARTERIAL AND/OR VENOUS) SITE, AND (2) WHETHER AN UNDERLYING BLEEDING OR CLOTTING TENDENCY WAS ENHANCED BY ANOTHER MEDICAL CONDITION OR THE INTRODUCTION OF MEDICATIONS OR DIETARY SUPPLEMENTS.

**HISTORY OF BLEEDING** A HISTORY OF BLEEDING IS THE MOST IMPORTANT PREDICTOR OF BLEEDING RISK. IN EVALUATING A PATIENT FOR A BLEEDING DISORDER, A HISTORY OF AT-RISK SITUATIONS, INCLUDING THE RESPONSE TO PAST SURGERIES, SHOULD BE ASSESSED. DOES THE PATIENT HAVE A HISTORY OF SPONTANEOUS OR TRAUMA/SURGERY-INDUCED BLEEDING? SPONTANEOUS HEMARTHROSES ARE A HALLMARK OF MODERATE AND SEVERE FACTORS VIII AND IX DEFICIENCY AND, IN RARE CIRCUMSTANCES, OF OTHER CLOTTING FACTOR DEFICIENCIES. MUCOSAL BLEEDING SYMPTOMS ARE MORE SUGGESTIVE OF UNDERLYING PLATELET DISORDERS OR VON WILLEBRAND DISEASE (VWD), TERMED DISORDERS OF PRIMARY HEMOSTASIS OR PLATELET PLUG FORMATION. DISORDERS AFFECTING PRIMARY HEMOSTASIS ARE SHOWN IN TABLE 59-1. THE DEVELOPMENT OF BRUISES (ECCHYMOSES) AFTER TRAUMA IS NORMAL; HOWEVER, AN EXAGGERATED RESPONSE TO TRAUMA MAY BE AN INDICATION OF AN UNDERLYING BLEEDING DISORDER. ECCHYMOSES PRESENTING WITHOUT KNOWN TRAUMA, PARTICULARLY ON THE TRUNK, AND ESPECIALLY LARGE ECCHYMOSES, >2 IN. IN DIAMETER, MAY BE A SIGN OF AN UNDERLYING BLEEDING DISORDER. THE INTRODUCTION OF MEDICATIONS OR NUTRITIONAL SUPPLEMENTS WITH PLATELET INHIBITORY ACTIVITY OFTEN ENHANCE BRUISING AND BLEEDING IN A PATIENT WITH AN UNDERLYING BLEEDING DISORDER. EASY BRUISING CAN ALSO BE A SIGN OF MEDICAL CONDITIONS IN WHICH THERE IS NO IDENTIFIABLE COAGULOPATHY; INSTEAD, THE CONDITIONS ARE CAUSED BY AN ABNORMALITY OF BLOOD VESSELS OR THEIR SUPPORTING TISSUES. IN EHLERS-DANLOS SYNDROME THERE MAY BE POSTTRAUMATIC BLEEDING AND A HISTORY OF JOINT HYPEREXTENSIBILITY. CUSHING’S SYNDROME, CHRONIC STEROID USE, AND AGING RESULT IN CHANGES IN SKIN AND SUBCUTANEOUS TISSUE, AND SUBCUTANEOUS BLEEDING OCCURS IN RESPONSE TO MINOR TRAUMA. THE LATTER HAS BEEN TERMED SENILE PURPURA.
EPISTAXIS IS A COMMON SYMPTOM, PARTICULARLY IN CHILDREN AND IN DRY CLIMATES, AND MAY NOT REFLECT AN UNDERLYING BLEEDING DISORDER. HOWEVER, IT IS THE MOST COMMON SYMPTOM IN HEREDITARY HEMORRHAGIC TELANGIECTASIA AND IN BOYS WITH VWD. CLUES THAT EPISTAXIS IS A SYMPTOM OF AN UNDERLYING BLEEDING DISORDER INCLUDE LACK OF SEASONAL VARIATION AND BLEEDING THAT REQUIRE MEDICAL EVALUATION OR TREATMENT, INCLUDING CAUTERIZATION. BLEEDING WITH ERUPTION OF PRIMARY TEETH IS SEEN IN CHILDREN WITH MORE SEVERE BLEEDING DISORDERS, SUCH AS MODERATE AND SEVERE HEMOPHILIA. IT IS UNCOMMON IN CHILDREN WITH MILD BLEEDING DISORDERS. PATIENTS WITH DISORDERS OF PRIMARY HEMOSTASIS (PLATELET ADHESION) DO HAVE INCREASED BLEEDING AFTER DENTAL CLEANINGS AND OTHER PROCEDURES THAT INVOLVE GUM MANIPULATION.

MENORRHAGIA IS DEFINED QUANTITATIVELY AS A LOSS OF >80 CC OF BLOOD PER CYCLE, BASED ON BLOOD LOSS REQUIRED TO PRODUCE IRON-DEFICIENCY ANEMIA. A COMPLAINT OF HEAVY MENSES IS SUBJECTIVE AND HAS A POOR CORRELATION WITH EXCESSIVE BLOOD LOSS. PREDICTORS OF MENORRHAGIA INCLUDE BLEEDING RESULTING IN IRON-DEFICIENCY ANEMIA OR A NEED FOR BLOOD TRANSFUSION, EXCESSIVE PAD OR TAMpon USE, MENSES LASTING LONGER THAN 8 DAYS, PASSAGE OF CLOTS, BLEEDING THROUGH PROTECTION, OR FLOODED AT NIGHT. MENORRHAGIA IS A COMMON SYMPTOM IN WOMEN WITH UNDERLYING BLEEDING DISORDERS AND IS REPORTED IN THE MAJORITY OF WOMEN WITH VWD AND FACTOR XI DEFICIENCY AND IN SYMPTOMATIC CARRIERS OF HEMOPHILIA A. WOMEN WITH UNDERLYING BLEEDING DISORDERS ARE MORE LIKELY TO HAVE OTHER BLEEDING SYMPTOMS, INCLUDING BLEEDING AFTER DENTAL EXTRACTIONS, POSTOPERATIVE BLEEDING, AND POSTPARTUM BLEEDING, AND ARE MUCH MORE LIKELY TO HAVE MENORRHAGIA BEGINNING AT MENARCHE THAN WOMEN WITH MENORRHAGIA DUE TO OTHER CAUSES. POSTPARTUM HEMORRHAGE IS A COMMON SYMPTOM IN WOMEN WITH UNDERLYING BLEEDING DISORDERS. THIS OCCURS MOST COMMONLY IN THE FIRST 48 H AFTER DELIVERY, BUT IT MAY ALSO BE MANIFEST BY PROLONGED OR EXCESSIVE BLEEDING AFTER DISCHARGE FROM THE HOSPITAL. WOMEN WITH A HISTORY OF POSTPARTUM HEMORRHAGE HAVE A HIGH RISK OF RECURRENCE WITH SUBSEQUENT PREGNANCIES. RUPTURE OF OVARIAN CYSTS WITH INTRA-ABDOMINAL HEMORRHAGE HAS ALSO BEEN REPORTED IN WOMEN WITH UNDERLYING BLEEDING DISORDERS. TONSILLECTOMY IS A MAJOR HEMOSTATIC CHALLENGE, AS INTACT HEMOSTATIC MECHANISMS ARE ESSENTIAL TO PREVENT EXCESSIVE BLEEDING FROM THE TONSILLAR BED. BLEEDING MAY OCCUR EARLY AFTER SURGERY OR AFTER APPROXIMATELY 7 DAYS POSTOPERATIVELY, WITH LOSS OF THE ESCHAR AT THE OPERATIVE SITE. SIMILAR DELAYED BLEEDING IS SEEN AFTER COLONIC POLYP RESECTION BY

**Table 59-1 PRIMARY HEMOSTATIC (PLATELET PLUG) DISORDERS**

| DEFECTS OF PLATELET ADHESION |
| VON WILLEBRAND DISEASE |
| BERNArd-SouLiER SYNDROME (ABSENCE OF DYSFUNCTION OFGP*B-IX-V) |
| DEFECTS OF PLATELET AGRGATION |
| GLANZMANN'S THROMbastHENIA (ABSENCE OR DYSFUNCTION OF GP**B***A) |
| AFIBRINOGENEMIA |
| DEFECTS OF PLATELET SECRETION |
| DECREASED CYCLOOXYGENASE ACTIVITY |
| DRUG-INDUCED (ASPIRIN, NONSTEROIDAL ANTI-INFLAMMATORY AGENTS) |
| INHERITED |
GRANULE STORAGE POOL DEFECTS
INHERITED
ACQUIRED
NONSPECIFIC DRUG EFFECTS
UREMIA
PLATELET COATING (EG, PARAPROTEIN, PENICILLIN)
DEFECT OF PLATELET COAGULANT ACTIVITY
SCOTT’S SYNDROME

CAUTERY. GASTROINTESTINAL (GI) BLEEDING AND HEMATURIA ARE USUALLY DUE TO UNDERLYING PATHOLOGY AND PROCEDURES TO IDENTIFY AND TREAT THE BLEEDING SITE SHOULD BE UNDERTAKEN, EVEN IN PATIENTS WITH KNOWN BLEEDING DISORDERS. VWD, PARTICULARLY TYPES 2 AND 3, HAS BEEN ASSOCIATED WITH ANGIODYSPLASIA OF THE BOWEL AND GI BLEEDING. HEMARTHROSES AND SPONTANEOUS MUSCLE HEMATOMAS ARE CHARACTERISTIC OF MODERATE OR SEVERE CONGENITAL FACTOR VIII OR IX DEFICIENCY. THEY CAN ALSO BE SEEN IN MODERATE AND SEVERE DEFICIENCIES OF FIBRINOGEN, PROTHROMBIN, AND OF FACTORS V, VII, AND X. SPONTANEOUS HEMARTHROSES OCCUR RARELY IN OTHER BLEEDING DISORDERS EXCEPT FOR SEVERE VWD, WITH ASSOCIATED FVIII LEVELS <5%. MUSCLE AND SOFT TISSUE BLEEDS ARE ALSO COMMON IN ACQUIRED FVIII DEFICIENCY. BLEEDING INTO A JOINT RESULTS IN SEVERE PAIN AND SWELLING, AS WELL AS LOSS OF FUNCTION, BUT IS RARELY ASSOCIATED WITH DISCOLORATION FROM BRUISING AROUND THE JOINT. LIFE-THREATENING SITES OF BLEEDING INCLUDE BLEEDING INTO THE OROPHARYNX, WHERE BLEEDING CAN OBSTRUCT THE AIRWAY, INTO THE CENTRAL NERVOUS SYSTEM, AND INTO THE RETROPERITONEUM. CENTRAL NERVOUS SYSTEM BLEEDING IS THE MAJOR CAUSE OF BLEEDING-RELATED DEATHS IN PATIENTS WITH SEVERE CONGENITAL FACTOR DEFICIENCIES.

PROHEMORRHAGIC EFFECTS OF MEDICATIONS AND DIETARY SUPPLEMENTS

ASPIRIN AND OTHER NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) THAT INHIBIT CYCLOOXYGENASE 1 IMPAIR PRIMARY HEMOSTASIS AND MAY EXACERBATE BLEEDING FROM ANOTHER CAUSE OR EVEN UNMASK A PREVIOUSLY OCCULT MILD BLEEDING DISORDER SUCH AS VWD. ALL NSAIDS, HOWEVER, CAN PRECIPITATE GASTROINTESTINAL BLEEDING, WHICH MAY BE MORE SEVERE IN PATIENTS WITH UNDERLYING BLEEDING DISORDERS. THE ASPIRIN EFFECT ON PLATELET FUNCTION AS ASSESSED BY AGGREGOMETRY CAN PERSIST FOR UP TO 7 DAYS, ALTHOUGH IT HAS FREQUENTLY RETURNED TO NORMAL BY 3 DAYS AFTER THE LAST DOSE. THE EFFECT OF OTHER NSAIDS IS SHORTER, AS THE INHIBITOR EFFECT IS REVERSED WHEN THE DRUG IS REMOVED. MANY HERBAL SUPPLEMENTS CAN IMPAIR HEMOSTATIC FUNCTION (TABLE 59-2). SOME HAVE BEEN MORE CONVINCINGLY ASSOCIATED WITH A BLEEDING RISK THAN OTHERS. FISH OIL OR CONCENTRATED OMEGA 3 FATTY ACID SUPPLEMENTS IMPAIR PLATELET ACTIVATION. THEY ALTER PLATELET BIOCHEMISTRY TO PRODUCE MORE PG13, A MORE POTENT PLATELET INHIBITOR THAN PROSTACYCLIN (PGI2), AND MORE THROMBOXANE A3, A LESS POTENT PLATELET ACTIVATOR THAN THROMBOXANE A2. IN FACT, DIETS NATURALLY RICH IN OMEGA 3 FATTY ACIDS CAN RESULT IN A PROLONGED BLEEDING TIME AND ABNORMAL PLATELET AGGREGATION STUDIES, BUT THE ACTUAL ASSOCIATED BLEEDING RISK IS UNCLEAR. VITAMIN E APPEARS TO INHIBIT PROTEIN KINASE C-MEDIATED PLATELET AGGREGATION AND NITRIC OXIDE PRODUCTION. IN PATIENTS WITH UNEXPLAINED BRUISING OR BLEEDING, IT IS PRUDENT TO REVIEW ANY NEW MEDICATIONS OR SUPPLEMENTS AND DISCONTINUE THOSE
THAT MAY BE ASSOCIATED WITH BLEEDING.

UNDERLYING SYSTEMIC DISEASES THAT CAUSE OR EXACERBATE A BLEEDING TENDENCY  ACQUIRED BLEEDING DISORDERS ARE COMMONLY SECONDARY TO, OR ASSOCIATED WITH, SYSTEMIC DISEASE. THE CLINICAL EVALUATION OF A PATIENT WITH A BLEEDING TENDENCY MUST THEREFORE INCLUDE A THOROUGH ASSESSMENT FOR EVIDENCE OF UNDERLYING DISEASE. BRUISING OR MUCOSAL BLEEDING MAY BE THE PRESENTING COMPLAINT IN LIVER DISEASE, SEVERE RENAL IMPAIRMENT, HYPOTHYROIDISM, PARAPROTEINEMIAS OR AMYLOIDOSIS, AND CONDITIONS CAUSING BONE MARROW FAILURE. ALL COAGULATION FACTORS ARE SYNTHESIZED IN THE LIVER AND HEPATIC FAILURE RESULTS IN COMBINED FACTOR DEFICIENCIES. THIS IS OFTEN COMPOUNDED BY THROMBOCYTOPENIA FROM SPLENOMEGALY DUE TO PORTAL HYPERTENSION. COAGULATION FACTORS II, VII, IX, X AND PROTEINS C, S, AND Z ARE DEPENDENT ON VITAMIN K FOR POSTTRANSLATIONAL MODIFICATION. ALTHOUGH VITAMIN K IS REQUIRED IN BOTH PROCOAGULANT AND ANTICOAGULANT PROCESSES, THE PHENOTYPE OF VITAMIN K DEFICIENCY OR THE WARFARIN EFFECT ON COAGULATION IS BLEEDING. THE NORMAL BLOOD PLATELET COUNT IS 150,000-450,000/µL. THROMBOCYTOPENIA RESULTS FROM DECREASED PRODUCTION, INCREASED DESTRUCTION, AND/OR SEQUESTRATION. ALTHOUGH THE BLEEDING RISK VARIES SOMEWHAT BY THE REASON FOR THE THROMBOCYTOPENIA, BLEEDING RARELY OCCURS IN ISOLATED THROMBOCYTOPENIA AT COUNTS <50,000/µL AND USUALLY NOT UNTIL <10,000-20,000/µL. COEXISTING COAGULOPATHIES, AS SEEN IN LIVER FAILURE OR DISSEMINATED COAGULATION; INFECTION; PLATELET-

367 CHAPTER 59 BLEEDING AND THROMBOSIS

TABLE 59-2 HERBAL SUPPLEMENTS ASSOCIATED WITH INCREASED BLEEDING

HERBS WITH POTENTIAL ANTI-PLATELET ACTIVITY
GINKGO (GINKGO BILOBA L)
GARLIC (ALLIUM SATIVUM)
BILBERRY (VACCINIUM MYRTILLUS)
GINGER (GINGIBER OFFICINALE)
DONG QUAI (ANGELICA SINENSIS)
FEVERFEW (TANACETUM PARTHENIUM)
ASIAN GINSENG (PANAX GINSENG)
AMERICAN GINSENG (PANAX QUINQUEFOLIUS)
SIBERIAN GINSENG/ELEUTHERO (ELEUTHEROCOCCUS SENTICOSUS)
TUMERIC (CIRCUMA LONGA)
MEADOWSWEET (FILIPENDUIA ULMARIA)
WILLOW (SALIX SPP.)
COUMARIN-CONTAINING HERBS
MOTHERWORTH (LEONURUS CARDIACA)
CHAMOMILE (MATRICARIA RECUTITA, CHAMAEMELUM MOBILE)
HORSE CHESTNUT (AESCULUS HIPPOCASTANUM)
RED CLOVER (TRIFOLIUM PRATENSE)
FENUGREEK (TRIGONELLA FOENUM-GRAECUM)

COEXISTING COAGULOPATHIES, AS SEEN IN LIVER FAILURE OR DISSEMINATED COAGULATION; INFECTION; PLATELET-

ASSESSMENT FOR EVIDENCE OF UNDERLYING DISEASE. BRUISING OR MUCOSAL BLEEDING MAY BE THE PRESENTING COMPLAINT IN LIVER DISEASE, SEVERE RENAL IMPAIRMENT, HYPOTHYROIDISM, PARAPROTEINEMIAS OR AMYLOIDOSIS, AND CONDITIONS CAUSING BONE MARROW FAILURE. ALL COAGULATION FACTORS ARE SYNTHESIZED IN THE LIVER AND HEPATIC FAILURE RESULTS IN COMBINED FACTOR DEFICIENCIES. THIS IS OFTEN COMPOUNDED BY THROMBOCYTOPENIA FROM SPLENOMEGALY DUE TO PORTAL HYPERTENSION. COAGULATION FACTORS II, VII, IX, X AND PROTEINS C, S, AND Z ARE DEPENDENT ON VITAMIN K FOR POSTTRANSLATIONAL MODIFICATION. ALTHOUGH VITAMIN K IS REQUIRED IN BOTH PROCOAGULANT AND ANTICOAGULANT PROCESSES, THE PHENOTYPE OF VITAMIN K DEFICIENCY OR THE WARFARIN EFFECT ON COAGULATION IS BLEEDING. THE NORMAL BLOOD PLATELET COUNT IS 150,000-450,000/µL. THROMBOCYTOPENIA RESULTS FROM DECREASED PRODUCTION, INCREASED DESTRUCTION, AND/OR SEQUESTRATION. ALTHOUGH THE BLEEDING RISK VARIES SOMEWHAT BY THE REASON FOR THE THROMBOCYTOPENIA, BLEEDING RARELY OCCURS IN ISOLATED THROMBOCYTOPENIA AT COUNTS <50,000/µL AND USUALLY NOT UNTIL <10,000-20,000/µL. COEXISTING COAGULOPATHIES, AS SEEN IN LIVER FAILURE OR DISSEMINATED COAGULATION; INFECTION; PLATELET-
INHIBITORY DRUGS; AND UNDERLYING MEDICAL CONDITIONS CAN ALL INCREASE THE RISK OF BLEEDING IN THE THROMBOCYTOPENIC PATIENT.  MOST PROCEDURES CAN BE PERFORMED IN PATIENTS WITH A PLATELET COUNT OF 50,000/*L.  THE LEVEL NEEDED FOR MAJOR SURGERY WILL DEPEND ON THE TYPE OF SURGERY AND THE PATIENTS’ UNDERLYING MEDICAL STATE, ALTHOUGH A COUNT OF APPROXIMATELY 80,000/*L IS LIKELY SUFFICIENT.

HISTORY OF THROMBOSIS  THE RISK OF THROMBOSIS, LIKE THAT OF BLEEDING, IS INFLUENCED BY BOTH GENETIC AND ENVIRONMENTAL INFLUENCES. THE MAJOR RISK FACTOR FOR ARTERIAL THROMBOSIS IS ATHEROSCLEROSIS, WHILE THOSE FOR VENOUS THROMBOSIS ARE IMMOBILITY, SURGERY, UNDERLYING MEDICAL CONDITIONS SUCH AS MALIGNANCY, MEDICATIONS SUCH AS HORMONAL THERAPY, OBESITY, AND GENETIC PREDISPOSITIONS.  FACTORS THAT INCREASE RISKS FOR VENOUS AND BOTH VENOUS AND ARTERIAL THROMBOSES ARE SHOWN IN TABLE 59-3.  THE MOST IMPORTANT POINT IN A HISTORY RELATED TO VENOUS THROMBOSIS IS WHETHER THE THROMBOTIC EVENT WAS IDIOPATHIC (MEANING THERE WAS NO CLEAR PRECIPITATING FACTOR) OR WAS A PRECIPITATED EVENT. IN PATIENTS WITHOUT UNDERLYING MALIGNANCY, HAVING AN IDIOPATHIC EVENT IS THE STRONGEST PREDICTOR OF RECURRENCE OF VENOUS THROMBOEMBOLISM.  IN PATIENTS WHO HAVE A VAGUE HISTORY OF THROMBOSIS, A HISTORY OF BEING TREATED WITH WARFARIN SUGGESTS A PAST DVT.  AGE IS AN IMPORTANT RISK FACTOR FOR VENOUS THROMBOSIS; THE RISK OF DVT INCREASES PER DECADE, WITH AN APPROXIMATE INCIDENCE OF 1/100,000 PER YEAR IN EARLY CHILDHOOD TO 1/200 PER YEAR AMONG OCTOGENARIANS. FAMILY HISTORY IS HELPFUL IN DETERMINING IF THERE IS A GENETIC PREDISPOSITION AND HOW STRONG THAT PREDISPOSITION APPEARS TO BE. A GENETIC THROMBOPHILIA THAT CONFERS A RELATIVELY SMALL INCREASED RISK, SUCH AS BEING A HETEROZYGOE FOR THE PROTHROMBIN G20210A OR FACTOR V LEIDEN MUTATION, MAY BE A RELATIVELY MINOR DETERMINANT OF

TABLE 59-3 RISK FACTORS FOR THROMBOSIS

VENOUS

INHERITED
FACTOR V LEIDEN
PROTHROMBIN G20210A
ANTITHROMBIN DEFICIENCY
PROTEIN C DEFICIENCY
PROTEIN SDEFICIENCY
ELEVATED FVIII

ACQUIRED
AGE
PREVIOUS THROMBOSIS
IMMOBILIZATION
MAJOR SURGERY
PREGNANCY & PUERPERIUM
HOSPITALIZATION
OBESITY
INFECTION
APC RESISTANCE, NONGENETIC
ELEVATED FACTOR II, IX, XI
ELEVATED TAFI LEVELS
LOW LEVELS OF TFPI

VENOUS AND ARTERIAL

INHERITED
HOMOCYSTINURIA
DYSFIBRINOGENEMIA

MIXED (INHERITED AND ACQUIRED)
HYPERHOMOCYSTEINEMIA

ACQUIRED
MALIGNANCY
ANTIPHOSPHOLIPID ANTIBODY SYNDROME
HORMONAL THERAPY
POLYCYTHEMIA VERA
ESSENTIAL THROMBOCYTHEMIA
PAROXYSMAL NOCTURAL HEMOGLOBINURIA
THROMBOTIC THROMBOCYTOPENIC PURPURA
HEPARIN-INDUCED THROMBOCYTOPENIA
DISSEMINATED INTRAVASCULAR COAGULATION

###UNKNOWN WHETHER RISK IS INHERITED OR ACQUIRED.

**NOTE:** APC, ACTIVATED PROTEIN C; TAFI, THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR; TFPI, TISSUE FACTOR PATHWAY INHIBITOR.

RISK IN AN ELDERLY INDIVIDUAL UNDERGOING A HIGH RISK SURGICAL PROCEDURE. AS SHOWN IN FIG. 59-5, A THROMBOTIC EVENT OFTEN HAS MORE THAN ONE CONTRIBUTING FACTOR. PREDISPOSING FACTORS MUST BE CAREFULLY ASSESSED TO DETERMINE THE RISK OF RECURRENT THROMBOSIS, AND WITH CONSIDERATION OF THE PATIENT’S BLEEDING RISK, DETERMINE THE LENGTH OF ANTICOAGULATION. SIMILAR CONSIDERATION SHOULD BE GIVEN TO DETERMINING THE NEED TO TEST THE PATIENT AND FAMILY MEMBERS FOR GENETIC THROMBOPHILIAS.

**FIGURE 59-5 THROMBOTIC RISK OVER TIME.** SHOWN SCHEMATICALLY IS AN INDIVIDUAL’S THROMBOTIC RISK OVER TIME. AN UNDERLYING FACTOR V LEIDEN MUTATION PROVIDES A “THEORETICALLY” CONSTANT INCREASED RISK. THE THROMBOTIC RISK INCREASES WITH AGE AND, INTERMITTENTLY, WITH ORAL CONTRACEPTIVE (OCP) OR HORMONE REPLACEMENT (HRT) USE, OTHER EVENTS MAY INCREASE THE RISK FURTHER. AT SOME POINT THE CUMULATIVE RISK MAY INCREASE TO THE THRESHOLD FOR THROMBOSIS AND RESULT IN DEEP VENOUS THROMBOSIS (DVT). NOTE: THE MAGNITUDE AND DURATION OF RISK PORTRAYED IN THE FIGURE IS MEANT FOR EXAMPLE ONLY AND MAY NOT PRECISELY REFLECT THE RELATIVE RISK DETERMINED BY CLINICAL STUDY. 

*FROM BA KONKLE, A SCHAFER, IN DP ZIPES ET AL (EDS): BRAUNWALD’S HEART DISEASE, 7TH ED. PHILADELPHIA, SAUNDERS, 2005; MODIFIED WITH PERMISSION FROM FR ROSENDAAL: VENOUS THROMBOSIS: A MULTICAUSAL DISEASE. LANCET 353:1167, 1999*
368 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

LABORATORY EVALUATION  CAREFUL HISTORY TAKING AND CLINICAL EXAMINATION ARE ESSENTIAL COMPONENTS IN THE ASSESSMENT OF BLEEDING AND THROMBOTIC RISK. THE USE OF LABORATORY TESTS OF COAGULATION COMPLEMENT, BUT CANNOT SUBSTITUTE FOR, CLINICAL ASSESSMENT. NO TEST PROVIDES A GLOBAL ASSESSMENT OF HEMOSTASIS. THE BLEEDING TIME HAS BEEN USED TO ASSESS BLEEDING RISK; HOWEVER, IT DOES NOT PREDICT BLEEDING RISK WITH SURGERY AND IS NOT RECOMMENDED FOR THIS INDICATION. THE PFA-100, AN INSTRUMENT THAT MEASURES PLATELET-DEPENDENT COAGULATION UNDER FLOW CONDITIONS, IS MORE SENSITIVE AND SPECIFIC FOR PLATELET DISORDERS AND VWD THAN THE BLEEDING TIME; HOWEVER, IT IS NOT SENSITIVE ENOUGH TO RULE OUT UNDERLYING MILD BLEEDING DISORDERS. ALSO, IT HAS NOT BEEN EVALUATED PROSPECTIVELY TO DETERMINE ITS UTILITY IN PREDICTING BLEEDING RISK, ALTHOUGH SUCH STUDIES ARE UNDERWAY.

FOR ROUTINE PREOPERATIVE AND PREPROCEDURE TESTING, AN ABNORMAL PROTHROMBIN TIME (PT) MAY DETECT LIVER DISEASE OR VITAMIN K DEFICIENCY THAT HAD NOT BEEN PREVIOUSLY APPRECIATED. STUDIES HAVE NOT CONFIRMED THE USEFULNESS OF AN ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) IN PREOPERATIVE EVALUATIONS IN PATIENTS WITH A NEGATIVE BLEEDING HISTORY. THE PRIMARY USE OF COAGULATION TESTING SHOULD BE TO CONFIRM THE PRESENCE AND TYPE OF BLEEDING DISORDER IN A PATIENT WITH A SUSPICIOUS CLINICAL HISTORY.

BECAUSE OF THE NATURE OF COAGULATION ASSAYS, PROPER SAMPLE ACQUISITION AND HANDLING IS CRITICAL TO OBTAINING VALID RESULTS. IN PATIENTS WITH ABNORMAL COAGULATION ASSAYS WHO HAVE NO BLEEDING HISTORY, REPEAT STUDIES WITH ATTENTION TO THESE FACTORS FREQUENTLY RESULT IN NORMAL VALUES. MOST COAGULATION ASSAYS ARE PERFORMED IN SODIUM CITRATE ANTICOAGULATED PLASMA THAT IS RECALCIFIED FOR THE ASSAY. BECAUSE THE ANTICOAGULANT IS IN LIQUID SOLUTION AND NEEDS TO BE ADDED TO BLOOD IN PROPORTION TO THE PLASMA VOLUME, INCORRECTLY FILLED OR INADEQUATELY MIXED BLOOD COLLECTION TUBES WILL GIVE ERRORNEOUS RESULTS. VACUTAINER TUBES SHOULD BE FILLED TO >90% OF THE RECOMMENDED FILL, WHICH IS USUALLY DENOTED BY A LINE ON THE TUBE. AN ELEVATED HEMATOCRIT (>55%) CAN RESULT IN A FALSE VALUE DUE TO A DECREASED PLASMA TO ANTICOAGULANT RATIO.

II, VII, IX, and X in anticoagulated patients is now expressed as the international sensitivity index (ISI). An inverse relationship exists between the ISI and thromboplastin sensitivity. The international normalized ratio (INR) is then determined based on the formula: 

INR = (PT###PATIENT/PT###NORMAL MEAN)###SI.

While the INR was developed to assess anticoagulation due to reduction of vitamin K-dependent coagulation factors, it is commonly used in the evaluation of patients with liver disease. This measure provides a system for comparing values from testing performed at different laboratories. However, as progressive liver failure is associated with variable changes in coagulation factors, the degree of prolongation of either the PT or the INR only roughly predicts the bleeding risk. Thrombin generation has been shown to be normal in many patients with mild to moderate liver dysfunction. As the PT only measures one aspect of hemostasis affected by liver dysfunction, we likely overestimate the bleeding risk of a mildly elevated INR in this setting.

The APTT assesses the intrinsic and common coagulation pathways, factors XI, IX, VIII, V, II, fibrinogen, and also prekallikrein, high molecular weight kininogen and factor XII (Fig. 59-6). The APTT reagent contains phospholipids derived from either animal or vegetable sources that function as a platelet substitute in the coagulation pathways and includes an activator of the intrinsic coagulation system, such as ellagic acid or the particulate activators kaolin, celite, or micronized silica.

The phospholipid composition of APTT reagents varies, which influences the sensitivity of individual reagents to clotting factor deficiencies and to inhibitors such as heparin and lupus anticoagulants. Thus, APTT results will vary from one laboratory to another, and the normal range in the laboratory where the testing occurs should be used in the interpretation. Local laboratories can relate their APTT values to therapeutic heparin anticoagulation by correlating APTT values with direct measurements of heparin activity (anti-Xa or protamine titration assays) in samples from heparinized patients, although correlation between these assays is often poor. The APTT reagent will vary in sensitivity to individual factor deficiencies and usually becomes prolonged with individual factor deficiencies of 30-50%. The relationship between defects in secondary hemostasis (fibrin formation) and coagulation test abnormalities is shown in Table 59-4.

**Figure 59-6 Coagulation Factor Activity** Tested in the activated partial thromboplastin time (APTT) in red and prothrombin time (PT) in green, or both. HMWK, high-molecular-weight kininogen; PK, prekallikrein; F, factor.

**Mixing Studies** Mixing studies are used to evaluate a prolonged APTT or, less commonly PT, to distinguish between a factor deficiency and an inhibitor. In this assay, normal plasma and patient plasma are mixed in a 50:50 ratio, and the APTT or PT is determined immediately and after incubation at 37°C for varying times, typically 30, 60, and/or 120 min. With isolated factor deficiencies, the APTT will correct with mixing and stay corrected with incubation. With APTT prolongation due to a lupus anticoagulant, the
MIXING AND INCUBATION WILL SHOW NO CORRECTION. IN ACQUIRED NEUTRALIZING FACTOR ANTIBodies, SUCH AS AN ACQUIRED FACTOR VIII INHIBITOR, THE INITIAL ASSAY MAY OR MAY NOT CORRECT IMMEDIATELY AFTER MIXING BUT WILL PROLONG OR REMAIN PROLONGED WITH INCUBATION AT 37°C. FAILURE TO CORRECT WITH MIXING CAN ALSO BE DUE TO THE PRESENCE OF OTHER INHIBITORS OR INTERFERING SUBSTANCES SUCH AS HEPARIN, FIBRIN SPLIT PRODUCTS, AND PARAPROTEINS.

SPECIFIC FACTOR ASSAYS DECISIONS TO PROCEED WITH SPECIFIC CLOTTING FACTOR ASSAYS WILL BE INFLUENCED BY THE CLINICAL SITUATION AND THE RESULTS OF COAGULATION SCREENING TESTS. PRECISE DIAGNOSIS AND EFFECTIVE MANAGEMENT OF INHERITED AND ACQUIRED COAGULATION DEFICIENCIES NECESSITATE QUANTITATION OF THE RELEVANT FACTORS. WHEN BLEEDING IS SEVERE, SPECIFIC ASSAYS ARE OFTEN URGENTLY REQUIRED TO GUIDE APPROPRIATE THERAPY. INDIVIDUAL FACTOR ASSAYS ARE USUALLY PERFORMED AS MODIFICATIONS OF THE MIXING STUDY, WHERE THE PATIENT’S PLASMA IS

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369 CHAPTER 59 BLEEDING AND THROMBOSIS

TABLE 59-4 HEMOSTATIC DISORDERS AND COAGULATION TEST ABNORMALITIES

- **PROLONGED ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)**
  - NO CLINICAL BLEEDING - * FACTORS XII, HIGH-MOLECULAR-WEIGHT KININOGEN, PROTEIN KINASE
  - VARIABLE, BUT USUALLY MILD, BLEEDING - * FACTOR XI, MILD * FVIII AND FIX
  - FREQUENT, SEVERE BLEEDING - SEVERE DEFICIENCIES OF FVIII AND FIX

- **HEPARIN**
  - PROLONGED PROTHROMBIN TIME (PT)

- **FACTOR VII DEFICIENCY**

- **VITAMIN K DEFICIENCY - EARLY**

- **WARFARIN ANTICOAGULATION**

- **PROLONGED APTT AND PT**

- **FACTOR II, VOR X DEFICIENCY**

- **VITAMIN K DEFICIENCY - LATE**

- **DIRECT THROMBIN INHIBITORS**

- **PROLONGED THROMBIN TIME**

- **HEPARIN OR HEPARIN-LIKE INHIBITORS**

- **MILD OR NO BLEEDING - DYSFIBRINOGENEMIA**

- **FREQUENT, SEVERE BLEEDING - AFIBRINOGENEMIA**

- **PROLONGED PT AND/OR APTT NOT CORRECT WITH MIXING WITH NORMAL PLASMA**

- **BLEEDING - SPECIFIC FACTOR INHIBITOR**

- **MO SYMPTOMS, OR CLOTTING AND/OR PREGNANCY LOSS - LUPUS ANTICOAGULANT**

- **DISSEMINATED INTRAVASCULAR COAGULATION**

- **HEPARIN OR DIRECT THROMBIN INHIBITOR**

- **ABNORMAL CLOT SOLUBILITY**

- **FACTOR XIII DEFICIENCY**

- **INHIBITORS OR DEFECTIVE CROSS-LINKING R APID CLOT LYSIS**

- **DEFICIENCY OF *###2-ANTIPLASMIN OR PLASMINOGEN ACTIVATOR INHIBITOR 1**
TREATMENT WITH FIBRINOLYTIC THERAPY

MIXED WITH PLASMA DEFICIENT IN THE FACTOR BEING STUDIED. THIS WILL CORRECT ALL FACTOR DEFICIENCIES TO >50%, THUS MAKING PROLONGATION OF CLOT FORMATION DUE TO A FACTOR DEFICIENCY DEPENDENT ON THE FACTOR MISSING FROM THE ADDED PLASMA.

TESTING FOR ANTIPHOSPHOLIPID ANTIBODIES  ANTIBODIES TO PHOSPHOLIPIDS (CARDIOLIPIN) OR PHOSPHOLIPID-BINDING PROTEINS (*###2-MICROGLOBULIN AND OTHERS) ARE DETECTED BY ELISA. WHEN THESE ANTIBODIES INTERFERE WITH PHOSPHOLIPID-DEPENDENT COAGULATION TESTS, THEY ARE TERMED LUPUS ANTICOAGULANTS. THE APTT HAS VARIABLE SENSITIVITY TO LUPUS ANTICOAGULANTS, DEPENDING IN PART ON THE APTT REAGENTS USED. AN ASSAY UTILIZING A SENSITIVE REAGENT HAS BEEN TERMED AN LA-PTT. THE DILUTE RUSSELL VIPER VENOM TEST (DRVVT) AND THE TISSUE THROMBOPLASTIN TIME (TTI) ARE MODIFICATIONS OF STANDARD TESTS WITH THE PHOSPHOLIPID REAGENT DECREASED, THUS INCREASING THE SENSITIVITY TO ANTIBODIES THAT INTERFERE WITH THE PHOSPHOLIPID COMPONENT. THE TESTS, HOWEVER, ARE NOT SPECIFIC FOR LUPUS ANTICOAGULANTS, AS FACTOR DEFICIENCIES OR OTHER INHIBITORS ALSO RESULT IN PROLONGATION. DOCUMENTATION OF A LUPUS ANTICOAGULANT REQUIRES NOT ONLY PROLONGATION OF A PHOSPHOLIPID-DEPENDENT COAGULATION TEST BUT ALSO LACK OF CORRECTION WHEN MIXED WITH NORMAL PLASMA AND CORRECTION WITH THE ADDITION OF ACTIVATED PLATELET MEMBRANES OR CERTAIN PHOSPHOLIPIDS, E.G.,HEXAGONAL PHASE.

OTHER COAGULATION TESTS  THE THROMBIN TIME AND THE REPTILASE TIME MEASURE FIBRINOGEN CONVERSION TO FIBRIN AND ARE PROLONGED WHEN THE FIBRINOGEN LEVEL IS LOW (USUALLY <80-100 MG/DL); QUALITATIVELY ABNORMAL, AS SEEN IN INHERITED OR ACQUIRED DYSFIBRINOGENEMIAS; OR WHEN FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS INTERFERE. THE THROMBIN TIME, BUT NOT THE REPTILASE TIME, IS PROLONGED IN THE PRESENCE OF HEPARIN. MEASUREMENT OF ANTI-FACTOR XA PLASMA INHIBITORY ACTIVITY IS A TEST FREQUENTLY USED TO ASSESS LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) ACTIVITY OR AS A DIRECT MEASUREMENT OF UNFRACTIONATED HEPARIN (UFH) ACTIVITY. HEPARIN IN THE PATIENT SAMPLE INHIBITS THE ENZYMATIC CONVERSION OF AN XA-SPECIFIC CHROMOGENIC SUBSTRATE TO COLORED PRODUCT BY FACTOR XA. STANDARD CURVES ARE CREATED USING MULTIPLE CONCENTRATIONS OF UFH AND LMWH AND ARE USED TO CALCULATE THE CONCENTRATION OF ANTI-XA ACTIVITY IN THE PATIENT PLASMA.

LABORATORY TESTING FOR THROMBOPHILIA  LABORATORY ASSAYS TO DETECT THROMBOPHILIC STATES INCLUDE MOLECULAR DIAGNOSTIC, IMMUNOLOGIC AND FUNCTIONAL ASSAYS. THESE ASSAYS VARY IN THEIR SENSITIVITY AND SPECIFICITY FOR THE CONDITION BEING TESTED. FURTHERMORE, ACUTE THROMBOSIS, ACUTE ILLNESSES, INFLAMMATORY CONDITIONS, PREGNANCY, AND MEDICATIONS AFFECT LEVELS OF MANY COAGULATION FACTORS AND THEIR INHIBITORS. ANTITHROMBIN IS DECREASED BY HEPARIN AND IN THE SETTING OF ACUTE THROMBOSIS. PROTEIN C AND S LEVELS MAY BE INCREASED IN THE SETTING OF ACUTE THROMBOSIS AND ARE DECREASED BY WARFARIN. ANTIPHOSPHOLIPID ANTIBODIES ARE FREQUENTLY TRANSIENTLY POSITIVE IN ACUTE ILLNESS. AS THROMBOPHILIA EVALUATIONS ARE USUALLY PERFORMED TO ASSESS THE NEED TO EXTEND ANTICOAGULATION, TESTING SHOULD BE PERFORMED IN A STEADY STATE, REMOTE FROM THE ACUTE
EVENT. IN MOST INSTANCES WARFARIN ANTICOAGULATION CAN BE STOPPED AFTER THE INITIAL 3-6 MONTHS OF TREATMENT, AND TESTING IS PERFORMED AT LEAST 3 WEEKS LATER. FURTHERMORE, SENSITIVE MARKERS OF COAGULATION ACTIVATION, NOTABLY THE D-DIMER ASSAY AND THE THROMBIN GENERATION TEST, HOLD PROMISE AS PREDICTORS, WHEN ELEVATED, OF RECURRENT THROMBOSIS WHEN MEASURED AT LEAST 1 MONTH FROM DISCONTINUATION OF WARFARIN, ALTHOUGH FURTHER STUDY IS NEEDED TO BETTER SUPPORT THIS APPLICATION.

MEASURES OF PLATELET FUNCTION THE BLEEDING TIME HAS BEEN USED TO ASSESS BLEEDING RISK; HOWEVER, IT HAS NOT BEEN FOUND TO PREDICT BLEEDING RISK WITH SURGERY, AND IT IS NOT RECOMMENDED FOR USE FOR THIS INDICATION. THE PFA-100 AND SIMILAR INSTRUMENTS THAT MEASURE PLATELET-DEPENDENT COAGULATION UNDER FLOW CONDITIONS ARE GENERALLY MORE SENSITIVE AND SPECIFIC FOR PLATELET DISORDERS AND VWD THAN THE BLEEDING TIME; HOWEVER, DATA ARE INSUFFICIENT TO SUPPORT THEIR USE TO PREDICT BLEEDING RISK OR MONITOR RESPONSE TO THERAPY. WHEN THEY ARE USED IN THE EVALUATION OF A PATIENT WITH BLEEDING SYMPTOMS, ABNORMAL RESULTS, AS WITH THE BLEEDING TIME, REQUIRE SPECIFIC TESTING, SUCH AS VWF ASSAYS AND/OR PLATELET AGGREGATION STUDIES. SINCE ALL OF THESE “SCREENING” ASSAYS MAY MISS PATIENTS WITH MILD BLEEDING DISORDERS, FURTHER STUDIES ARE NEEDED TO DEFINE THEIR ROLE IN HEMOSTASIS TESTING.

FOR CLASSIC PLATELET AGGREGOMETRY, VARIOUS AGONISTS ARE ADDED TO THE PATIENT’S PLATELET-RICH PLASMA, AND PLATELET AGGLUTINATION AND AGGREGATION ARE OBSERVED. TESTS OF PLATELET SECRETION IN RESPONSE TO AGONISTS CAN ALSO BE MEASURED. THESE TESTS ARE AFFECTED BY MANY FACTORS, INCLUDING NUMEROUS MEDICATIONS, AND THE ASSOCIATION BETWEEN MINOR DEFECTS IN AGGREGATION OR SECRETION IN THESE ASSAYS AND BLEEDING RISK IS NOT CLEARLY ESTABLISHED.

ACKNOWLEDGMENT
ROBERT I. HANDIN, MD, CONTRIBUTED THIS CHAPTER IN THE 16TH EDITION AND SOME MATERIAL FROM THAT CHAPTER HAVE BEEN RETAINED HERE.

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370 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

60 ENLARGEMENT OF LYMPH NODES AND SPLEEN
PATRICK H. HENRY, DAN L. LONGO

This chapter is intended to serve as a guide to the evaluation of patients who present with enlargement of the lymph nodes (lymphadenopathy) or the spleen (splenomegaly). Lymphadenopathy is a rather common clinical finding in primary care settings, whereas palpable splenomegaly is less so.

LYMPHADENOPATHY

Lymphadenopathy may be an incidental finding in patients being examined for various reasons, or it may be a presenting sign or symptom of the patient’s illness. The physician must eventually decide whether the lymphadenopathy is a normal finding or one that requires further study, up to and including biopsy. Soft, flat, submandibular nodes (<1 cm) are often palpable in healthy children and young adults, and healthy adults may have palpable inguinal nodes of up to 2 cm, which are considered normal. Further evaluation of these normal nodes is not warranted. In contrast, if the physician believes the node(s) to be abnormal, then pursuit of a more precise diagnosis is needed.

APPROACH TO THE PATIENT: LYMPHADENOPATHY

Lymphadenopathy may be a primary or secondary manifestation of numerous disorders, as shown in Table 60-1. Many of these disorders are infrequent causes of lymphadenopathy. In primary care practice, more than two-thirds of patients with lymphadenopathy have nonspecific causes or upper respiratory illnesses (viral or bacterial), and <1% have a malignancy. In one study, 84% of patients referred for evaluation of lymphadenopathy had a “benign” diagnosis. The remaining 16% had a malignancy (lymphoma or metastatic adenocarcinoma). Of the patients with benign lymphadenopathy, 63% had a nonspecific or reactive etiology (no causative agent found), and the remainder had a specific cause demonstrated, most commonly infectious mononucleosis, toxoplasmosis, or tuberculosis. Thus, the vast majority of patients with lymphadenopathy will have a nonspecific etiology requiring few diagnostic tests.

CLINICAL ASSESSMENT  The physician will be aided in the pursuit of an explanation for the lymphadenopathy by a careful medical history, physical examination, selected laboratory tests, and perhaps
AN EXCISIONAL LYMPH NODE BIOPSY. THE MEDICAL HISTORY SHOULD REVEAL THE SETTING IN WHICH LYMPHADENOPATHY IS OCCURRING. SYMPTOMS SUCH AS SORE THROAT, COUGH, FEVER, NIGHT SWEATS, FATIGUE, WEIGHT LOSS, OR PAIN IN THE NODES SHOULD BE SOUGHT. THE PATIENT’S AGE, SEX, OCCUPATION, EXPOSURE TO PETS, SEXUAL BEHAVIOR, AND USE OF DRUGS SUCH AS DIPHENYLHYDANTOIN ARE OTHER IMPORTANT HISTORIC POINTS. FOR EXAMPLE, CHILDREN AND YOUNG ADULTS USUALLY HAVE BENIGN (I.E., NONMALIGNANT) DISORDERS, SUCH AS VIRAL OR BACTERIAL UPPER RESPIRATORY INFECTIONS, INFECTIOUS MONONUCLEOSIS, TOXOPLASMOSIS, AND, IN SOME COUNTRIES, TUBERCULOSIS, WHICH ACCOUNT FOR THE OBSERVED LYMPHADENOPATHY. IN CONTRAST, AFTER AGE 50 THE INCIDENCE OF MALIGNANT DISORDERS INCREASES AND THAT OF BENIGN DISORDERS DECREASES.

THE PHYSICAL EXAMINATION CAN PROVIDE USEFUL CLUES SUCH AS THE EXTENT OF LYMPHADENOPATHY (LOCALIZED OR GENERALIZED), SIZE OF NODES, TEXTURE, PRESENCE OR ABSENCE OF NODAL TENDERNESS, SIGNS OF INFLAMMATION OVER THE NODE, SKIN LESIONS, AND SPLENOMEGALY. A THOROUGH EAR, NOSE, AND THROAT (ENT) EXAMINATION IS INDICATED IN ADULT PATIENTS WITH CERVICAL ADENOPATHY AND A HISTORY OF TOBACCO USE. LOCALIZED OR REGIONAL ADENOPATHY IMPLIES INVOLVEMENT OF A SINGLE ANATOMIC AREA. GENERALIZED ADENOPATHY HAS BEEN DEFINED AS INVOLVEMENT OF THREE OR

**TABLE 60-1 DISEASES ASSOCIATED WITH LYMPHADENOPATHY**

1. INFECTIOUS DISEASES
   A. VIRAL-INFECTIOUS MONONUCLEOSIS SYNDROMES (EBV, CMV), INFECTIOUS HEPATITIS, HERPES SIMPLEX, HERPESVIRUS-6, VARICELLA-ZOSTER VIRUS, RUBELLA, MEASLES, ADENOVIRUS, HRV, EPIDEMIC KERATOCONJUNCTIVITIS, VACCINIA, HERPESVIRUS-8
   B. BACTERIAL-STREPTOCOCCI, STAPHYLOCOCCI, CAT-SCRATCH DISEASE, BRUCELLOSIS, TULAREMIA, SCALE, BRUCELLOSIS, TULAREMIA, CHANCROID, MELIOIDOSIS, GLANDERS, TUBERCULOSIS, ATYPICAL MYCOBACTERIAL INFECTION, PRIMARY AND SECONDARY SYPHILIS, DIPHTHERIA, LEPROSY
   C. FUNGAL-HISTOPLASMOSIS, COCCIDIOIDOMYCOSIS, PARACOCIDIOIDOMYCOSIS
   D. CHLAMYDIAL-LYMPHOGRAVLUMA VENEREUM, TRACHOMA
   E. PARASITIC-TOXOPLASMOSIS, LEISHMANIASIS, TRYPANOSOMIASIS, FILARIASIS
   F. RICKETTSIAL-SCRUB TYPHUS, RICKETTSIALPOX, Q FEVER

2. IMMUNOLOGIC DISEASES
   A. RHEUMATOID ARTHRITIS
   B. JUVENILE RHEUMATOID ARTHRITIS
   C. MIXED CONNECTIVE TISSUE DISEASE
   D. SYSTEMIC LUPUS ERYTHEMATOSUS
   E. DERMATOMYOSITIS
   F. SJOGREN'S SYNDROME
   G. SERUM SICKNESS
   H. DRUG HYPERSENSITIVITY-DIPHENYLHYDANTOIN, HYDRALAZINE, ALLOPURINOL, PRIMIDONE, GOLD, CARBAMAZEPINE, ETC.
   I. ANGIOIMMUNOBLASTIC LYMPHADENOPATHY
   J. PRIMARY BILIARY CIRRHOSIS
   K. GRAFT-VERS.-HOST DISEASE
   L. SILICONE-ASSOCIATED
   M. AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME

3. MALIGNANT DISEASES
A. HEMATOLOGIC-HODGKIN'S DISEASE, NON-HODGKIN'S LYMPHOMAS, ACUTE OR CHRONIC LYMPHOCYTIC LEUKEMIA, HAIRY CELL LEUKEMIA, MALIGNANT HISTIOCYTOSIS, AMYLOIDOSIS
B. METASTATIC-FROM NUMEROUS PRIMARY SITES
4. LIPID STORAGE DISEASES-GAUCHERS, NIEMANN-PICK FABRY, TANGIER
5. ENDOCRINE DISEASES-HYPERTHYROIDISM
6. OTHER DISORDERS
A. CASTLEMAN'S DISEASE (GIANT LYMPH NODE HYPERPLASIA)
B. SARCOIDOSIS
C. DERMATOPATHIC LYMPHADENITIS
D. LYMPHOMATOID GRANULOMATOSIS
E. HISTIOCYTIC NECROTIZING LYMPHADENITIS (KIKUCHI'S DISEASE)
F. SINUS HISTIOCYTOSIS WITH MASSIVE LYMPHADENOPATHY (ROSAI-DORFMAN DISEASE)
G. MUCOCUTANEOUS LYMPH NODE SYNDROME (KAWASAKI'S DISEASE)
H. HISTIOCYTOSIS X
I. FAMILIAL MEDITERRANEAN FEVER
J. SEVERE HYPERTRIGLYCERYDERIDEMIA
K. VASCULAR TRANSFORMATION OF SINUSES
L. INFLAMMATORY PSEUDOTUMOR OF LYMPH NODE
M. CONGESTIVE HEART FAILURE

NOTE: EBV, EPSTEIN-BARR VIRUS; CMV, CYTOMEGALOVIRUS.

MORE NONCONTIGUOUS LYMPH NODE AREAS. MANY OF THE CAUSES OF LYMPHADENOPATHY (TABLE 60-1) CAN PRODUCE LOCALIZED OR GENERALIZED ADENOPATHY, SO THIS DISTINCTION IS OF LIMITED UTILITY IN THE DIFFERENTIAL DIAGNOSIS. NEVERTHELESS, GENERALIZED LYMPHADENOPATHY IS FREQUENTLY ASSOCIATED WITH NONMALIGNANT DISORDERS SUCH AS INFECTIOUS MONONUCLEOSIS [EPSTEIN-BARR VIRUS (EBV) OR CYTOMEGALOVIRUS (CMV)], TOXOPLASMOSIS, AIDS, OTHER VIRAL INFECTIONS, SYSTEMIC LUPUS ERYTHEMATOSUS (SLE), AND MIXED CONNECTIVE TISSUE DISEASE. ACUTE AND CHRONIC LYMPHOCYTIC LEUKEMIAS AND MALIGNANT LYMPHOMAS ALSO PRODUCE GENERALIZED ADENOPATHY IN ADULTS.


PAGE NO. 17

371 CHAPTER 60 ENLARGEMENT OF LYMPH NODES AND SPLEEN

NODES IS ALWAYS ABNORMAL. BECAUSE THESE NODES DRAIN REGIONS OF THE LUNG AND RETROPERITONEAL SPACE, THEY CAN REFLECT LYMPHOMAS, OTHER CANCERS, OR INFECTIOUS PROCESSES ARISING IN THESE AREAS. VIRCHOW'S NODE IS AN ENLARGED LEFT SUPRACLAVICULAR NODE INFILTRATED WITH META-
STATIC CANCER FROM A GASTROINTESTINAL PRIMARY. METASTASES TO SUPRACLAVICULAR NODES ALSO OCCUR FROM LUNG, BREAST, TESTIS, OR OVARIAN CANCERS. TUBERCULOSIS, SARCOIDOSIS, AND TOXOPLASMOsis ARE NONNEOPLASTIC CAUSES OF SUPRACLAVICULAR ADENOPATHY. AXILLARY ADENOPATHY IS USUALLYDUE TO INJURIES OR LOCALIZED INFECTIONS OF THE IPSILATERAL UPPER EXTREMITY. MALIGNANT CAUSES INCLUDE MELANOMA OR LYMPHOMA AND, IN WOMEN, BREAST CANCER. INGUINAL LYMPHADENOPATHY IS USUALLY SECONDARY TO INFECTIONS OR TRAUMA OF THE LOWER EXTREMITIES AND MAY ACCOMPANY SEXUALLY TRANSMITTED DISEASES SUCH AS LYMPHOGANULOMA VENEREUM, PRIMARY SYPHILIS, GENITAL HERPES, OR CHANCROID. THESE NODES MAY ALSO BE INVOLVED BY LYMPHOMAS AND METASTATIC CANCER FROM PRIMARY LESIONS OF THE RECTUM, GENITALIA, OR LOWER EXTREMITIES (MELANOMA).

THE SIZE AND TEXTURE OF THE LYMPH NODE(S) AND THE PRESENCE OF PAIN ARE USEFUL PARAMETERS IN EVALUATING A PATIENT WITH LYMPHADENOPATHY. NODES <1.0 CM##2 IN AREA (1.0 CM X 1.0 CM OR LESS) ARE ALMOST ALWAYS SECONDARY TO BENIGN, NONSPECIFIC REACTIVE CAUSES. IN ONE RETROSPECTIVE ANALYSIS OF YOUNGER PATIENTS (9-25 YEARS) WHO HAD A LYMPH NODE BIOPSY, A MAXIMUM DIAMETER OF >2 CM SERVED AS ONE DISCRIMINANT FOR PREDICTING THAT THE BIOPSY WOULD REVEAL MALIGNANT OR GRANULOMATOUS DISEASE. ANOTHER STUDY SHOWED THAT A LYMPH NODE SIZE OF 2.25 CM##2 (1.5 CM X 1.5 CM) WAS THE BEST SIZE LIMIT FOR DISTINGUISHING MALIGNANT OR GRANULOMATOUS LYMPHADENOPATHY FROM OTHER CAUSES OF LYMPHADENOPATHY. PATIENTS WITH NODE(S) *1.0 CM##2 SHOULD BE OBSERVED AFTER EXCLUDING INFECTIOUS MONONUCLEOSIS AND/OR TOXOPLASMOsis UNLESS THERE ARE SYMPTOMS AND SIGNS OF AN UNDERLYING SYSTEMIC ILLNESS.

THE TEXTURE OF LYMPH NODES MAY BE DESCRIBED AS SOFT, FIRM, RUBBERY, HARD, DISCRETE, MATTED, TENDER, MOVABLE, OR FIXED. TENDERNESS IS FOUND WHEN THE CAPSULE IS STRETCHED DURING RAPID ENLARGEMENT, USUALLY SECONDARY TO AN INFLAMMATORY PROCESS. SOME MALIGNANT DISEASES SUCH AS ACUTE LEUKEMIA MAY PRODUCE RAPID ENLARGEMENT AND PAIN IN THE NODES. NODES INVOLVED BY LYMPHOMA TEND TO BE LARGE, DISCRETE, SYMMETRIC, RUBBERY, FIRM, MOBILE, AND NONTENDER. NODES CONTAINING METASTATIC CANCER ARE OFTEN HARD, NONTENDER, AND NONMOVABLE BECAUSE OF FIXATION TO SURROUNDING TISSUES. THE COEXISTENCE OF SPLENOMEGALY IN THE PATIENT WITH LYMPHADENOPATHY IMPLIES A SYSTEMIC ILLNESS SUCH AS INFECTIOUS MONONUCLEOSIS, LYMPHOMA, ACUTE OR CHRONIC LEUKEMIA, SLE, SARCOIDOSIS, TOXOPLASMOsis, CAT-SCRATCH DISEASE, OR OTHER LESS COMMON HEMATOLOGIC DISORDERS. THE PATIENT’S STORY SHOULD PROVIDE HELPFUL CLUES ABOUT THE UNDERLYING SYSTEMIC ILLNESS.

NONSUPERFICIAL PRESENTATIONS (THORACIC OR ABDOMINAL) OF ADE- NOPATHY ARE USUALLY DETECTED AS THE RESULT OF A SYMPTOM-DIRECTED DIAGNOSTIC WORKUP. THORACIC ADENOPATHY MAY BE DETECTED BY ROUTINE CHEST RADIOGRAPHY OR DURING THE WORKUP FOR SUPERFICIAL ADE- NOPATHY. IT MAY ALSO BE FOUND BECAUSE THE PATIENT COMPLAINS OF A COUGH OR WHEEZING FROM AIRWAY COMPRESSION; HOARSENESS FROM RECURRENT LARYNGEAL NERVE INVOLVEMENT; DYSPHAGIA FROM ESOPHAGEAL COMPRESSION; OR SWELLING OF THE NECK, FACE, OR ARMS SECONDARY TO COMPRESSION OF THE SUPERIOR VENA CAVA OR SUBCLAVIAN VEIN. THE DIFFERENTIAL DIAGNOSIS OF MEDIASTINAL AND HILAR ADENOPATHY INCLUDES PRIMARY LUNG DISORDERS AND SYSTEMIC ILLNESSES THAT CHARACTERISTICALLY INVOLVE MEDIASTINAL OR HILAR NODES. IN THE YOUNG, MEDIASTINAL ADE-
Nopathy is associated with infectious mononucleosis and sarcoidosis. In endemic regions, histoplasmosis can cause unilateral paratracheal lymph node involvement that mimics lymphoma. Tuberculosis can also cause unilateral adenopathy. In older patients, the differential diagnosis includes primary lung cancer (especially among smokers), lymphomas, metastatic carcinoma (usually lung), tuberculosis, fungal infection, and sarcoidosis. Enlarged intraabdominal or retroperitoneal nodes are usually malignant. Although tuberculosis may present as mesenteric lymphadenitis, these masses usually contain lymphomas or, in young men, germ cell tumors.

LABORATORY INVESTIGATION

The laboratory investigation of patients with lymphadenopathy must be tailored to elucidate the etiology suspected from the patient's history and physical findings. One study from a family practice clinic evaluated 249 younger patients with “enlarged lymph nodes, not infected” or “lymphadenitis.” No laboratory studies were obtained in 51%. When studies were performed, the most common were a complete blood count (CBC) (33%), throat culture (16%), chest X-ray (12%), or monospot test (10%). Only eight patients (3%) had a node biopsy, and half of those were normal or reactive. The CBC can provide useful data for the diagnosis of acute or chronic leukemias, EBV or CMV mononucleosis, lymphoma with a leukemic component, pyogenic infections, or immune cytopenias in illnesses such as SLE. Serologic studies may demonstrate antibodies specific to components of EBV, CMV, HIV, and other viruses; Toxoplasma gondii; Brucella; etc. If SLE is suspected, then antinuclear and anti-DNA antibody studies are warranted.

The chest X-ray is usually negative, but the presence of a pulmonary infiltrate or mediastinal lymphadenopathy would suggest tuberculosis, histoplasmosis, sarcoidosis, lymphoma, primary lung cancer, or metastatic cancer and demands further investigation. A variety of imaging techniques (CT, MRI, ultrasound, color Doppler ultrasonography) have been employed to differentiate benign from malignant lymph nodes, especially in patients with head and neck cancer. CT and MRI are comparably accurate (65-90%) in the diagnosis of metastases to cervical lymph nodes. Ultrasonography has been used to determine the long (L) axis, short (S) axis, and a ratio of long to short axis in cervical nodes. An L/S ratio of <2.0 has a sensitivity and a specificity of 95% for distinguishing benign and malignant nodes in patients with head and neck cancer. This ratio has greater specificity and sensitivity than palpation or measurement of either the long or the short axis alone.

The indications for lymph node biopsy are imprecise, yet it is a valuable diagnostic tool. The decision to biopsy may be made early in a patient’s evaluation or delayed for up to 2 weeks. Prompt biopsy should occur if the patient’s history and physical findings suggest a malignancy; examples include a solitary, hard, nontender cervical node in an older patient who is a chronic user of tobacco; supraclavicular adenopathy; and solitary or generalized adenopathy that is firm, movable, and suggestive of lymphoma. If a primary head and neck cancer is suspected as the basis of a solitary, hard cervical node, then a careful ENT examination should be performed. Any mucosal lesion that is suspicious for a primary neo-
PLASTIC PROCESS SHOULD BE BIOPSIED FIRST. IF NO MUCOSAL LESION IS
DETECTED, AN EXCISIONAL BIOPSY OF THE LARGEST NODE SHOULD BE PER-
FORMED. FINE-NEEDLE ASPIRATION SHOULD NOT BE PERFORMED AS THE
FIRST DIAGNOSTIC PROCEDURE. MOST DIAGNOSES REQUIRE MORE TISSUE THAN
SUCH ASPIRATION CAN PROVIDE, AND IT OFTEN DELAYS A DEFINITIVE DIAGNO-
SIS. FINE-NEEDLE ASPIRATION SHOULD BE RESERVED FOR THYROID NODULES
AND FOR CONFIRMATION OF RELAPSE IN PATIENTS WHOSE PRIMARY DIAGNO-
SIS IS KNOWN. IF THE PRIMARY PHYSICIAN IS UNCERTAIN ABOUT WHETHER
TO PROCEED TO BIOPSY, CONSULTATION WITH A HEMATOLOGIST OR MEDICAL
ONCOLOGIST SHOULD BE HELPFUL. IN PRIMARY CARE PRACTICES, <5% OF
LYMPHADENOPATHY PATIENTS WILL REQUIRE A BIOPSY. THAT PERCENTAGE
WILL BE CONSIDERABLY LARGER IN REFERRAL PRACTICES, I.E., HEMATOLOGY,
ONCOLOGY, OR ENT.
TWO GROUPS HAVE REPORTED ALGORITHMS THAT THEY CLAIM WILL IDENTIFY
MORE PRECISELY THOSE LYMPHADENOPATHY PATIENTS WHO SHOULD HAVE A
BIOPSY. BOTH REPORTS WERE RETROSPECTIVE ANALYSES IN REFERRAL
PRACTICES.
THE FIRST STUDY INVOLVED PATIENTS 9-25 YEARS OF AGE WHO HAD A NODE
BIOPSY PERFORMED. THREE VARIABLES WERE IDENTIFIED THAT PREDICTED
THOSE YOUNG PATIENTS WITH PERIPHERAL LYMPHADENOPATHY WHO SHOULD
UNDERGO BIOPSY; LYMPH NODE SIZE >2 CM IN DIAMETER AND ABNORMAL
CHEST X-RAY HAD POSITIVE PREDICTIVE VALUES, WHEREAS RECENT ENT SYM-P

372 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF
DISEASES

TOMS HAD NEGATIVE PREDICTIVE VALUES. THE SECOND STUDY EVALUATED 220
LYMPHADENOPATHY PATIENTS IN A HEMATOLOGY UNIT AND IDENTIFIED FIVE
VARIABLES [LYMPH NODE SIZE, LOCATION (SUPRACLAVICULAR OR NONSUPRA-
CLAVICULAR), AGE (>40 YEARS OR <40 YEARS), TEXTURE (NONHARD OR HARD),
AND TENDERNESS] THAT WERE UTILIZED IN A MATHEMATICAL MODEL TO IDEN-
TIFY THOSE PATIENTS REQUIRING A BIOPSY. POSITIVE PREDICTIVE VALUE WAS
 FOUND FOR AGE >40 YEARS, SUPRACLAVICULAR LOCATION, NODE SIZE >2.25
 CM###2, HARD TEXTURE, AND LACK OF PAIN OR TENDERNESS. NEGATIVE
PREDICTIVE
VALUE WAS EVIDENT FOR AGE <40 YEARS, NODE SIZE <1.0 CM###2, NONHARD
TEXTURE, AND TENDER OR PAINFUL NODES. NINETY-ONE PERCENT OF THOSE
WHO REQUIRED BIOPSY WERE CORRECTLY CLASSIFIED BY THIS MODEL. SINCE
BOTH OF THESE STUDIES WERE RETROSPECTIVE ANALYSES AND ONE WAS LIMITED
TO YOUNG PATIENTS, IT IS NOT KNOWN HOW USEFUL THESE MODELS WOULD BE
IF APPLIED PROSPECTIVELY IN A PRIMARY CARE SETTING.
MOST LYMPHADENOPATHY PATIENTS DO NOT REQUIRE A BIOPSY, AND AT
LEAST HALF REQUIRE NO LABORATORY STUDIES. IF THE PATIENT’S HISTORY AND
PHYSICAL FINDINGS POINT TO A BENIGN CAUSE FOR LYMPHADENOPATHY,
THEN CAREFUL FOLLOW-UP AT A 2- TO 4-WEEK INTERVAL CAN BE EMPLOYED.
THE PATIENT SHOULD BE INSTRUCTED TO RETURN FOR REEVALUATION IF THE
NODE(S) INCREASE IN SIZE. ANTIBIOTICS ARE NOT INDICATED FOR LYMPHAD-
ENOPATHY UNLESS STRONG EVIDENCE OF A BACTERIAL INFECTION IS PRESENT.
GLUCOCORTICOIDS SHOULD NOT BE USED TO TREAT LYMPHADENOPATHY BE-
CAUSE THEIR LYMPOLYTIC EFFECT OBSCURES SOME DIAGNOSES (LYMHO-
MA, LEUKEMIA, CASTLEMAN’S DISEASE) AND THEY CONTRIBUTE TO DELAYED
HEALING OR ACTIVATION OF UNDERLYING INFECTIONS. AN EXCEPTION TO THIS STATEMENT IS THE LIFE-THREATENING PHARYNGEAL OBSTRUCTION BY ENLARGED LYMPHOID TISSUE IN WALDEYER’S RING THAT IS OCCASIONALLY SEEN IN INFECTIOUS MONONUCLEOSIS.

SPLENOMEGALY
STRUCTURE AND FUNCTION OF THE SPLEEN

THE SPLEEN IS A RETICULOENDOTHELIAL ORGAN THAT HAS ITS EMBRYOLOGIC ORIGIN IN THE DORSAL MESOGASTRİUM AT ABOUT 5 WEEKS’ GESTATION. IT ARISES IN A SERIES OF HILLOCKS, MIGRATES TO ITS NORMAL ADULT LOCATION IN THE LEFT UP- PER QUADRANT (LUQ), AND IS ATTACHED TO THE STOMACH VIA THE GASTROILI- NAL LIGAMENT AND TO THE KIDNEY VIA THE LIENORENAL LIGAMENT. WHEN THE HILLOCKS FAIL TO UNIFY INTO A SINGLE TISSUE MASS, ACCESSORY SPLEENS MAY DEVELOP IN AROUND 20% OF PERSONS. THE FUNCTION OF THE SPLEEN HAS BEEN ELUSIVE. GALEN BELIEVED IT WAS THE SOURCE OF “BLACK BILE” OR MELAN- CHOLIA, AND THE WORD HYPOCHONDRIA (LITERALLY, BENEATH THE RIBS) AND THE IDIOM “TO VENT ONE’S SPLEEN” ATTEST TO THE BELIEFS THAT THE SPLEEN HAD AN IMPORTANT INFLUENCE ON THE PSYCHE AND EMOTIONS. IN HUMANS ITS NORMAL PHYSIOLOGIC ROLES SEEM TO BE THE FOLLOWING:
1. MAINTENANCE OF QUALITY CONTROL OVER ERYTHROCYTES IN THE RED PULP BY REMOVAL OF SENESCENT AND DEFECTIVE RED BLOOD CELLS. THE SPLEEN AC- COMPLISHES THIS FUNCTION THROUGH A UNIQUE ORGANIZATION OF ITS PA- RENCHYMA AND VASCULATURE (FIG. 60-1).
2. SYNTHESIS OF ANTIBODIES IN THE WHITE PULP.
3. THE REMOVAL OF ANTIBODY-COATED BACTERIA AND ANTIBODY-COATED BLOOD CELLS FROM THE CIRCULATION.

AN INCREASE IN THESE NORMAL FUNCTIONS MAY RESULT IN SPLENOMEGALY. THE SPLEEN IS COMPOSED OF RED PULP AND WHITE PULPY WHICH ARE MAL- PIGHI’S TERMS FOR THE RED BLOOD-FILLED SINUSES AND RETICULOENDOTHELIAL CELL-LINED CORDS AND THE WHITE LYMPHOID FOLLICLES ARRAYED WITHIN THE RED PULP MATRIX. THE SPLEEN IS IN THE PORTAL CIRCULATION. THE REASON FOR THIS IS UNKNOWN BUT MAY RELATE TO THE FACT THAT LOWER BLOOD PRESSURE AL- LOWS LESS RAPID FLOW AND MINIMIZES DAMAGE TO NORMAL ERYTHROCYTES. BLOOD FLOWS INTO THE SPLEEN AT A RATE OF ABOUT 150 ML/MIN THROUGH THE SPLENIC ARTERY, WHICH ULTIMATELY RAMIFIES INTO CENTRAL ARTERIOLES. SOME BLOOD GOES FROM THE ARTERIOLES TO CAPILLARIES AND THEN TO SPLENIC VEINS AND OUT OF THE SPLEEN, BUT THE MAJORITY OF BLOOD FROM CENTRAL ARTERIOLES FLOWS INTO THE MACROPHAGE-LINED SINUSES AND CORDS. THE BLOOD ENTER- ING THE SINUSES REENTERS THE CIRCULATION THROUGH THE SPLENIC VENULES, BUT THE BLOOD ENTERING THE CORDS IS SUBJECTED TO AN INSPECTION OF SORTS.

FIGURE 60-1 SCHEMATIC SPLEEN STRUCTURE. THE SPLEEN COMPRISSES MANY UNITS OF RED AND WHITE PULP CENTERED AROUND SMALL BRANCHES OF THE SPLENIC ARTERY, CALLED CENTRAL ARTERIES. WHITE PULP IS LYMPHOID IN
NATURAL AND CONTAINS B CELL FOLLICLES, A MARGINAL ZONE AROUND THE FOLLICLES, AND T CELL-RICH AREAS SHEATHING ARTERIOLES. THE RED PULP AREAS INCLUDE PULP SINUSES AND PULP CORDS. THE CORDS ARE DEAD ENDS. IN ORDER TO REGAIN ACCESS TO THE CIRCULATION, RED BLOOD CELLS MUST TRAVERSE TINY OPENINGS IN THE SINUSOIDAL LINING. STIFF, DAMAGED, OR OLD RED CELLS CANNOT ENTER THE SINUSES. (TOP PORTION OF FIGURE FROM CA JANEWAY ET AL. IMMUNOBIOLOGY, 5TH ED., NEW YORK, GARLAND, 2001; BOTTOM PORTION OF FIGURE FROM RS HILLMAN, KA AULT: HEMATOLOGY IN CLINICAL PRACTICE, 4TH ED. NEW YORK, McGRRAW-HILL, 2005.)

IN ORDER TO RETURN TO THE CIRCULATION, THE BLOOD CELLS IN THE CORDS MUST SQUEEZE THROUGH SLITS IN THE CORD LINING TO ENTER THE SINUSES THAT LEAD TO THE VENULES. OLD AND DAMAGED ERYTHROCYTES ARE LESS DEFORMABLE AND ARE RETAINED IN THE CORDS, WHERE THEY ARE DESTROYED AND THEIR COMPONENTS RECYCLED. RED CELL INCLUSION BODIES SUCH AS PARASITES (CHAP. 203 AND EL8), NUCLEAR RESIDUA (HOWELL-JOLLY BODIES, FIG. 58-6), OR DENATURED HEMOGLOBIN (HEINZ BODIES) ARE PINCHED OFF IN THE PROCESS OF PASSING THROUGH THE SLITS, A PROCESS CALLED PITTING. THE CULLING OF DEAD AND DAMAGED CELLS AND THE PITTING OF CELLS WITH INCLUSIONS APPEAR TO OCCUR WITHOUT SIGNIFICANT DELAY SINCE THE BLOOD TRANSIT TIME THROUGH THE SPLEEN IS ONLY SLIGHTLY SLOWER THAN IN OTHER ORGANS. THE SPLEEN IS ALSO CAPABLE OF ASSISTING THE HOST IN ADAPTING TO ITS HOSTILE ENVIRONMENT. IT HAS AT LEAST THREE ADAPTIVE FUNCTIONS: (1) CLEARANCE OF BACTERIA AND PARTICULATES FROM THE BLOOD, (2) THE GENERATION OF IMMUNE RESPONSES TO CERTAIN PATHOGENS, AND (3) THE GENERATION OF CELLULAR COMPONENTS OF THE BLOOD UNDER CIRCUMSTANCES IN WHICH THE MARROW IS UNABLE TO MEET THE NEEDS (I.E., EXTRAMEDULLARY HEMATOPOIESIS). THE LATTER ADAPTATION IS A RECAPITULATION OF THE BLOOD-FORMING FUNCTION THE SPLEEN PLAYS DURING GESTATION. IN SOME ANIMALS, THE SPLEEN ALSO SERVES A ROLE IN THE VASCULAR ADAPTATION TO STRESS BECAUSE IT STORES RED BLOOD CELLS (OFTEN HEMOCENTRATED TO HIGHER HEMATOCRITS THAN NORMAL) UNDER NORMAL CIRCUMSTANCES AND CONTRACTS UNDER THE INFLUENCE OF *-ADRENERGIC STIMULATION TO PROVIDE THE ANIMAL WITH AN AUTOTRANSFUSION AND IMPROVED OXYGEN-CARRYING CAPACITY. HOWEVER, THE NORMAL HUMAN SPLEEN DOES NOT

373 CHAPTER 60 ENLARGEMENT OF LYMPH NODES AND SPLEEN
SEQUESTER OR STORE RED BLOOD CELLS AND DOES NOT CONTRACT IN RESPONSE TO SYMPATHETIC STIMULI. THE NORMAL HUMAN SPLEEN CONTAINS APPROXIMATELY ONE-THIRD OF THE TOTAL BODY PLATELETS AND A SIGNIFICANT NUMBER OF MARGINATED NEUTROPHILS. THESE SEQUESTERED CELLS ARE AVAILABLE WHEN NEEDED TO RESPOND TO BLEEDING OR INFECTION.

**APPROACH TO THE PATIENT:**

**Splenomegaly**

**CLINICAL ASSESSMENT**  THE MOST COMMON SYMPTOMS PRODUCED BY DISEASES INVOLVING THE SPLEEN ARE PAIN AND A HEAVY SENSATION IN THE LUQ. MASSIVE SPLENOMEGALY MAY CAUSE EARLY SATIETY. PAIN MAY RESULT FROM ACUTE SWELLING OF THE SPLEEN WITH STRETCHING OF THE CAPSULE, INFARCTION, OR INFLAMMATION OF THE CAPSULE. FOR MANY YEARS IT WAS BELIEVED THAT SPLENIC INFARCTION WAS CLINICALLY SILENT, WHICH AT TIMES IS TRUE. HOWEVER, SOMA WEISS, IN HIS CLASSIC 1942 REPORT OF THE SELF-OBSERVATIONS BY A HARVARD MEDICAL STUDENT ON THE CLINICAL COURSE OF SUBACUTE BACTERIAL ENDOCARDITIS, DOCUMENTED THAT SEVERE LUQ AND PLEURITIC CHEST PAIN MAY ACCOMPANY THROMBOEMBOLIC OCCLUSION OF SPLENIC BLOOD FLOW. VASCULAR OCCLUSION, WITH INFARCTION AND PAIN, IS COMMONLY SEEN IN CHILDREN WITH SICKLE CELL CRISIS. RUPTURE OF THE SPLEEN, FROM EITHER TRAUMA OR INFILTRATIVE DISEASE THAT BREAKS THE CAPSULE, MAY RESULT IN INTRAPERITONEAL BLEEDING, SHOCK, AND DEATH. THE RUPTURE ITSELF MAY BE PAINLESS.

A PALPABLE SPLEEN IS THE MAJOR PHYSICAL SIGN PRODUCED BY DISEASES AFFECTING THE SPLEEN AND SUGGESTS ENLARGEMENT OF THE ORGAN. THE NORMAL SPLEEN IS SAID TO WEIGH <250 G, DECREASES IN SIZE WITH AGE, NORMALLY LIES ENTIRELY WITHIN THE RIB CAGE, HAS A MAXIMUM CEPHALOCAUDAD DIAMETER OF 13 CM BY ULTRASONOGRAPHY OR MAXIMUM LENGTH OF 12 CM AND/OR WIDTH OF 7 CM BY RADIONUCLIDE SCAN, AND IS USUALLY NOT PALPABLE. HOWEVER, A PALPABLE SPLEEN WAS FOUND IN 3% OF 2200 ASYMPTOMATIC, MALE, FRESHMAN COLLEGE STUDENTS. FOLLOW-UP AT 3 YEARS REVEALED THAT 30% OF THOSE STUDENTS STILL HAD A PALPABLE SPLEEN WITHOUT ANY INCREASE IN DISEASE PREVALENCE. TEN-YEAR FOLLOW-UP FOUND NO EVIDENCE FOR LYMPHOID MALIGNANCIES. FURTHERMORE, IN SOME TROPICAL COUNTRIES (E.G., NEW GUINEA) THE INCIDENCE OF SPLENOMEGALY MAY REACH 60%. THUS, THE PRESENCE OF A PALPABLE SPLEEN DOES NOT ALWAYS EQUATE WITH PRESENCE OF DISEASE. EVEN WHEN DISEASE IS PRESENT, SPLENOMEGALY MAY NOT REFLECT THE PRIMARY DISEASE BUT RATHER A REACTION TO IT. FOR EXAMPLE, IN PATIENTS WITH HODGKIN’S DISEASE, ONLY TWO-THIRDS OF THE PALPABLE SpleENS SHOW INVOLVEMENT BY THE CANCER.

PHYSICAL EXAMINATION OF THE SPLEEN UTILIZES PRIMARILY THE TECHNIQUES OF PALPATION AND PERCUSSION. INSPECTION MAY REVEAL FULLNESS IN THE LUQ THAT DESCENDS ON INSPIRATION, A FINDING ASSOCIATED WITH A MASSIVELY ENLARGED SPLEEN. AUSCULTATION MAY REVEAL A VENOUS HUM OR FRICTION RUB. PALPATION CAN BE ACCOMPLISHED BY BIMANUAL PALPATION, BALLOTMENT, AND PALPATION FROM ABOVE (MIDDLETON MANEUVER). FOR BI-MANUAL PALPATION, WHICH IS AT LEAST AS RELIABLE AS THE OTHER TECHNIQUES, THE PATIENT IS SUPINE WITH FLEXED KNEES. THE EXAMINER’S

**PERCUSSION** FOR SPLENIC DULLNESS IS ACCOMPLISHED WITH ANY OF THREE TECHNIQUES DESCRIBED BY NIXON, CASTELL, OR BARKUN:

1. **NIXON'S METHOD:** THE PATIENT IS PLACED ON THE RIGHT SIDE SO THAT THE SPLEEN LIES ABOVE THE COLON AND STOMACH. PERCUSSION BEGINS AT THE LOWER LEVEL OF PULMONARY RESONANCE IN THE POSTERIOR AXILLARY LINE AND PROCEEDS DIAGONALLY ALONG A PERPENDICULAR LINE TOWARD THE LOWER MIDANTERIOR COSTAL MARGIN. THE UPPER BORDER OF DULLNESS IS NORMALLY 6-8 CM ABOVE THE COSTAL MARGIN. DULLNESS >8 CM IN AN ADULT IS PRESUMED TO INDICATE SPLENIC ENLARGEMENT.

2. **CASTELL'S METHOD:** WITH THE PATIENT SUPINE, PERCUSSION IN THE LOWEST INTERCOSTAL SPACE IN THE ANTERIOR AXILLARY LINE (8TH OR 9TH) PRODUCES A RESONANT NOTE IF THE SPLEEN IS NORMAL IN SIZE. THIS IS TRUE DURING EXPIRATION OR FULL INSPIRATION. A DULL PERCUSSION NOTE ON FULL INSPIRATION SUGGESTS SPLENOMEGALY.

3. **PERCUSSION OF TRAUBE'S SEMILUNAR SPACE:** THE BORDERS OF TRAUBE'S SPACE ARE THE SIXTH RIB SUPERIORLY, THE LEFT MIDAXILLARY LINE LATERALLY, AND THE LEFT COSTAL MARGIN INFERIORLY. THE PATIENT IS SUPINE WITH THE LEFT ARM SLIGHTLY ABDUCTED. DURING NORMAL BREATHING, THIS SPACE IS PERCUSSED FROM MEDIAL TO LATERAL MARGINS, YIELDING A NORMAL RESONANT SOUND. A DULL PERCUSSION NOTE SUGGESTS SPLENOMEGALY.

STUDIES COMPARING METHODS OF PERCUSSION AND PALPATION WITH A STANDARD OF ULTRASONOGRAPHY OR SCINTIGRAPHY HAVE REVEALED SENSITIVITY OF 56-71% FOR PALPATION AND 59-82% FOR PERCUSSION. REPRODUCIBILITY AMONG EXAMINERS IS BETTER FOR PALPATION THAN PERCUSSION. BOTH TECHNIQUES ARE LESS RELIABLE IN OBESE PATIENTS OR PATIENTS WHO HAVE JUST EATEN. THUS, THE PHYSICAL EXAMINATION TECHNIQUES OF PALPATION AND PERCUSSION ARE IMPRECISE AT BEST. IT HAS BEEN SUGGESTED THAT THE EXAMINER PERFORM PERCUSSION FIRST AND, IF POSITIVE, PROCEED TO PALPATION; IF THE SPLEEN IS PALPABLE, THEN ONE CAN BE REASONABLY CONFIDENT THAT SPLENOMEGALY EXISTS. HOWEVER, NOT ALL LUQ MASSES ARE ENLARGED SPLEENS; GASTRIC OR COLON TUMORS AND PANCREATIC OR RENAL CYSTS OR TUMORS CAN MIMIC SPLENOMEGALY.

THE PRESENCE OF AN ENLARGED SPLEEN CAN BE MORE PRECISELY DETERMINED, IF NECESSARY, BY LIVER-SPLEEN RADIONUCLIDE SCAN, CT, MR, OR ULTRASONOGRAPHY. THE LATTER TECHNIQUE IS THE CURRENT PROCEDURE OF CHOICE FOR ROUTINE ASSESSMENT OF SPLEEN SIZE (NORMAL = A MAXIMUM CEPHALOCAUDAL DIAMETER OF 13 CM) BECAUSE IT HAS HIGH SENSITIVITY AND SPECIFICITY AND IS SAFE, NONINVASIVE, QUICK, MOBILE, AND LESS COSTLY. NUCLEAR MEDICINE SCANS ARE ACCURATE, SENSITIVE, AND RELIABLE BUT ARE COSTLY, REQUIRE GREATER TIME TO GENERATE DATA, AND UTILIZE IM-
MOBILE EQUIPMENT. THEY HAVE THE ADVANTAGE OF DEMONSTRATING ACCESSORY SPLENIC TISSUE. CT AND MRI PROVIDE ACCURATE DETERMINATION OF SPLEEN SIZE, BUT THE EQUIPMENT IS IMMOBILE AND THE PROCEDURES ARE EXPENSIVE. MRI APPEARS TO OFFER NO ADVANTAGE OVER CT. CHANGES IN SPLEEN STRUCTURE SUCH AS MASS LESIONS, INFARCTS, INHOMOGENEOUS INFILTRATES, AND CYSTS ARE MORE READILY ASSESSED BY CT, MRI, OR ULTRASONOGRAPHY. NONE OF THESE TECHNIQUES IS VERY RELIABLE IN THE DETECTION OF PATCHY INFILTRATION (E.G., HODGKIN’S DISEASE).

DIFFERENTIAL DIAGNOSIS MANY OF THE DISEASES ASSOCIATED WITH SPLENOMEGALY ARE LISTED IN TABLE 60-2. THEY ARE GROUPED ACCORDING TO THE PRESUMED BASIC MECHANISMS RESPONSIBLE FOR ORGAN ENLARGEMENT:

1. HYPERPLASIA OR HYPERTROPHY RELATED TO A PARTICULAR SPLENIC FUNCTION SUCH AS RETICULOENDOTHELIAL HYPERPLASIA (WORK HYPERTROPHY) IN DISEASES SUCH AS HEREDITARY SPHEROCYTOSIS OR THALASSEMIA SYNDROMES THAT REQUIRE REMOVAL OF LARGE NUMBERS OF DEFECTIVE RED BLOOD CELLS; IMMUNE HYPERPLASIA IN RESPONSE TO SYSTEMIC INFECTION (INFECTIOUS MONONUCLEOSIS, SUBACUTE BACTERIAL ENDOCARDITIS) OR TO IMMUNOLOGIC DISEASES (IMMUNE THROMBOCYTOPENIA, SLE, FELTY’S SYNDROME).
2. PASSIVE CONGESTION DUE TO DECREASED BLOOD FLOW FROM THE SPLEEN IN CONDITIONS THAT PRODUCE PORTAL HYPERTENSION (CIRRHOSIS, BUDD-CHIARI SYNDROME, CONGESTIVE HEART FAILURE).
3. INFILTRATIVE DISEASES OF THE SPLEEN (LYMPHOMAS, METASTATIC CANCER, AMYLOIDOSIS, GAUCHER’S DISEASE, MYELOPROLIFERATIVE DISORDERS WITH EXTRAMEDULLARY HEMATOPOIESIS).

374 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

TABLE 60-2 DISEASES ASSOCIATED WITH SPLENOMEGALY GROUPED BY PATHOGENIC MECHANISM

ENLARGEMENT DUE TO INCREASED DEMAND FOR SPLENIC FUNCTION

RETICULOENDOTHELIAL SYSTEM HYPERPLASIA (FOR REMOVAL OF DEFECTIVE ERYTHROCYTES)
SPHEROCYTOSIS
EARLY SICKLE CELL ANEMIA
OVALOCYTOSIS
THALASSEMIA MAJOR
HEMOGLOBINOPATHIES
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
PERNICIOUS ANEMIA
IMMUNE HYPERPLASIA
RESPONSE TO INFECTION (VIRAL BACTERIAL FUNGAL PARASITIC)
INFECTIOUS MONONUCLEOSIS
AIDS
VIRAL HEPATITIS
CYTOMEGALOVIRUS
SUBACUTE BACTERIAL ENDOCARDITIS
BACTERIAL SEPTICEMIA
CONGENITAL SYPHILIS
SPLENIC ABSCESS
TUBERCULOSIS
HISTOPLASMOSIS
MALARIA
LEISHMANIASIS
TRYPANOSOMIASIS
EHRLICHIOSIS
DISORDERED IMMUNOREGULATION
RHEUMATOID ARTHRITIS (FELTY’S SYNDROME)
SYSTEMIC LUPUS ERYTHEMATOSUS
COLLAGEN VASCULAR DISEASES
SERUM SICKNESS
IMMUNE HEMOLYTIC ANEMIAS
IMMUNE THROMBOCYTOPENIAS
IMMUNE NEUTROPENIAS
DRUG REACTIONS
ANGIOIMMUNOBLASTIC LYMPHADENOPATHY
SARCIOIDOSIS
THYROTOXICOSIS (BENIGN LYMPHOID HYPERTROPHY)
INTERLEUKIN 2 THERAPY
EXTRAMEDULLARY HEMATOPOIESIS
MYELOFIBROSIS
MARROW DAMAGE BY TOXINS, RADIATION, STRONTIUM
MARROW INFILTRATION BY TUMORS, LEUKEMIAS, GAUCHER’S DISEASE

ENLARGEMENT DUE TO ABNORMAL
SPLENIC OR PORTAL BLOOD FLOW

CIRRHOSIS
HEPATIC VEIN OBSTRUCTION
PORTAL VEIN OBSTRUCTION, INTRAHEPATIC OR EXTRAHEPATIC
CAVERNOUS TRANSFORMATION OF THE PORTAL VEIN
SPLENIC VEIN OBSTRUCTION
SPLENIC ARTERY ANEURYSM
HEPATIC SCHISTOSOMIASIS
CONGESTIVE HEART FAILURE
HEPATIC ECHINOCOCCOSIS
PORTAL HYPERTENSION (ANY CAUSE INCLUDING THE ABOVE): “BANTI’S DISEASE”

INFECTION OF THE SPLEEN

IN TRACELLULAR OR EXTRACELLULAR DEPOSITIONS
AMYLOIDOSIS
GAUCHER’S DISEASE
NIEMANN-PICK DISEASE
TANGIER DISEASE
HURLER’S SYNDROME AND OTHER MUCOPOLYSACCHARIDOSES
HYPERLIPIDEMIAS

BENIGN AND MALIGNANT CELLULAR INFILTRATIONS
LEUKEMIAS (ACUTE, CHRONIC, LYMPHOID, MYELOID, MONOCYTIC)
LYMPHOMAS
HODGKIN’S DISEASE
MYELOPROLIFERATIVE SYNDROMES (EG, POLYCYTHEMIA VERA, ESSENTIAL THROMBOCYTOSIS)
ANGIOSARCOMAS
METASTATIC TUMORS (MELANOMA IS MOST COMMON)
EOSINOPHILIC GRANULOMA
HISTIOCYTOSIS X
HAMARTOMAS
HEMANGIOMAS, FIBROMAS, LYMPHANGIOMAS
SPLENIC CYSTS

UNKNOWN ETIOLOGY

IDIOPATHIC SPLENOMEGALY
BERYLLIOSIS
IRON-DEFICIENCY ANEMIA

THE DIFFERENTIAL DIAGNOSTIC POSSIBILITIES ARE MUCH FEWER WHEN THE SPLEEN IS “MASSIVELY ENLARGED,” PALPABLE MORE THAN 8 CM BELOW THE LEFT COSTAL MARGIN OR ITS DRAINED WEIGHT IS >1000 G (TABLE 60-3). THE VAST MAJORITY OF SUCH PATIENTS WILL HAVE NON-HODGKIN’S LYMPHOMA, CHRONIC LYMPHOCYTIC LEUKEMIA, HAIRY CELL LEUKEMIA, CHRONIC MYELOGENOUS LEUKEMIA, MYELOFIBROSIS WITH MYELOID METAPLASIA, OR POLYCYTHEMIA VERA.

LABORATORY ASSESSMENT  THE MAJOR LABORATORY ABNORMALITIES ACCOMPANYING SPLENOMEGALY ARE DETERMINED BY THE UNDERLYING SYSTEMIC ILLNESS. ERYTHROCYTE COUNTS MAY BE NORMAL, DECREASED (THALASSEMIA MAJOR SYNDROMES, SLE, CIRRHOSIS WITH PORTAL HYPERTENSION), OR INCREASED (POLYCYTHEMIA VERA). GRANULOCYTE COUNTS MAY BE NORMAL, DECREASED (FELTY’S SYNDROME, CONGESTIVE SPLENOMEGALY, LEUKEMIAS), OR INCREASED (INFECTIONS OR INFLAMMATORY DIS

TABLE 60-3 DISEASES ASSOCIATED WITH MASSIVE SPLENOMEGALY###A

CHRONIC MYELOGENOUS LEUKEMIA
LYMPHOMAS
HAIRY CELL LEUKEMIA
MYELOFIBROSIS WITH MYELOID METAPLASIA
POLYCYTHEMIA VERA

GAUCHER’S DISEASE
CHRONIC LYMPHOCYTIC LEUKEMIA
SARCOIDOSIS
AUTOIMMUNE HEMOLYTIC ANEMIA
DIFFUSE SPLENIC HEMANGIOMATOSIS

###ATHE SPLEEN EXTENDS GREATER THAN 8 CM BELOW LEFT COSTAL MARGIN AND/OR WEIGHS MORE THAN 1000G.
EASE, MYELOPROLIFERATIVE DISORDERS). SIMILARLY, THE PLATELET COUNT MAY BE NORMAL, DECREASED WHEN THERE IS ENHANCED SEQUESTREATION OR DESTRUCTION OF PLATELETS IN AN ENLARGED SPLEEN (CONGESTIVE SPLENOMEGALY, GAUCHER’S DISEASE, IMMUNE THROMBOCYTOPENIA), OR INCREASED IN THE MYELOPROLIFERATIVE DISORDERS SUCH AS POLYCYTHEMIA VERA.

THE CBC MAY REVEAL CYTOPENIA OF ONE OR MORE BLOOD CELL TYPES, WHICH SHOULD SUGGEST HYPERSPLENISM. THIS CONDITION IS CHARACTERIZED BY SPLENOMEGALY, CYTOPENIA(S), NORMAL OR HYPERPLASTIC BONE MARROW, AND A RESPONSE TO SPLENECTOMY. THE LATTER CHARACTERISTIC IS LESS PRECISE BECAUSE REVERSAL OF CYTOPENIA, PARTICULARLY GRANULOCYTOPENIA, IS SOMETIMES NOT SUSTAINED AFTER SPLENECTOMY. THE CYTOPENIAS RESULT FROM INCREASED DESTRUCTION OF THE CELLULAR ELEMENTS SECONDARY TO REDUCED FLOW OF BLOOD THROUGH ENLARGED AND CONGESTED CORDS (CONGESTIVE SPLENOMEGALY) OR TO IMMUNE-MEDIATED MECHANISMS. IN HYPERSPLENISM, VARIOUS CELL TYPES USUALLY HAVE NORMAL MORPHOLOGY ON THE PERIPHERAL BLOOD SMEAR, ALTHOUGH THE RED CELLS MAY BE S HEROCYTIC DUE TO LOSS OF SURFACE AREA DURING THEIR LONGER TRANSIT THROUGH THE ENLARGED SPLEEN. THE INCREASED MARROW PRODUCTION OF RED CELLS SHOULD BE REFLECTED AS AN INCREASED RETICULOCYTE PRODUCTION INDEX, ALTHOUGH THE VALUE MAY BE LESS THAN EXPECTED DUE TO INCREASED SEQUESTRA TION OF RETICULOCYTES IN THE SPLEEN. THE NEED FOR ADDITIONAL LABORATORY STUDIES IS DICTATED BY THE DIFFERENTIAL DIAGNOSIS OF THE UNDERLYING ILLNESS OF WHICH SPLENOMEGALY IS A MANIFESTATION.

SPLENECTOMY

SPLENECTOMY IS INFREQUENTLY PERFORMED FOR DIAGNOSTIC PURPOSES, ESPECIALLY IN THE ABSENCE OF CLINICAL ILLNESS OR OTHER DIAGNOSTIC TESTS THAT SUGGEST UNDERLYING DISEASE. MORE OFTEN SPLENECTOMY IS PERFORMED FOR SYMPTOM CONTROL IN PATIENTS WITH MASSIVE SPLENOMEGALY, FOR DISEASE CONTROL IN PATIENTS WITH TRAUMATIC SPLENIC RUPTURE, OR FOR CORRECTION OF CYTOPENIAS IN PATIENTS WITH HYPERSPLENISM OR IMMUNE-MEDIATED DE-
STRUCTION OF ONE OR MORE CELLULAR BLOOD ELEMENTS. SPLENECTOMY IS NEEDED FOR STAGING OF PATIENTS WITH HODGKIN’S DISEASE ONLY IN THOSE WITH CLINICAL STAGE I OR II DISEASE IN WHOM RADIATION THERAPY ALONE IS CONTEMPLATED AS THE TREATMENT. NONINVASIVE STAGING OF THE SPLEEN IN HODGKIN’S DISEASE IS NOT A SUFFICIENTLY RELIABLE BASIS FOR TREATMENT DECISIONS BECAUSE ONE-THIRD OF NORMAL-SIZED SPEENS WILL BE INVOLVED WITH HODGKIN’S DISEASE AND ONE-THIRD OF ENLARGED SPEENS WILL BE TUMOR-FREE. ALTHOUGH SPLENECTOMY IN CHRONIC MYELOGENOUS LEUKEMIA DOES NOT AFFECT THE NATURAL HISTORY OF DISEASE, REMOVAL OF THE MASSIVE SPLEEN USUALLY MAKES PATIENTS SIGNIFICANTLY MORE COMFORTABLE AND SIMPLIFIES THEIR MANAGEMENT BY SIGNIFICANTLY REDUCING TRANSFUSION REQUIREMENTS. SPLENECTOMY IS AN EFFECTIVE SECONDARY OR TERTIARY TREATMENT FOR TWO CHRONIC B CELL LEUKEMIAS, HAIRY CELL LEUKEMIA AND PROLYMPHOCYTIC LEUKEMIA, AND FOR THE VERY RARE SPLENIC MANTLE CELL OR MARGINAL ZONE LYMPHOMA. SPLENECTOMY IN THESE DISEASES MAY BE ASSOCIATED WITH SIGNIFICANT TUMOR REGRESSION IN BONE MARROW AND OTHER SITES OF DISEASE.

PAGE NO. 21

375 CHAPTER 61 DISORDERS OF GRANULOCYTES AND MONOCYTES

SIMILAR REGRESSIONS OF SYSTEMIC DISEASE HAVE BEEN NOTED AFTER SPLENIC IR- RADIATION IN SOME TYPES OF LYMPHOID TUMORS, ESPECIALLY CHRONIC LYMPHOCYTIC LEUKEMIA AND PROLYMPHOCYTIC LEUKEMIA. THIS HAS BEEN TERMED THE ABSCOPAL EFFECT. SUCH SYSTEMIC TUMOR RESPONSES TO LOCAL THERAPY DIRECTED AT THE SPLEEN SUGGEST THAT SOME HORMONE OR GROWTH FACTOR PRODUCED BY THE SPLEEN MAY AFFECT TUMOR CELL PROLIFERATION, BUT THIS CONJECTURE IS NOT YET SUBSTANTIATED. A COMMON THERAPEUTIC INDICATION FOR SPLENECTOMY IS TRAUMATIC OR IATROGENIC SPLENIC RUPTURE. IN A FRACTION OF PATIENTS WITH SPLENIC RUPTURE, PERITONEAL SEEDING OF SPLENIC FRAGMENTS CAN LEAD TO SPLENOSIS-THE PRESENCE OF MULTIPLE RESTS OF SPLEEN TISSUE NOT CONNECTED TO THE PORTAL CIRCULATION. THIS ECTOPIC SPLEEN TISSUE MAY CAUSE PAIN OR GASTROINTESTINAL OBSTRUCTION, AS IN ENDOMETRIOSIS. A LARGE NUMBER OF HEMATOLOGIC, IMMUNOLOGIC, AND CONGESTIVE CAUSES OF SPLENOMEGALY CAN LEAD TO DESTRUCTION OF ONE OR MORE CELLULAR BLOOD ELEMENTS. IN THE MAJORITY OF SUCH CASES, SPLENECTOMY CAN CORRECT THE CYTOPENIAS, PARTICULARLY ANEMIA AND THROMBOCYTOPENIA. IN A LARGE SERIES OF PATIENTS SEEN IN TWO TERTIARY CARE CENTERS, THE INDICATION FOR SPLENECTOMY WAS DIAGNOSTIC IN 10% OF PATIENTS, THERAPEUTIC IN 44%, STAGING FOR HODGKIN’S DISEASE IN 20%, AND INCIDENTAL TO ANOTHER PROCEDURE IN 26%. PERHAPS THE ONLY CONTRAINDICATION TO SPLENECTOMY IS THE PRESENCE OF MARROW FAILURE, IN WHICH THE ENLARGED SPLEEN IS THE
ONLY SOURCE OF HEMATOPOIETIC TISSUE.
THE ABSENCE OF THE SPLEEN HAS MINIMAL LONG-TERM EFFECTS ON THE HEMATOLOGIC PROFILE. IN THE IMMEDIATE POSTSplenectomy PERIOD, Leukocytosis (up to 25,000/*L) and Thrombocytosis (up to 1 X 10###6/*L) MAY DEVELOP, BUT WITHIN 2-3 WEEKS, BLOOD CELL COUNTS AND SURVIVAL OF EACH CELL LINEAGE ARE USUALLY NORMAL. THE CHRONIC MANIFESTATIONS OF Splenectomy ARE MARKED VARIATION IN SIZE AND SHAPE OF Erythrocytes (Anisocytosis, Poikilocytosis) AND THE PRESENCE OF Howell-Jolly Bodies (Nuclear Remnants), Heinz Bodies (Denatured HEMOGLOBIN), Basophilic Stippling, AND AN OCCASIONAL NUCLEATED Erythrocyte IN THE PERIPHERAL BLOOD. WHEN SUCH Erythrocyte Abnormalities APPEAR IN A PATIENT WHOSE SPLEEN HAS NOT BEEN REMOVED, ONE SHOULD SUSPECT Splenic Infiltration BY Tumor THAT HAS INTERFERED WITH ITS NORMAL CULLING AND Pitting FUNCTION. THE MOST SERIOUS CONSEQUENCE OF SpleNECTOMY IS INCREASED SUSCEPTIBILITY TO BACTERIAL INFECTIONS, PARTICULARLY THOSE WITH CAPSULES SUCH AS Streptococcus Pneumoniae, Haemophilus influenzae, AND SOME GRAM-NEGATIVE ENTERIC ORGANISMS. Patients UNDER AGE 20 YEARS ARE PARTICULARLY SUSCEPTIBLE TO OVERTWELMING SEPSIS WITH S. Pneumoniae, AND THE OVERALL ActuARIal RISK OF SEPSIS IN PATIENTS WHO HAVE HAD THEIR SPLEENS REMOVED IS ABOUT 7% IN 10 YEARS. THE CASE-FATALITY RISK FOR Pneumococcal SEPSIS IN Splenectomized Patients IS 50-80%. About 25% OF Patients WITHOUT Spleens WILL DEVELOP A SERIOUS INFECTION AT SOME TIME IN THEIR LIFE. THE FREQUENCY IS HIGHEST WITHIN THE FIRST 3 YEARS AFTER SpleNectomy. ABOUT 15% OF THE INFECTIONS ARE POLYMICROBIAL, AND LUNG, SKIN, AND BLOOD ARE THE MOST COMMON SITES. NO INCREASED RISK OF VIRAL INFECTION HAS BEEN NOTED IN PATIENTS WHO HAVE NO SPLEEN. THE SUSCEPTIBILITY TO BACTERIAL INFECTIONS RELATES TO THE INABILITY TO REMOVE OPSONIZED BACTERIA FROM THE BLOODSTREAM AND A DEFECT IN MAKING ANTIBODIES TO T CELL-INDEPENDENT ANTIgENS SUCH AS THE POLYsACcharIDE COMPONENTS OF BACTERIAL CAPSULES. Pneumococcal VACCINE (23-VALENT POLYsACcharIDE VACCINE) SHOULD BE ADMINISTERED TO ALL PATIENTS 2 WEEKS BEFORE ELECTIVE SpleNECTOMY. THE ADVISORY COMMITTEE ON IMMUNIZATION PRACTICES RECOMMENDS THAT EVEN SpleNECTOMIZED PATIENTS RECEIVE Pneumococcal VACCINE WITH A REPEAT VACCINATION 5 YEARS LATER. EFFICACY HAS NOT BEEN PROVEN IN THIS SETTING, AND THE RECOMMENDATION DISCOUNTS THE POSSI-
ABILITY THAT ADMINISTRATION OF THE VACCINE MAY ACTUALLY LOWER THE TITER OF SPECIFIC PNEUMOCOCCAL ANTIBODIES. A MORE EFFECTIVE PNEUMOCOCCAL CONJUGATE VACCINE THAT INVOLVES T CELLS IN THE RESPONSE IS NOW AVAILABLE (PREVENAR, 7-VALENT). THE VACCINE TO NEISSERIA MENINGITIDIS SHOULD ALSO BE GIVEN TO PATIENTS IN WHOM ELECTIVE SPLENECTOMY IS PLANNED. ALTHOUGH EFFICACY DATA FOR HAEMOPHILUS INFLUENZAE TYPE B VACCINE ARE NOT AVAILABLE FOR OLDER CHILDREN OR ADULTS, IT MAY BE GIVEN TO PATIENTS WHO HAVE HAD A SPLENECTOMY. SPLENECTOMIZED PATIENTS SHOULD BE EDUCATED TO CONSIDER ANY UNEXPLAINED FEVER AS A MEDICAL EMERGENCY. PROMPT MEDICAL ATTENTION WITH EVALUATION AND TREATMENT OF SUSPECTED BACTEREMIA MAY BE LIFE-SAVING. ROUTINE CHEMOPROPHYLAXIS WITH ORAL PENICILLIN CAN RESULT IN THE EMERGENCE OF DRUG-RESISTANT STRAINS AND IS NOT RECOMMENDED. IN ADDITION TO AN INCREASED SUSCEPTIBILITY TO BACTERIAL INFECTIONS, SPLENECTOMIZED PATIENTS ARE ALSO MORE SUSCEPTIBLE TO THE PARASITIC DISEASE BABESIOSIS. THE SPLENECTOMIZED PATIENT SHOULD AVOID AREAS WHERE THE PARASITE BABESIA IS ENDEMIC (E.G., CAPE COD, MA). SURGICAL REMOVAL OF THE SPLEEN IS AN OBVIOUS CAUSE OF HYPOSPLENISM. PATIENTS WITH SICKLE CELL DISEASE OFTEN SUFFER FROM AUTOSPLENECTOMY AS A RESULT OF SPLENIC DESTRUCTION BY THE NUMEROUS INFARCTS ASSOCIATED WITH SICKLE CELL CRISSES DURING CHILDHOOD. INDEED, THE PRESENCE OF A PALPABLE SPLEEN IN A PATIENT WITH SICKLE CELL DISEASE AFTER AGE 5 SUGGESTS A COEXISTING HEMOGLOBINOPATHY, E.G., THALASSEMIA OR HEMOGLOBIN C. IN ADDITION, PATIENTS WHO RECEIVE SPLENIC IRRADIATION FOR A NEOPLASTIC OR AUTOIMMUNE DISEASE ARE ALSO FUNCTIONALLY HYPOSPLENIC. THE TERM HYPOSPLENISM IS PRE-FERRED TO ASPLENISM IN REFERRING TO THE PHYSIOLOGIC CONSEQUENCES OF SPLENECTOMY BECAUSE ASPLENA IS A RARE, SPECIFIC, AND FATAL CONGENITAL ABNORMALITY IN WHICH THERE IS A FAILURE OF THE LEFT SIDE OF THE COELOMIC CAVITY (WHICH INCLUDES THE SPLENIC ANLAGEN) TO DEVELOP NORMALLY. INFANTS WITH ASPLENA HAVE NO SPLEENS, BUT THAT IS THE LEAST OF THEIR PROBLEMS. THE RIGHT SIDE OF THE DEVELOPING EMBRYO IS DUPLICATED ON THE LEFT SO THERE IS LIVER WHERE THE SPLEEN SHOULD BE, THERE ARE TWO RIGHT LUNGS, AND THE HEART COMPRISSES TWO RIGHT ATRIA AND TWO RIGHT VENTRICLES.

FURTHER READINGS
BARKUN AN ET AL: THE BEDSIDE ASSESSMENT OF SPLENIC ENLARGEMENT. AM J MED 91:512, 1991
GRAVES SA ET AL: DOES THIS PATIENT HAVE SPLENOMEGALY? JAMA 270:2218, 1993
MCINTYRE OR, EBAUGH FG JR: PALPABLE SPLEENS: TEN YEAR FOLLOW-UP. ANN INTERN MED 90:130, 1979
EASE IS THE EXACT ROLE OF THE CELL TYPES COMPLETELY ESTABLISHED. THUS, WHEREAS NEUTROPHILS ARE CLASSICALLY THOUGHT TO BE CRITICAL TO HOST DEFENSE AGAINST BACTERIA, THEY MAY ALSO PLAY IMPORTANT ROLES IN DEFENSE AGAINST VIRAL INFECTIONS.

THE BLOOD DELIVERS LEUKOCYTES TO THE VARIOUS TISSUES FROM THE BONE MARROW, WHERE THEY ARE PRODUCED. NORMAL BLOOD LEUKOCYTE COUNTS ARE 4.3-10.8 X 10^9/L, WITH NEUTROPHILS REPRESENTING 45-74% OF THE CELLS, BANDS 0-4%, LYMPHOCYTES 16-45%, MONOCYTES 4-10%, EOSINOPHILS 0-7%, AND BASOPHILS 0-2%. VARIATION AMONG INDIVIDUALS AND AMONG DIFFERENT ETHNIC GROUPS CAN BE SUBSTANTIAL WITH LOWER LEUKOCYTE NUMBERS FOR CERTAIN AFRICAN-AMERICAN ETHNIC GROUPS. THE VARIOUS LEUKOCYTES ARE DERIVED FROM A COMMON STEM CELL IN THE BONE MARROW. THREE-

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376 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

FIGURE 61-1 SCHEMATIC EVENTS IN NEUTROPHIL PRODUCTION, RECRUITMENT, AND INFLAMMATION. THE FOUR CARDINAL SIGNS OF INFLAMMATION (RUBOR, TUMOR, CALOR, DOLOR) ARE INDICATED, AS ARE THE INTERACTIONS OF NEUTROPHILS WITH OTHER CELLS AND CYTOKINES. PMN, POLYMORPHONUCLEAR LEUKOCYTES; G-CSF, GRANULOCYTE COLONY-STIMULATING FACTOR; IL, INTERLEUKIN; TNF-*, TUMOR NECROSIS FACTOR *.

FOURTHS OF THE NUCLEATED CELLS OF BONE MARROW ARE COMMITTED TO THE PRODUCTION OF LEUKOCYTES. LEUKOCYTE MATURATION IN THE MARROW IS UNDER THE REGULATORY CONTROL OF A NUMBER OF DIFFERENT FACTORS, KNOWN AS COLONY-STIMULATING FACTORS (CSFS) AND INTERLEUKINS (ILS). BECAUSE AN ALTERATION IN THE NUMBER AND TYPE OF LEUKOCYTES IS OFTEN ASSOCIATED WITH DISEASE PROCESSES, TOTAL WHITE BLOOD COUNT (WBC) (CELLS PER *L) AND DIFFERENTIAL COUNTS ARE INFORMATIVE. THIS CHAPTER FOCUSES ON NEUTROPHILS, MONOCYTES, AND EOSINOPHILS. LYMPHOCYTES AND BASOPHILS ARE DISCUSSED IN CHAPS. 308 AND 311, RESPECTIVELY.

NEUTROPHILS MATURATION

IMPORTANT EVENTS IN NEUTROPHIL LIFE ARE SUMMARIZED IN FIG. 61-1. IN NORMAL HUMANS, NEUTROPHILS ARE PRODUCED ONLY IN THE BONE MARROW. THE MINIMUM NUMBER OF STEM CELLS NECESSARY TO SUPPORT HEMATOPOIE-
SIS IS ESTIMATED TO BE 400-500 AT ANY ONE TIME. HUMAN BLOOD MONOCYTES, TISSUE MACROPHAGES, AND STROMAL CELLS PRODUCE CSFS, HORMONES REQUIRED FOR THE GROWTH OF MONOCYTES AND NEUTROPHILS IN THE BONE MARROW. THE HEMATOPOIETIC SYSTEM NOT ONLY PRODUCES ENOUGH NEUTROPHILS (-1.3 X 10^11 CELLS PER 80-KG PERSON PER DAY) TO CARRY OUT PHYSIOLOGIC FUNCTIONS BUT ALSO HAS A LARGE RESERVE STORED IN THE MARROW, WHICH CAN BE MOBILIZED IN RESPONSE TO INFLAMMATION OR INFECTION. AN INCREASE IN THE NUMBER OF BLOOD NEUTROPHILS IS CALLED NEUTROPHILIA, AND THE PRESENCE OF IMMATURE CELLS IS TERMED A SHIFT TO THE LEFT. A DECREASE IN THE NUMBER OF BLOOD NEUTROPHILS IS CALLED NEUTROPENIA. NEUTROPHILS AND MONOCYTES EVOLVE FROM PLURIPOTENT STEM CELLS UNDER THE INFLUENCE OF CYTOKINES AND CSFS (FIG. 61-2). THE PROLIFERATION PHASE THROUGH THE METAMYELOCYTE TAKES ABOUT 1 WEEK, WHILE THE MATURATION PHASE FROM METAMYELOCYTE TO MATURE NEUTROPHIL TAKES ANOTHER WEEK. THE MYELOBLAST IS THE FIRST RECOGNIZABLE PRECURSOR CELL AND IS FOLLOWED BY THE PROMYELOCYTE. THE PROMYELOCYTE EVOLVES WHEN THE CLASSIC LYSOSONAL GRANULES, CALLED THE PRIMARY, OR AZUROPHIL, GRANULES ARE PRODUCED. THE PRIMARY GRANULES CONTAIN HYDROLASES, ELASTASE, MYELOPEROXIDASE, CATHEPSIN G, CATIONIC PROTEINS, AND BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN, WHICH IS IMPORTANT FOR KILLING GRAM-NEGATIVE BACTERIA. AZUROPHIL GRANULES ALSO CONTAIN DEFENSINS, A FAMILY OF CYSTEINE-RICH POLYPEPTIDES WITH BROAD ANTIMICROBIAL ACTIVITY AGAINST BACTERIA, FUNGI, AND CERTAIN ENVELOPED VIRUSES. THE PROMYELOCYTE DIVIDES TO PRODUCE THE MYELOCYTE, A CELL RESPONSIBLE FOR THE SYNTHESIS OF THE SPECIFIC, OR SECONDARY, GRANULES, WHICH CONTAIN UNIQUE (SPECIFIC) CONSTITUENTS SUCH AS LACTOFERRIN, VITAMIN B12-BINDING PROTEIN, MEMBRANE COMPONENTS OF THE REDUCED NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) OXIDASE REQUIRED FOR HYDROGEN PEROXIDE PRODUCTION, HISTAMINASE, AND RECEPTORS FOR CERTAIN CHEMOTRATRACTANTS AND ADHERENCE-PROMOTING FACTORS (CR3) AS WELL AS RECEPTORS FOR THE BASEMENT MEMBRANE COMPONENT, LAMININ. THE SECONDARY GRANULES DO NOT CONTAIN ACID HYDROLASES AND THEREFORE ARE NOT CLASSIC LYSOSONES. PACKAGING OF SECONDARY GRANULE CONTENTS DURING MYELOPOIESIS IS CONTROLLED BY CCAAT/ENHANCER BINDING PROTEIN.* SECONDARY GRANULE CONTENTS ARE READILY RELEASED EXTRACELLULARLY, AND THEIR MOBILIZATION IS IMPORTANT IN MODULATING INFLAMMATION. DURING THE FINAL STAGES OF MATURATION, NO CELL DIVISION OCCURS, AND THE CELL PASSES THROUGH THE METAMYELOCYTE STAGE AND THEN TO THE BAND NEUTROPHIL WITH A SAUSAGE-SHAPED NUCLEUS (FIG. 61-3). AS THE BAND CELL MATURES, THE NUCLEUS ASSUMES A LOBULATED CONFIGURATION. THE NUCLEUS OF NEUTROPHILS NORMALLY CONTAINS UP TO FOUR SEGMENTS (FIG. 61-4). EXCESSIVE SEGMENTATION (MORE THAN FIVE NUCLEAR LOBES) MAY BE A MANIFESTATION OF
FOLATE OR VITAMIN B12 DEFICIENCY (SEE FIG. 100-4) AND THE CONGENITAL NEUTROPENIA SYNDROME OF WARTS, HYPOGAMMAGLOBULINEMIA, INFECTIONS, AND MYELOKATHEXIS (WHIM) DESCRIBED BELOW. THE PELGER-HUET ANOMALY (FIG. 61-5), AN INFREQUENT DOMINANT BENIGN INHERITED TRAIT, RESULTS IN NEUTROPHILS WITH DISTINCTIVE BILOBED NUCLEI THAT MUST BE DISTINGUISHED FROM BAND FORMS. ACQUIRED FIGURE 61-2 STAGES OF NEUTROPHIL DEVELOPMENT SHOWN SCHEMATICALLY. G-CSF (GRANULOCYTE COLONY-STIMULATING FACTOR) AND GM-CSF (GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR) ARE CRITICAL TO THIS PROCESS. IDENTIFYING CELLULAR CHARACTERISTICS AND SPECIFIC CELL-SURFACE MARKERS ARE LISTED FOR EACH MATURATIONAL STAGE.

377 CHAPTER 61 DISORDERS OF GRANULOCYTES AND MONOCYTES

FIGURE 61-3 NEUTROPHIL BAND WITH DOHLE BODY. THE NEUTROPHIL WITH A SAUSAGE-SHAPED NUCLEUS IN THE CENTER OF THE FIELD IS A BAND FORM. DOHLE BODIES ARE DISCRETE, BLUE-STAINING NONGRANULAR AREAS FOUND IN THE PERIPHERY OF THE CYTOPLASM OF THE NEUTROPHIL IN INFECTIONS AND OTHER TOXIC STATES. THEY REPRESENT AGGREGATES OF ROUGH ENDOPLASMIC RETICULUM.

BILOBED NUCLEI, PSEUDO PELGER-HUET ANOMALY, CAN OCCUR WITH ACUTE INFECTIONS OR IN MYELODYSPLASTIC SYNDROMES. THE PHYSIOLOGIC ROLE OF THE NORMAL MULTILOBED NUCLEUS OF NEUTROPHILS IS UNKNOWN, BUT IT MAY ALLOW GREAT DEFORMATION OF NEUTROPHILS DURING MIGRATION INTO TISSUES AT SITES OF INFLAMMATION.

IN SEVERE ACUTE BACTERIAL INFECTION, PROMINENT NEUTROPHIL CYTOPLASMIC GRANULES, CALLED TOXIC GRANULATIONS, ARE OCCASIONALLY SEEN. TOXIC GRANULATIONS ARE IMMATURE OR ABNORMALLY STAINING AZUROPHIL GRANULES. CYTOPLASMIC INCLUSIONS, ALSO CALLED DOHLE BODIES (FIG. 61-3), CAN BE SEEN DURING INFECTION AND ARE FRAGMENTS OF RIBOSOME-RICH ENDOPLASMIC RETICULUM. LARGE NEUTROPHIL VACUOLES ARE OFTEN PRESENT IN ACUTE BACTERIAL INFECTION AND PROBABLY REPRESENT PINOCYTOSED (INTERNALIZED) MEMBRANE. NEUTROPHILS ARE HETEROGENEOUS IN FUNCTION. MONOCLONAL ANTIBODIES HAVE BEEN DEVELOPED THAT RECOGNIZE ONLY A SUBSET OF MATURE NEUTROPHILS. THE MEANING OF NEUTROPHIL HETEROGENEITY IS NOT KNOWN. THE MORPHOLOGY OF EOSINOPHILS AND BASOPHILS IS SHOWN IN FIG. 61-6.
MARROW RELEASE AND CIRCULATING COMPARTMENTS

SPECIFIC SIGNALS, INCLUDING IL-1, TUMOR NECROSIS FACTOR * (TNF-*), THE CSFS, COMPLEMENT FRAGMENTS, AND CHEMOKINES, MOBILIZE LEUKOCYTES FROM THE BONE MARROW AND DELIVER THEM TO THE BLOOD IN AN UNSTIMULATED STATE. UNDER NORMAL CONDITIONS, ~90% OF THE NEUTROPHIL POOL IS IN THE BONE MARROW, 2-3% IN THE CIRCULATION, AND THE REMAINDER IN THE TISSUES (FIG. 61-7).

THE CIRCULATING POOL EXISTS IN TWO DYNAMIC COMPARTMENTS: ONE FREE-LEY FLOWING AND ONE MARGINATED. THE FREELY FLOWING POOL IS ABOUT ONE-HALF THE NEUTROPHILS IN THE BASAL STATE AND IS COMPOSED OF THOSE CELLS THAT ARE IN THE BLOOD AND NOT IN CONTACT WITH THE ENDOTHELIUM. MARGINATED LEUKOCYTES ARE THOSE THAT ARE IN CLOSE PHYSICAL CONTACT WITH THE ENDOTHELIUM (FIG. 61-8). IN THE PULMONARY CIRCULATION, WHERE AN EXTENSIVE CAPILLARY BED (~ 1000 CAPILLARIES PER ALVEOLUS) EXISTS, MARGINATION OCCURS BECAUSE THE CAPILLARIES ARE ABOUT THE SAME SIZE AS A MATURE NEUTROPHIL. THEREFORE, NEUTROPHIL FLUIDITY AND DEFORMABILITY ARE NECES-

FIGURE 61-4 NORMAL GRANULOCYTE. THE NORMAL GRANULOCYTE HAS A SEGMENTED NUCLEUS WITH HEAVY, CLUMPED CHROMATIN; FINE NEUTROPHILIC GRANULES ARE DISPERSED THROUGHOUT THE CYTOPLASM.

FIGURE 61-5 PELGER-HUET ANOMALY. IN THIS BENIGN DISORDER, THE MAJORITY OF GRANULOCYTES ARE BILOBED. THE NUCLEUS FREQUENTLY HAS A SPECTACLE-LIKE, OR "PINCE-NEZ," CONFIGURATION.

FIGURE 61-6 NORMAL EOSINOPHIL AND BASOPHIL. THE EOSINOPHIL CONTAINS LARGE, BRIGHT ORANGE GRANULES AND USUALLY A BILOBED NUCLEUS. THE BASOPHIL CONTAINS LARGE PURPLE-BLACK GRANULES THAT FILL THE CELL AND OBSCURE THE NUCLEUS.

SARY TO MAKE THE TRANSIT THROUGH THE PULMONARY BED. INCREASED NEUTROPHIL RIGIDITY AND DECREASED DEFORMABILITY LEAD TO AUGMENTED NEUTROPHIL TRAPPING AND MARGINATION IN THE LUNG. IN CONTRAST, IN THE SYSTEMIC POSTCAPILLARY VENULES, MARGINATION IS MEDIATED BY THE INTERACTION OF SPECIFIC CELL-SURFACE MOLECULES CALLED SELECTINS. SELECTINS ARE GLYCOPROTEINS EXPRESSED ON NEUTROPHILS AND ENDOTHELIAL CELLS, AMONG OTHERS, THAT CAUSE A LOW-AFFINITY INTERACTION, RESULTING IN "ROLLING" OF THE NEUTROPHIL ALONG THE ENDOTHELIAL SURFACE. ON NEUTROPHILS, THE MOLECULE L-SELECTIN [CLUSTER DETERMINANT (CD) 62L] BINDS TO GLYCOSYLATED PROTEINS ON ENDOTHELIAL CELLS [E.G., GLYCOSYLATION-DEPENDENT CELL ADHE-
SION MOLECULE (GLYCAM 1) AND CD34. GLYCOPROTEINS ON NEUTROPHILS, MOST IMPORTANTLY SIALYL-LEWIS##X (SLE##X, CD 15S), ARE TARGETS FOR BINDING OF SELECTINS EXPRESSED ON ENDOTHELIAL CELLS [E-SELECTIN (CD62E) AND P-SELECTIN (CD62P)] AND OTHER LEUKOCYTES. IN RESPONSE TO CHEMOTACTIC STIMULI FROM INJURED TISSUES (E.G., COMPLEMENT PRODUCT C5A, LEUKOTRIENE B###4, IL-8) OR BACTERIAL PRODUCTS [E.G., N-FORMVLMETHIONYL-LEUCYLPHENYLALANINE (F-METLEUPHE)], NEUTROPHIL ADHESIVENESS INCREASES, AND THE CELLS “STICK” TO THE ENDOTHELIUM THROUGH INTEGRINS. THE INTEGRINS ARE LEUKOCYTE GLYCOPROTEINS THAT EXIST AS COMPLEXES OF A COMMON CD18 * CHAIN WITH CD11A (LFA-1), CD11B (CALLED MAC-1, CR3, OR THE C3BI RECEPTOR), AND CD11C (CALLED P150, 95 OR CR4). CD11A/CD18 AND CD11B/CD18 BIND TO SPECIFIC ENDOTHELIAL RECEPTORS [INTERCELLULAR ADHESION MOLECULES (ICAM ) 1 AND 2]. ON CELL STIMULATION, L-SELECTIN IS SHED FROM NEUTROPHILS, AND E-SELECTIN INCREASES IN THE BLOOD, PRESUMABLY BECAUSE IT IS SHED FROM ENDOTHELIAL CELLS; RECEPTORS FOR CHEMOATTRACTANTS AND OPSONINS ARE MOBILIZED; AND THE PHAGOCYTES ORIENT TOWARD THE CHEMOATTRACTANT SOURCE IN THE EXTRAVASCULAR SPACE, INCREASE THEIR MOTILE ACTIVITY (CHEMOKINESIS), AND MIGRATE DIRECTIONALLY (CHEMOTAXIS) INTO TISSUES. THE PROCESS OF MIGRATION INTO TISSUES IS CALLED DIAPEDESIS AND INVOLVES THE CRAWLING OF NEUTROPHILS BETWEEN POSTCAPILLARY ENDOTHELIAL CELLS THAT OPEN JUNCTIONS BETWEEN AD-

FIGURE 61-7 SCHEMATIC NEUTROPHIL DISTRIBUTION AND KINETICS BETWEEN THE DIFFERENT ANATOMIC AND FUNCTIONAL POOLS.

378 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

FIGURE 61-8 NEUTROPHIL TRAVEL THROUGH THE PULMONARY CAPILLARIES IS DEPENDENT ON NEUTROPHIL DEFORMABILITY. NEUTROPHIL RIGIDITY (EG, CAUSED BY C5A) ENHANCES PULMONARY TRAPPING AND RESPONSE TO PULMONARY PATHOGENS IN A WAY THAT IS NOT SO DEPENDENT ON CELL-SURFACE RECEPTORS. INTRAALVEOLAR CHEMOTACTIC FACTORS, SUCH AS THOSE CAUSED BY CERTAIN BACTERIA (E.G., STREPTOCOCCUS PNEUMONIAE) LEAD TO DIAPEDESIS OF NEUTROPHILS FROM THE PULMONARY CAPILLARIES INTO THE ALVEOLAR SPACE. NEUTROPHIL INTERACTION WITH THE ENDOTHELIUM OF THE SYSTEMIC POSTCAPILLARY VENULES IS DEPENDENT ON MOLECULES OF ATTACHMENT. THE NEUTROPHIL “ROLLS” ALONG THE
ENDOTHELIUM USING SELECTINS: NEUTROPHIL CD1 5S (SIALYL-LEWIS###X) BINDS TO CD62E (E-SELECTIN) AND CD62P (P-SELECTIN) ON ENDOTHELIAL CELLS. CD62L (L-SELECTIN) ON NEUTROPHILS BINDS TO CD34 AND OTHER MOLECULES (E.G., GLYCAM-1) EXPRESSED ON ENDO-
THELIUM. CHEMOKINES OR OTHER ACTIVATION FACTORS STIMULATE INTEGRIN-MEDIATED “TIGHT ADHESION”: CD11LA/CD18 (LFA-1) AND CD11 B/CD18 (MAC-1, CR3) BIND TO CD54 (ICAM-1) AND CD102 (ICAM-2) ON THE ENDOTHELIUM. DIAPEDESIS OCCURS BETWEEN ENDOTHELIAL CELLS: CD31 (PECAM-1) EXPRESSED BY THE EMIGRATING NEUTROPHIL INTERACTS WITH CD31 EXPRESSED AT THE ENDOTHELIAL CELL-CELL JUNCTION.

JACENT CELLS TO PERMIT LEUKOCYTE PASSAGE. DIAPEDESIS INVOLVES PLATELET/
ENDOTHELIAL CELL ADHESION MOLECULE (PECAM) 1 (CD31), WHICH IS EX-
PRESSED ON BOTH THE EMIGRATING LEUKOCYTE AND THE ENDOTHELIAL CELLS. THE ENDOTHELIAL RESPONSES (INCREASED BLOOD FLOW FROM INCREASED VASODILATION AND PERMEABILITY) ARE MEDIATED BY ANAPHYLATOXINS (E.G., C3A AND C5A) AS WELL AS VASODILATORS SUCH AS HISTAMINE, BRADYKININ, SEROTONIN, NITRIC OXIDE, VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), AND PROSTAGLANDINS E AND I. CYTOKINES REGULATE SOME OF THESE PROCESSES [E.G., TNF-* INDUC-
TION OF VEGF, INTERFERON (IFN) * INHIBITION OF PROSTAGLANDIN E]. IN THE HEALTHY ADULT, MOST NEUTROPHILS LEAVE THE BODY BY MIGRATION THROUGH THE MUCOUS MEMBRANE OF THE GASTROINTESTINAL TRACT. NORMALLY, NEUTROPHILS SPEND A SHORT TIME IN THE CIRCULATION (HALF-LIFE, 6-7 H). SE-
NESCENT NEUTROPHILS ARE CLEARED FROM THE CIRCULATION BY MACR
OPHAGES IN THE LUNG AND SPLEEN. ONCE IN THE TISSUES, NEUTROPHILS RELEASE ENZYMES, SUCH AS COLLAGENASE AND ELASTASE, WHICH HELP ESTABLISH ABSCESS CAVITIES.

NEUTROPHILS INGEST PATHOGENIC MATERIALS THAT HAVE BEEN OPSONIZED BY IGG AND C3B. FIBRONEDIN AND THE TETRAPEPTIDE TUFTSIN ALSO FACILITATE PHAGOCYTOSIS. WITH PHAGOCYTOSIS COMES A BURST OF OXYGEN CONSUMPTION AND ACTIVA-
TION OF THE HEXOSE-MONOPHOSPHATE SHUNT. A MEMBRANE-ASSOCIATED
NADPH OXIDASE, CONSISTING OF MEMBRANE AND CYTOSOLIC COMPONENTS, IS ASSEMBLED AND CATALYZES THE REDUCTION OF OXYGEN TO SUPEROXIDE ANION, WHICH IS THEN CONVERTED TO HYDROGEN PEROXIDE AND OTHER TOXIC OXYGEN PRODUCTS (E.G., HYDROXYL RADICAL). HYDROGEN PEROXIDE + CHLORIDE + NEUTROPHIL MYELOPEROXIDASE GENERATE HYPOCHLOROUS ACID (BLEACH), HY-
POCHLORITE, AND CHLORINE. THESE PRODUCTS OXIDIZE AND HALOGENATE MICRO-
ORGANISMS AND TUMOR CELLS AND, WHEN UNCONTROLLED, CAN DAMAGE HOST TISSUE. STRONGLY CATIONIC PROTEINS, DEFENSINS, AND PROBABLY NITRIC OXIDE ALSO PARTICIPATE IN MICROBIAL KILLING. LADOFERRIN CHELATES IRON, AN IMPOR-
TANT GROWTH FACTOR FOR MICROORGANISMS, ESPECIALLY FUNGI. OTHER
ENZYMES, SUCH AS LYSOZYME AND ACID PROTEASES, HELP DIGEST MICROBIAL DEBRIS. AFTER 1-4 DAYS IN TISSUES, NEUTROPHILS DIE. THE APOPTOSIS OF NEUTROPHILS IS ALSO CYTOKINE-REGULATED; GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) AND IFN-* PROLONG THEIR LIFE SPAN. UNDER CERTAIN CONDITIONS, SUCH AS IN DELAYED-TYPE HYPERSENSITIVITY, MONOCYTE ACCUMULATION OCCURS WITHIN 6-12 H OF INITIATION OF INFLAMMATION. NEUTROPHILS, MONOCYTES, MICROORGANISMS IN VARIOUS STATES OF DIGESTION, AND ALTERED LOCAL TISSUE CELLS MAKE UP THE INFLAMMATORY EXUDATE, PUS. MYELOPEROXIDASE CONFERS THE CHARACTERISTIC GREEN COLOR TO PUS AND MAY PARTICIPATE IN TURNING OFF THE INFLAMMATORY PROCESS BY INACTIVATING CHEMOATTRACTANTS AND IMMOBILIZING PHAGOCYTIC CELLS. NEUTROPHILS RESPOND TO CERTAIN CYTOKINES [IFN-* , GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF), IL-8] AND PRODUCE CYTOKINES AND CHEMOTACTIC SIGNALS [TNF-* , IL-8, MACROPHAGE INFLAMMATORY PROTEIN (MIP) 1] THAT MODULATE THE INFLAMMATORY RESPONSE. IN THE PRESENCE OF FIBRINOGEN, F-MET LEU PHE OR LEUKOTRIENE B4 INDUCES IL-8 PRODUCTION BY NEUTROPHILS, PROVIDING AUTOCRINE AMPLIFICATION OF INFLAMMATION. CHEMOKINES (CHEMOATTRACTANT CYTOKINES) ARE SMALL PROTEINS PRODUCED BY MANY DIFFERENT CELL TYPES, INCLUDING ENDOTHELIAL CELLS, FIBROBLASTS, EPITHELIAL CELLS, NEUTROPHILS, AND MONOCYTES, THAT REGULATE NEUTROPHIL, MONOCYTE, EOSINOPHIL, AND LYMPHOCYTE RECRUITMENT AND ACTIVATION. CHEMOKINES TRANSDUCE THEIR SIGNALS THROUGH HETEROTRIMERIC G PROTEIN-LINKED RECEPTORS THAT HAVE SEVEN CELL MEMBRANE-SPANNING DOMAINS, THE SAME TYPE OF CELL-SURFACE RECEPTOR THAT MEDIATES THE RESPONSE TO THE CLASSIC CHEMOATTRACTANTS F-METLEUПHE AND C5A. FOUR MAJOR GROUPS OF CHEMOKINES ARE RECOGNIZED BASED ON THE CYSTEINE STRUCTURE NEAR THE N TERMINUS: C, CC, CXC, AND CXXXC. THE CXC CYTOKINES SUCH AS IL-8 MAINLY ATTRACT NEUTROPHILS; CC CHEMOKINES SUCH AS MIP-1 ATTRACT LYMPHOCYTES, MONOCYTES, EOSINOPHILS, AND BASOPHILS; THE C CHEMOKINE LYMPHOTACTIN IS T CELL TROPIC; THE CXXXC CHEMOKINE FRACTALKINE ATTRACTS NEUTROPHILS, MONOCYTES, AND T CELLS. THESE MOLECULES AND THEIR RECEPTORS NOT ONLY REGULATE THE TRAFFICKING AND ACTIVATION OF INFLAMMATORY CELLS, BUT SPECIFIC CHEMOKINE RECEPTORS SERVE AS CO-RECEPTORS FOR HIV INFECTION (CHAP. 182) AND HAVE A ROLE IN ATHEROGENESIS.
Neutrophil Abnormalities

A defect in the neutrophil life cycle can lead to dysfunction and compromised host defenses. Inflammation is often depressed, and the clinical result is often recurrent with severe bacterial and fungal infections. Aphthous ulcers of mucous membranes (gray ulcers without pus) and gingivitis and periodontal disease suggest a phagocytic cell disorder. Patients with congenital phagocyte defects can have infections within the first few days of life. Skin, ear, upper and lower respiratory tract, and bone infections are common. Sepsis and meningitis are rare. In some disorders the frequency of infection is variable, and patients can go for months or even years without major infection. Aggressive management of these congenital diseases has extended the life span of patients well beyond 30 years.

Neutropenia

The consequences of absent neutrophils are dramatic. Susceptibility to infectious diseases increases sharply when neutrophil counts fall below 1000 cells/μL. When the absolute neutrophil count (ANC; band forms and mature neutrophils combined) falls to <500 cells/μL, control of endogenous microbial flora (e.g., mouth, gut) is impaired; when the ANC is <200/μL, the inflammatory process is absent. Neutropenia can be due to depressed production, increased peripheral destruction, or excessive peripheral pooling. A falling
RATION OF MYELOID PRECURSORS. THE MARROW SUPPRESSION IS GENERALLY DOSE-RELATED AND DEPENDENT ON CONTINUED ADMINISTRATION OF THE DRUG. RECOMBINANT HUMAN G-CSF USUALLY REVERSES THIS FORM OF NEUTROPENIA. ANOTHER IMPORTANT MECHANISM FOR IATROGENIC NEUTROPENIA IS THE EFFECT OF DRUGS THAT SERVE AS IMMUNE HAPTENS AND SENSITIZE NEUTROPHILS OR NEUTROPHIL PRECURSORS TO IMMUNE-MEDIATED PERIPHERAL DESTRUCTION. THIS FORM OF DRUG-INDUCED NEUTROPENIA CAN BE SEEN WITHIN 7 DAYS OF EXPOSURE TO THE DRUG, WITH PREVIOUS DRUG EXPOSURE, RESULTING IN PREEXISTING ANTIBODIES. NEUTROPENIA MAY OCCUR A FEW HOURS AFTER ADMINISTRATION OF THE DRUG. ALTHOUGH ANY DRUG CAN CAUSE THIS FORM OF NEUTROPENIA, THE MOST FREQUENT CAUSES ARE COMMONLY USED ANTIBIOTICS, SUCH AS SULFA-CONTAINING COMPOUNDS, PENICILLINS, AND CEPIHALOSPORINS. FEVER AND EOSINOPHILIA MAY ALSO BE ASSOCIATED WITH DRUG REACTIONS, BUT OFTEN THESE SIGNS ARE NOT PRESENT. DRUG-INDUCED NEUTROPENIA CAN BE SEVERE, BUT DISCONTINUATION OF THE SENSITIZING DRUG IS SUFFICIENT FOR RECOVERY, WHICH IS USUALLY SEEN WITHIN 5-7 DAYS AND IS COMPLETE BY 10 DAYS. READMINISTRATION OF THE SENSITIZING DRUG SHOULD BE AVOIDED, SINCE ABRUPT NEUTROPENIA WILL OF-TEN RESULT. FOR THIS REASON, DIAGNOSTIC CHALLENGE SHOULD BE AVOIDED.

AUTOIMMUNE NEUTROPENIAS CAUSED BY CIRCULATING ANTIETROPHIL ANTIBODIES ARE ANOTHER FORM OF ACQUIRED NEUTROPENIA THAT RESULTS IN INCREASED DESTRUCTION OF NEUTROPHILS. ACQUIRED NEUTROPENIA MAY ALSO BE SEEN WITH VIRAL INFECTIONS, INCLUDING INFECTION WITH HIV. ACQUIRED NEU-TROPENIA MAY BE CYCLIC IN NATURE, OCCURRING AT INTERVALS OF SEVERAL WEEKS. ACQUIRED CYCLIC OR STABLE NEUTROPENIA MAY BE ASSOCIATED WITH AN EXPANSION OF LARGE GRANULAR LYMPHOCYTES (LGLS), WHICH MAY BE T CELLS, NK CELLS, OR NK-LIKE CELLS. PATIENTS WITH LGL LYMPHOCYTOSIS MAY HAVE MODERATE BLOOD AND BONE MARROW LYMPHOCYTOSIS, NEUTROPENIA, POLYCLONAL HYPERGAMMAGLOBULINEMIA, SPLENOMEGALY, RHEUMATOID ARTHRITIS, AND AB-

**TABLE 61-1 CAUSES OF NEUTROPENIA**

**DECREASED PRODUCTION**

DRUG-INDUCED-ALKYLATING AGENTS (NITROGEN MUSTARD, BUSULFAN, CHLORAM-BUCIL, CYCLOPHOSPHAMIDE); ANTIMETABOLITES (METHOTREXATE, 6-MERCAPTO-PURINE, 5-FLUCYTOSINE); NONCYTOTOXIC AGENTS [ANTIBIOTICS (CHLORAMPHENICOL, PENICILLINS, SULFONAMIDES), PHENOTHIAZINES, TRANQUILIZERS (MEPROBAMATE), ANTICONVULSANTS (CARBAMAZEPINE), ANTIPSYCHOTICS (CLOZAPINE), CERTAIN DIURETICS, ANTI-INFLAMMATORY AGENTS, ANTITHYROID DRUGS, MANY OTHERS] HEMATOLOGIC DISEASES-IDIOPATHIC, CYCLIC NEUTROPENIA, CHEDIAK-HIGASHI SYNDROME, APLASTIC ANEMIA, INFANTILE GENETIC DISORDERS (SEE TEXT) TUMOR INVASION, MYELOFIBROSIS
NUTRITIONAL DEFICIENCY - VITAMIN B12, FOLATE (ESPECIALLY ALCOHOLICS) INFECTION - TUBERCULOSIS, TYPHOID FEVER, BRUCELLOSIS, TULAREMIA, MEASLES, INFECTIOUS MONONUCLEOSIS, MALARIA, VIRAL HEPATITIS, LEISHMANIASIS, AIDS

PERIPHERAL DESTRUCTION

ANTEUROPHIL ANTIBODIES AND/OR SPLENIC OR LUNG TRAPPING AUTOIMMUNE DISORDERS - FELTY'S SYNDROME, RHEUMATOID ARTHRITIS, LUPUS ERYTHEMATOSUS DRUGS AS HAPTONS - AMINOPYRINE, *-METHYLDopa, PHENYLButAZONE, MERCURIAL DIURETICS, SOME PHENOTHIAZINES WEGENER'S GRANULOMATOSIS

PERIPHERAL POOLING (TRANSIENT NEUTROPENIA)

OVERWHELMING BACTERIAL INFECTION (ACUTE ENDOTOXEMIA) HEMODIALYSIS CARDIOPULMONARY BYPASS

SENSE OF LYMPHADENOPATHY. SUCH PATIENTS MAY HAVE A CHRONIC AND RELATIVELY STABLE COURSE. RECURRENT BACTERIAL INFECTIONS ARE FREquent. BENIGN AND MALIGNANT FORMS OF THIS SYNDROME OCCUR. IN SOME PATIENTS, A SPONTANEOUS REGRESSION HAS OCCURRED EVEN AFTER 11 YEARS, SUGGESTING AN IMMUNOREGULATORY DEFECT AS THE BASIS FOR AT LEAST ONE FORM OF THE DISORDER.

GLUCOCORTICOIDS, CYCLOSPORINE, IFN-* , AND NUCLEOSIDES SUCH AS 2-CHLOROIDEOXYADENOSINE EACH HAVE INDUCED REMISSION.

HEREDITARY NEUTROPENIAS HEREDITARY NEUTROPENIAS ARE RARE AND MAY MANIFEST IN EARLY CHILDHOOD AS A PROFOUND CONSTANT NEUTROPENIA OR AGRANULOCYTOSIS. CONGENITAL FORMS OF NEUTROPENIA INCLUDE KOSTMANN’S SYNDROME (NEUTROPHIL COUNT <100/*L), WHICH IS OFTEN FATAL DUE TO MUTATIONS IN THE ANTI-APOPTOSIS GENE HAX-1; SEVERE CHRONIC NEUTROPENIA (NEUTROPHIL COUNT OF 300-1500/*L) DUE TO MUTATIONS IN NEUTROPHIL ELASTASE; HEREDITARY CYCLIC NEUTROPENIA, OR, MORE APPROPRIATELY, CYCLIC HEMATOPOIESIS, ALSO DUE TO MUTATIONS IN NEUTROPHIL ELASTASE; THE CARTILAGE-HAIR HYPOPLASIA SYNDROME DUE TO MUTATIONS IN THE MITOCHONDRIAL RNA-PROCESSING ENDOBRIBONUCLEASE RMRP; SHWACHMAN-DIAMOND SYNDROME ASSOCIATED WITH PANCREATIC INSUFFICIENCY DUE TO MUTATIONS IN THE SHWACHMAN-BODIAN-DIAMOND SYNDROME GENE SBDS; THE WHIM [WARTS, HYPOGAMMAGLOBULINEMIA, INFECTIONS, MYELOKATHESIS (RETENTION OF WBCS IN THE MARROW)] SYNDROME, CHARACTERIZED BY NEUTROPHIL HYPERSEGMENTATION AND BONE MARROW MYELOID ARREST DUE TO MUTATIONS IN THE CHEMOKINE RECEPTOR CXCR4; AND NEUTROPENIAS ASSOCIATED WITH OTHER IMMUNE DEFECTS, SUCH AS X-LINKED AGAMMAGLOBULINEMIA, WISKOTT-ALDRICH SYNDROME, AND CD40 LIGAND DEFICIENCY. MUTATIONS IN THE G-CSF RECEPTOR CAN DEVELOP IN SEVERE CONGENITAL NEUTROPENIA AND ARE LINKED TO LEUKEMIA.
MATERNAL FACTORS CAN BE ASSOCIATED WITH NEUTROPENIA IN THE NEWBORN. TRANSPLACENTAL TRANSFER OF IGG DIRECTED AGAINST ANTIGENS ON FETAL NEUTROPHILS CAN RESULT IN PERIPHERAL DESTRUCTION. DRUGS (E.G., THIAZIDES) INGESTED DURING PREGNANCY CAN CAUSE NEUTROPENIA IN THE NEWBORN BY EITHER DEPRESSED PRODUCTION OR PERIPHERAL DESTRUCTION. IN FELTY’S SYNDROME-THE TRIAD OF RHEUMATOID ARTHRITIS, SPLENOMEGALY, AND NEUTROPENIA (CHAP. 314)-SPLEEN-PRODUCED ANTIBODIES CAN SHORTEN NEUTROPHIL LIFE SPAN, WHILE LGLS CAN ATTACK MARROW NEUTROPHIL PRECURSORS. SPLENECTOMY MAY INCREASE NEUTROPHIL COUNT IN FELTY’S SYNDROME AND LOWER SERUM NEUTROPHIL-BINDING IGG. SOME FELTY’S SYNDROME PATIENTS ALSO HAVE NEUTROPENIA ASSOCIATED WITH AN INCREASED NUMBER OF LGLS. SPLENOMEGALY WITH PERIPHERAL TRAPPING AND DESTRUCTION OF NEUTROPHILS IS ALSO SEEN IN LYSOSOMAL STORAGE DISEASES AND IN PORTAL HYPERTENSION.

NEUTROPHILIA

NEUTROPHILIA RESULTS FROM INCREASED NEUTROPHIL PRODUCTION, INCREASED MARROW RELEASE, OR DEFECTIVE MARGINATION (TABLE 61-2). THE MOST IMPORTANT ACUTE CAUSE OF NEUTROPHILIA IS INFECTION.

### TABLE 61-2 CAUSES OF NEUTROPHILIA

#### INCREASED PRODUCTION

IDIOPATHIC

DRUG-INDUCED-GLUCOCORTICOID, G-CSF

INFECTION-BACTERIAL, FUNGAL, SOMETIMES VIRAL

INFLAMMATION-THERMAL INJURY, TISSUE NECROSIS, MYOCARDIAL AND PULMONARY INFARCTION, HYPERSENSITIVITY STATES, COLLAGEN VASCULAR DISEASES

MYELOPROLIFERATIVE DISEASES-MYELOCYTIC LEUKEMIA, MYELOID METAPLASIA, POLYCYTHEMIA VERA

#### INCREASED MARROW RELEASE

GLUCOCORTICOID

ACUTE INFECTION (ENDOTOXIN)

INFLAMMATION-THERMAL INJURY

#### DECREASED OR DEFECTIVE MARGINATION

DRUGS-EPINEPHRINE, GLUCOCORTICOID, NONSTEROIDAL ANTI-INFLAMMATORY AGENTS

STRESS, EXCITEMENT, VIGOROUS EXERCISE

LEUKOCYTE ADHESION DEFICIENCY TYPE 1 (INTEGRIN * CHAIN, CD18); LEUKOCYTE ADHESION DEFICIENCY TYPE 2 (SELECTIN LIGAND, CD15S, SIALYL-LEWIS###X)

#### MISCELLANEOUS

METABOLIC DISORDERS-KETOACIDOSIS, ACUTE RENAL FAILURE, ECLAMPSIA, ACUTE POISONING

DRUGS-LITHIUM
TABLE 61-3 TYPES OF GRANULOCYTE AND MONOCYTE DISORDERS

FUNCTION

ADHERENCE-AGGREGATION

DEFORMABILITY

CHEMOKINESIS-
CHEMOTAXIS

MICROBICIDAL ACTIVITY

CAUSE OF INDICATED DYSFUNCTION

DRUG-INDUCED

ASPIRIN, COLCHICINE, ALCOHOL, GLUCOCORTICOIDS, IBUPROFEN, PIROXICAM

GLUCOCORTICOIDS (HIGH DOSE), AURANOFIN, COLCHICINE (WEAK EFFECT), PHENYL BUTAZONE, NAPROXEN, INDOMETHACIN, INTERLEUKIN 2

COLCHICINE, CYCLOPHOSPHAMIDE, GLUCOCORTICOIDS (HIGH DOSE), TNF- * BLOCKING ANTIBODIES

ACQUIRED

NEONATAL STATE, HEMODIALYSIS

LEUKEMIA, NEONATAL STATE, DIABETES MELLITUS, IMMATURE NEUTROPHILS, THERMAL INJURY, MALIGNANCY, MALNUTRITION, PERIODONTAL DISEASE, NEONATAL STATE, SYSTEMIC LUPUS ERYTHEMATOSUS, RHEUMATOID ARTHRITIS, DIABETES MELLITUS, SEPSIS, INFLUENZA VIRUS INFECTION, HERPES SIMPLEX VIRUS INFECTION, ACRODERMATITIS ENTEROPATHICA, AIDS

LEUKEMIA, APLASTIC ANEMIA, CERTAIN
NEUTROPENIAS, TUFTSIN DEFICIENCY, THERMAL INJURY, SEPSIS, NEONATAL STATE, DIABETES MELLITUS, MALNUTRITION, AIDS

INHERITED

LEUKOCYTE ADHESION DEFICIENCY TYPES 1 AND 2

CHEDIAK-HIGASHI SYNDROME, NEUTROPHIL-SPECIFIC GRANULE DEFICIENCY, HYPER IGE-RECURRENT INFECTION (JOB'S) SYNDROME (IN SOME PATIENTS), DOWN SYNDROME, *-MANNOSIDASE DEFICIENCY, SEVERE COMBINED IMMUNODEFICIENCY, WISKOTT-ALDRICH SYNDROME

CHEDIAK-HIGASHI SYNDROME, NEUTROPHIL-SPECIFIC GRANULE DEFICIENCY, CHRONIC GRANULOMATOUS DISEASE, DEFECTS IN IFN-*//1L-12 AXIS

NEUTROPHILIA FROM ACUTE INFECTION REPRESENTS BOTH INCREASED PRODUCTION AND INCREASED MARROW RELEASE. INCREASED PRODUCTION IS ALSO ASSOCIATED WITH CHRONIC INFLAMMATION AND CERTAIN MYELOPROLIFERATIVE DISEASES. INCREASED MARROW RELEASE AND MOBILIZATION OF THE MARGINATED LEUKOCYTE POOL ARE INDUCED BY GLUCOCORTICOIDS. RELEASE OF EPINEPHRINE, AS WITH VIGOROUS EXERCISE, EXCITEMENT, OR STRESS, WILL DEMARGINATE NEUTROPHILS IN THE SPLEEN AND LUNGS AND DOUBLE THE NEUTROPHIL COUNT IN MINUTES. CIGARETTE SMOKING CAN INCREASE NEUTROPHIL COUNTS INTO THE ABNORMAL RANGE. LEUKOCYTOSIS WITH CELL COUNTS OF 10,000-25,000/*L OCCURS IN RESPONSE TO INFECTION AND OTHER FORMS OF ACUTE INFLAMMATION AND RESULTS FROM BOTH RELEASE OF THE MARGINATED POOL AND MOBILIZATION OF MARROW RESERVES. PERSISTENT NEUTROPHILIA WITH CELL COUNTS OF *30,000-50,000/*L IS CALLED A LEUKEMOID REACTION, A TERM OFTEN USED TO DISTINGUISH THIS DEGREE OF NEUTROPHILIA FROM LEUKEMIA. IN A LEUKEMOID REACTION, THE CIRCULATING NEUTROPHILS ARE USUALLY MATURE AND NOT DONALLY DERIVED.

ABNORMAL NEUTROPHIL FUNCTION INHERITED AND ACQUIRED ABNORMALITIES OF PHAGOCYTE FUNCTION ARE LISTED IN TABLE 61-3. THE RESULTING DISEASES ARE BEST CONSIDERED IN TERMS OF THE FUNCTIONAL DEFECTS OF ADHERENCE, CHEMOTAXIS, AND MICROBICIDAL ACTIVITY. THE DISTINGUISHING FEATURES OF THE IMPORTANT INHERITED DISORDERS OF PHAGOCYTE FUNCTION ARE SHOWN IN TABLE 61-4.

DISORDERS OF ADHESION TWO MAIN TYPES OF LEUKOCYTE ADHESION DEFICIENCY (LAD) HAVE BEEN DESCRIBED, LAD 1 AND LAD 2. BOTH ARE AUTO-SOMAL RECESSIVE TRAITS AND RESULT IN THE INABILITY OF NEUTROPHILS TO EXIT THE CIRCULATION TO SITES OF INFECTION, LEADING TO LEUKOCYTOSIS AND INCREASED SUSCEPTIBILITY TO INFECTION (FIG. 61-8). PATIENTS WITH LAD 1 HAVE MUTATIONS IN CD18, THE COMMON COMPONENT OF THE INTEGRINS LFA-1, MAC-1, AND PL50, 95, LEADING TO A DEFECT IN TIGHT ADHESION BE-
TWEEN NEUTROPHILS AND THE ENDOTHELIUM. THE HETERODIMER FORMED BY CD18/CD11b (MAC-1) IS ALSO THE RECEPTOR FOR THE COMPLEMENT-DE-RIVED OPSONIN C3BI (CR3). THE CD18 GENE IS LOCATED ON DISTAL CHO-ROMOSOME 21Q. THE SEVERITY OF THE DEFECT DETERMINES THE SEVERITY OF CLINICAL DISEASE. COMPLETE LACK OF EXPRESSION OF THE LEUKOCYTE INTEGRINS RESULTS IN A SEVERE PHENOTYPE IN WHICH INFLAMMATORY STIMULI DO NOT INCREASE THE EXPRESSION OF LEUKOCYTE INTEGRINS ON NEUTROPHILS OR ACTIVAT-ED T AND B CELLS. NEUTROPHILS (AND MONOCYTES) FROM PATIENTS WITH LAD 1 ADHERE POORLY TO ENDOTHELIAL CELLS AND PROTEIN-COATED SURFACES AND EXHIBIT DEFECTIVE SPREADING, AGGREGATION, AND CHEMOTAXIS. PATIENTS WITH LAD 1 HAVE RECURRENT BACTERIAL INFECTIONS INVOLVING THE SKIN, ORAL AND GENITAL MUCOSA, AND RESPIRATORY AND INTESTINAL TRACTS; PERSISTENT LEU-KOCYTOSIS (NEUTROPHIL COUNTS OF 15,000-20,000/*L) BECAUSE CELLS DO NOT MARGINATE; AND, IN SEVERE CASES, A HISTORY OF DELAYED SEPARATION OF THE UMBILICAL STUMP. INFECTIONS, ESPECIALLY OF THE SKIN, MAY BECOME NECROT-IC WITH PROGRESSIVELY ENLARGING BORDERS, SLOW HEALING, AND DEVELOPMENT OF DYSPLEASTIC SCARS. THE MOST COMMON BACTERIA ARE STAPHYLOCOCCUS AU-REUS AND ENTERIC GRAM-NEGATIVE BACTERIA. LAD 2 IS CAUSED BY AN ABNOR-MALITY OF FUCOSYLATION OF SLE###X (CD 15S), THE LIGAND ON NEUTROPHILS THAT INTERACTS WITH SELECTINS ON ENDOTHELIAL CELLS AND IS RESPONSIBLE FOR NEU-TROPHIL ROLLING ALONG THE ENDOTHELIUM. INFECTION SUSCEPTIBILITY IN LAD APPEARS TO BE LESS SEVERE THAN IN LAD 1. LAD 2 IS ALSO KNOWN AS CON-GENITAL DISORDER OF GLYCOSYLATION IIC (CDGIIC).

DISORDERS OF NEUTROPHIL GRANULES THE MOST COMMON NEUTROPHIL DEFECT IS MYELOPEROXIDASE DEFICIENCY, A PRIMARY GRANULE DEFECT INHERIT-ED AS AN AUTOSOMAL RECESSIVE TRAIT; THE INCIDENCE IS ~ 1 IN 2000 PERSONS. ISOLATED MYELOPEROXIDASE DEFICIENCY IS NOT ASSOCIATED WITH CLINICALLY COMPROMISED DEFENSES, PRESUMABLY BECAUSE OTHER DEFENSE SYSTEMS SUCH AS HYDROGEN PEROXIDE GENERATION ARE AMPLIFIED. MICROBICIDAL AC-TIVITY OF NEUTROPHILS IS DELAYED BUT NOT ABSENT. MYELOPEROXIDASE DEFI-CIENCY MAY MAKE OTHER ACQUIRED HOST DEFENSE DEFECTS MORE SERIOUS. AN ACQUIRED FORM OF MYELOPEROXIDASE DEFICIENCY OCCURS IN MYELOMONO-CYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA.

CHEDIAK-HIGASHI SYNDROME (CHS) IS A RARE DISEASE WITH AUTOSO-MAL RECESSIVE INHERITANCE DUE TO DEFECTS IN THE LYSOSOMAL TRANSPORT PROTEIN LYST, ENCODED BY THE GENE CHS1 AT 1Q42. THIS PROTEIN IS RE-QUIRED FOR NORMAL PACKAGING AND DISBURSEMENT OF GRANULES. NEUTRO-PHILS (AND ALL CELLS CONTAINING LYSOSOMES) FROM PATIENTS WITH CHS CHARACTERISTICALLY HAVE LARGE GRANULES (FIG. 61-9) MAKING IT A SYSTEMIC DISEASE. PATIENTS WITH CHS HAVE NYSTAGMUS, PARTIAL OCULOCUTANEOUS ALBINISM, AND AN INCREASED NUMBER OF INFECTIONS RESULTING FROM MANY BACTERIAL AGENTS. SOME CHS PATIENTS DEVELOP AN “ACCELERATED PHASE” IN CHILDHOOD WITH A HEMOPHAGOCYTIC SYNDROME AND AN AGGRESSIVE LYMPHOMA REQUIRING BONE MARROW TRANSPLANTATION. CHS NEUTROPHILS
AND MONOCYTES HAVE IMPAIRED CHEMOTAXIS AND ABNORMAL RATES OF MICROBIAL KILLING DUE TO SLOW RATES OF FUSION OF THE LYSOSOMAL GRANULES WITH PHAGOSOMES. NK CELL FUNCTION IS ALSO IMPAIRED. CHS PATIENTS MAY DEVELOP A SEVERE DISABLING PERIPHERAL NEUROPATHY IN ADULTHOOD THAT CAN LEAD TO BED CONFINEMENT. SPECIFIC GRANULE DEFICIENCY IS A RARE AUTOSOMAL RECESSIVE DISEASE IN WHICH THE PRODUCTION OF SECONDARY GRANULES AND THEIR CONTENTS, AS WELL AS THE PRIMARY GRANULE COMPONENT DEFENSINS, IS DEFECTIVE. THE DEFECT IN BACTERIAL KILLING LEADS TO SEVERE BACTERIAL INFECTIONS. ONE TYPE OF SPECIFIC GRANULE DEFICIENCY IS DUE TO A MUTATION IN THE CCAAT/ENHANCER BINDING PROTEIN-*, A REGULATOR OF EXPRESSION OF GRANULE COMPONENTS.

**CHRONIC GRANULOMATOUS DISEASE** CHRONIC GRANULOMATOUS DISEASE (CGD) IS A GROUP OF DISORDERS OF GRANULOCYTE AND MONOCYTE OXIDATIVE METABOLISM. ALTHOUGH CGD IS RARE, WITH AN INCIDENCE OF 1 IN 200,000 INDIVIDUALS, IT IS AN IMPORTANT MODEL OF DEFECTIVE NEUTROPHIL OXIDATIVE METABOLISM. MOST OFTEN CGD IS INHERITED AS AN X-LINKED RECESSIVE TRAIT; 30% OF PATIENTS INHERIT THE DISEASE IN AN AUTOSOMAL RECESSIVE PATTERN.

### TABLE 61-4 INHERITED DISORDERS OF PHAGOCYTE FUNCTION: DIFFERENTIAL FEATURES

**CLINICAL MANIFESTATIONS**

**CHRONIC GRANULOMATOUS DISEASES (70% X-LINKED, 30% AUTOSOMAL RECESSIVE)**
SEVERE INFECTIONS OF SKIN, EARS, LUNGS, LIVER, AND BONE WITH CATALYSE-POSITIVE MICROORGANISMS SUCH AS *S. AUREUS, BURKHOLDERIA CE-PACIA, ASPERGILLUS SPP.*, *CHROMOBACTERIUM VIOLACEUM*, OFTEN HARD TO CULTURE ORGANISM; EXCESSIVE INFLAMMATION WITH GRANULOMAS, FREQUENT LYMPH NODE SUPPURATION; GRANULOMAS CAN OBSTRUCT GI OR GU TRACTS, GINGIVITIS, APHTHOUS ULCERS, SEBORRHEIC DERMATITIS

**CHEDIAK-HIGASHI SYNDROME (AUTOSOMAL RECESSIVE)**
RECURRENT PYOGENIC INFECTIONS, ESPECIALLY WITH *S. AUREUS*; MANY PATIENTS GET LYMPHOMA-LIKE ILLNESS DURING ADOLESCENCE; PERIODONTAL DISEASE, PARTIAL OCULOCUTANEOUS ALBINISM, NYSTAGMUS, PROGRESSIVE PERIPHERAL NEUROPATHY, MENTAL RETARDATION IN SOME PATIENTS

**SPECIFIC GRANULE DEFICIENCY (AUTOSOMAL RECESSIVE)**
RECURRENT INFECTIONS OF SKIN, EARS, AND SINOPULMONARY TRACT; DELAYED WOUND HEALING; DECREASED INFLAMMATION; BLEEDING
DIATHESIS

MYELOPEROXIDASE DEFICIENCY (AUTOSOMAL RECESSIVE)

CLINICALLY NORMAL EXCEPT IN PATIENTS WITH UNDERLYING DISEASE SUCH AS DIABETES MELLITUS, THEN CANDIDIASIS OR OTHER FUNGAL INFECTIONS

LEUKOCYTE ADHESION DEFICIENCY

TYPE 1: DELAYED SEPARATION OF UMBILICAL CORD, SUSTAINED NEUTROPHILIA, RECURRENT INFECTIONS OF SKIN AND MUCOSA, GINGIVITIS, PERIODONTAL DISEASE

TYPE 2: MENTAL RETARDATION, SHORT STATURE, BOMBAY (HH) BLOOD PHENOTYPE, RECURRENT INFECTIONS, NEUTROPHILIA

PHAGOCYTE ACTIVATION DEFECTS (X-LINKED AND AUTOSOMAL RECESSIVE)

NEMO DEFICIENCY: MILD HYPOHIDROTIC ECTODERMAL DYSPLASIA; BROAD BASED IMMUNE DEFECT: PYOGENIC AND ENCAPSULATED BACTERIA, VIRUSES, PNEUMOCYSTIS, MYCOBACTERIA; X-LINKED

IRAK4 DEFICIENCY: SUSCEPTIBILITY TO PYOGENIC BACTERIA SUCH AS STAPHYLOCOCCI, STREPTOCOCCI, CLOSTRIDIA; RESISTANT TO MYCOBACTERIA, AUTOSOMAL RECESSIVE

HYPER IGE-RECURRENT INFECTION SYNDROME (AUTOSOMAL DOMINANT) (JOB'S SYNDROME)

ECZEMATOID OR PRURITIC DERMATITIS, “COLD” SKIN ABSCESSES, RECURRENT PNEUMONIAS WITH S. AUREUS WITH BRONCHOPLEURAL FISTULAE AND CYST FORMATION, MILD EOSINOPHILIA, MUCOCUTANEOUS CANDIDIASIS, CHARACTERISTIC FACIES, RESTRICTIVE LUNG DISEASE, SCOLIOSIS, DELAYED PRIMARY DENTAL DECIDUATION

MYCOBACTERIA SUSCEPTIBILITY (AUTOSOMAL DOMINANT AND RECESSIVE FORMS)

SEVERE LOCAL OR DISSEMINATED INFECTIONS WITH BACILLE CALMETTE-GUERIN (BCG), NONTUBERCULOUS MYCOBACTERIA, SALMONELLA, HISTOPLASMOSIS, POOR GRANULOMA FORMATION

CELLULAR OR MOLECULAR DEFECTS

NO RESPIRATORY BURST DUE TO THE LACK OF ONE OF FOUR NADPH OXIDASE SUBUNITS IN NEUTROPHILS, MONOCYTES, AND EOSINOPHILS

REDUCED CHEMOTAXIS AND PHAGOLYSOSOME FUSION, INCREASED RESPIRATORY BURST ACTIVITY, DEFECTIVE EGRESS FROM MARROW, ABNORMAL SKIN WINDOW; DEFECT IN LYST

ABNORMAL CHEMOTAXIS, IMPAIRED RESPIRATORY
BURST AND BACTERIAL KILLING, FAILURE TO UP-REGULATE CHEMOTACTIC AND ADHESION RECEPTORS WITH STIMULATION, DEFECT IN TRANSCRIPTION OF GRANULE PROTEINS, DEFECT IN C/EBP*

NO MYELOPEROXIDASE DUE TO PRE- AND POST-TRANSLATIONAL DEFECTS

IMPAIRED PHAGOCYTE ADHERENCE, AGGREGATION, SPREADING, CHEMOTAXIS, PHAGOCYTOSIS OF C3BI-COATED PARTICLES; DEFECTIVE PRODUCTION OF CD18 SUBUNIT COMMON TO LEUKOCYTE INTEGRINS
IMPAIRED PHAGOCYTE ROLLING ALONG ENDOTHELium

IMPAIRED PHAGOCYTE ACTIVATION BY IL-1, IL-18, TLR, CD40, TNF-* LEADING TO PROBLEMS WITH INFLAMMATION AND ANTIBODY PRODUCTION
IMPAIRED PHAGOCYTE ACTIVATION BY ENDO-TOXIN THROUGH TLR AND OTHER PATHWAYS; TNF-* SIGNALING PRESERVED

REDUCED CHEMOTAXIS IN SOME PATIENTS,
REDUCED SUPPRESSOR T CELL ACTIVITY

INABILITY TO KILL INTRACELLULAR ORGANISMS DUE TO LOW IFN-* PRODUCTION; MUTATIONS IN IFN-* RECEPTORS, IL-12 P40, STAT-1, NEMO

DIAGNOSIS

NBT OR DHR TEST, NO SUPEROXIDE AND H2O2 PRODUCTION BY NEUTROPHILS: IMMUNOBLOT FOR NADPH OXIDASE COMPONENTS GENETIC DETECTION

GIANT PRIMARY GRANULES IN NEUTROPHILS AND OTHER GRANULE-BEARING CELLS (WRIGHT'S STAIN), GENETIC DETECTION

LACK OF SECONDARY (SPECIFIC) GRANULES IN NEUTROPHILS (WRIGHT'S STAIN), NO NEUTROPHIL-SPECIFIC GRANULE CONTENTS (I.E., LACTOFERRIN), NO DEFENSINS, PLATELET * GRANULE ABNORMALITY; GENETIC DETECTION

NO PEROXIDASE IN NEUTROPHILS; GENETIC DETECTION

REDUCED PHAGOCYTE SURFACE EXPRESSION OF
THE CD18-CONTAINING INTEGRINS WITH MONOCLONAL ANTIBODIES AGAINST LFA-1 (CD18/CD11A), MAC-1 OR CR3 (CD18/CD11B), P150, 95 (CD 18/CD 11C); GENETIC DETECTION
REDUCED PHAGOCYTE SURFACE EXPRESSION OF SIALYL-LEWIS^X WITH MONOCLONAL ANTIBODIES AGAINST CD15S, GENETIC DETECTION

POOR IN VITRO RESPONSE TO ENDOTOXIN; LACK OF NF-*B ACTIVATION; GENETIC DETECTION

POOR IN VITRO RESPONSE TO ENDOTOXIN; LACK OF NF-*B ACTIVATION BY ENDOTOXIN GENETIC DETECTION

CLINICAL FEATURES, INVOLVING LUNGS, SKELETON, AND IMMUNE SYSTEM; SERUM IGE > 2000 IU/ML
LOW OR VERY HIGH LEVELS OF IFN-* RECEPTOR 1;
FUNCTIONAL ASSAYS OF CYTOKINE PRODUCTION AND RESPONSE, GENETIC DETECTION

**ABBREVIATIONS:** GL, GASTROINTESTINAL; GU, GENITOURINARY; NADPH, NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE, NBT, NITROBLUE TETRAZOLIUM (DYE-TEST), DHR, DIHYDRORHODAMINE (OXIDATION TEST); LYST, LYSOSOMAL TRANSPORT PROTEIN; C/EBP*, CCAAT/ENHANCER BINDING PROTEIN-*; NEMO, NF-*B ESSENTIAL MODULATOR; TLR, TOLL-LIKE RECEPTOR; IL, INTERLEUKIN; TNF, TUMOR NECROSIS FACTOR; IRAK4, IL-1 RECEPTOR-ASSOCIATED KINASE PROTEIN-*; NEMO 4, IFN, INTERFERON.

MUTATIONS IN THE GENES FOR THE FOUR PROTEINS THAT ASSEMBLE AT THE PLASMA MEMBRANE ACCOUNT FOR ALL PATIENTS WITH CGD. TWO PROTEINS (A 91-KDA PROTEIN, ABNORMAL IN X-LINKED CGD, AND A 22-KDA PROTEIN, ABSENT IN ONE FORM OF AUTOSOMAL RECESSIVE CGD) FORM THE HETERODIMER CYTOCHROME B-558 IN THE PLASMA MEMBRANE. TWO OTHER PROTEINS (47 AND 67 KDA, ABNORMAL IN THE OTHER AUTOSOMAL RECESSIVE FORMS OF CGD) ARE CYTOPLASMIC IN ORIGIN AND INTERACT WITH THE CYTOCHROME AFTER CELL ACTIVATION TO FORM NADPH OXIDASE, REQUIRED FOR HYDROGEN PEROXIDE PRODUCTION. LEUKOCYTES FROM PATIENTS WITH CGD HAVE SEVERELY DIMINISHED HYDROGEN PEROXIDE PRODUCTION. THE GENES INVOLVED IN EACH OF THE DEFECTS HAVE BEEN
382 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF DISEASES

FIGURE 61-9 CHEDIAK-HIGASHI SYNDROME. THE GRANULOCYTES CONTAIN HUGE CYTOPLASMIC GRANULES FORMED FROM AGGREGATION AND FUSION OF AZURO-PHILIC AND SPECIFIC GRANULES. LARGE ABNORMAL GRANULES ARE FOUND IN OTHER GRANULE-CONTAINING CELLS THROUGHOUT THE BODY.

CLONED AND SEQUENCED AND THE CHROMOSOME LOCATIONS IDENTIFIED. PATIENTS WITH CGD CHARACTERISTICALLY HAVE INCREASED NUMBERS OF INFECTIONS DUE TO CATALASE-POSITIVE MICROORGANISMS (ORGANISMS THAT DESTROY THEIR OWN HYDROGEN PEROXIDE). WHEN PATIENTS WITH CGD BECOME INFECTED, THEY OFTEN HAVE EXTENSIVE INFLAMMATORY REACTIONS, AND LYMPH NODE SUP-PURATION IS COMMON DESPITE THE ADMINISTRATION OF APPROPRIATE ANTIBIOTICS. APHTHOUS ULCERS AND CHRONIC INFLAMMATION OF THE NARES ARE OFTEN PRESENT. GRANULOMAS ARE FREQUENT AND CAN OBSTRUCT THE GASTROINTESTINAL OR GENITOURINARY TRACTS. THE EXCESSIVE INFLAMMATION PROBABLY REFLECTS FAILURE TO INHIBIT THE SYNTHESIS OR DEGRADATION OF CHEMOTRACTANTS AND ANTIGENS, LEADING TO PERSISTENT NEUTROPHIL ACCUMULATION. IMPAIRED KILLING OF INTRACELLULAR MICROORGANISMS BY MACROPHAGES MAY LEAD TO PERSISTENT CELL-MEDIATED IMMUNE ACTIVATION AND GRANULOMA FORMATION. AUTOIMMUNE COMPLICATIONS SUCH AS IMMUNE THROMBOCYTOPENIC PURPURA AND JUVENILE RHEUMATOID ARTHRITIS ARE ALSO INCREASED IN CGD. IN ADDITION, DISCOID LUPUS IS MORE COMMON IN X-LINKED CARRIERS.

DISORDERS OF PHAGOCYTE ACTIVATION PHAGOCYTES DEPEND ON CELL-SURFACE STIMULATION TO INDUCE SIGNALS THAT EVOKE MULTIPLE LEVELS OF THE INFLAMMATORY RESPONSE, INCLUDING CYTOKINE SYNTHESIS, CHEMOTAXIS, AND ANTIGEN PRESENTATION. MUTATIONS AFFECTING THE MAJOR PATHWAY THAT SIGNALS THROUGH NF-*B HAVE BEEN NOTED IN PATIENTS WITH A VARIETY OF INFECTION SUSCEPTIBILITY SYNDROMES. IF THE DEFECTS ARE AT A VERY LATE STAGE OF SIGNAL TRANSDUCTION, IN THE PROTEIN CRITICAL FOR NF-*B ACTIVATION KNOWN AS THE NF-*B ESSENTIAL MODULATOR (NEMO), THEN AFFECTED MALES DEVELOP ECTODERMAL DYSPLASIA AND SEVERE IMMUNE DEFICIENCY WITH SUSCEPTIBILITY TO BACTERIA, FUNGI, MYCOBACTERIA, AND VIRUSES. IF THE DEFECT IN NF-*B ACTIVATION IS CLOSER TO THE SIGNALING SOURCE, IN THE IL-1 RECEPTOR-ASSOCIATED KI-
NASE 4 (IRAk4), then children have a marked susceptibility to pyogenic infections early in life but develop resistance to infection later.

**MONONUCLEAR PHAGOCYTES**

The mononuclear phagocyte system is composed of monoblasts, promonocytes, and monocytes, in addition to the structurally diverse tissue macrophages that make up what was previously referred to as the reticuloendothelial system. Macrophages are long-lived phagocytic cells capable of many of the functions of neutrophils. They are also secretory cells that participate in many immunologic and inflammatory processes distinct from neutrophils. Monocytes leave the circulation by diapedesis more slowly than neutrophils and have a half-life in the blood of 12-24 h.

After blood monocytes arrive in the tissues, they differentiate into macrophages (“big eaters”) with specialized functions suited for specific anatomic locations. Macrophages are particularly abundant in capillary walls of the lung, spleen, liver, and bone marrow, where they function to remove microorganisms and other noxious elements from the blood. Alveolar macrophages, liver kupffer cells, splenic macrophages, peritoneal macrophages, bone marrow macrophages, lymphatic macrophages, brain microglial cells, and dendritic macrophages all have specialized functions. Macrophage-secreted products include lysozyme, neutral proteases, acid hydrolases, arginase, complement components, enzyme inhibitors (plasmin, *###2-macroglobulin), binding proteins (transferrin, fibronectin, transcobalamin II), nucleosides, and cytokines (TNF-*, IL-1, -8, -12, -18). IL-1 (Chaps. 17 and 308) has many functions, including initiating fever in the hypothalamus, mobilizing leukocytes from the bone marrow, and activating lymphocytes and neutrophils. TNF-* is a pyrogen that duplicates many of the actions of IL-1 and plays an important role in the pathogenesis of gram-negative shock (Chap. 265). TNF-* stimulates production of hydrogen peroxide and related toxic oxygen species by macrophages and neutrophils. In addition, TNF-* induces catabolic changes that contribute to the profound wasting (cachexia) associated with many chronic diseases.

Other macrophage-secreted products include reactive oxygen and nitrogen metabolites, bioactive lipids (arachidonic acid metabolites and platelet-activating factors), chemokines, CSFs, and factors stimulating fibroblast and vessel proliferation. Macrophages help regulate the replication of lymphocytes and participate in the killing of tumors, viruses, and certain bacteria (*Mycobacterium tuberculosis* and *Listeria monocytogenes*). Macrophages are key effector cells in the elimination of intracellular microorganisms. Their ability to fuse to form giant cells that coalesce into granulomas in response to some inflammatory stimuli is important in the elimination of intracellular microbes and is under the control of IFN-*.* Nitric oxide induced by IFN-* is an im-
PORTANT EFFECTOR AGAINST INTRACELLULAR PARASITES, INCLUDING TUBERCULOSIS AND LEISHMANIA.

MACROPHAGES PLAY AN IMPORTANT ROLE IN THE IMMUNE RESPONSE (CHAP. 308). THEY PROCESS AND PRESENT ANTIGEN TO LYMPHOCYTES AND SECRETE CYTOKINES THAT MODULATE AND DIRECT LYMPHOCYTE DEVELOPMENT AND FUNCTION. MACROPHAGES PARTICIPATE IN AUTOIMMUNE PHENOMENA BY REMOVING IMMUNE COMPLEXES AND OTHER SUBSTANCES FROM THE CIRCULATION. POLYMORPHISMS IN MACROPHAGE RECEPTORS FOR IMMUNOGLOBULIN (FC*RII) DETERMINE SUSCEPTIBILITY TO SOME INFECTIONS AND AUTOIMMUNE DISEASES. IN WOUND HEALING, THEY DISPOSE OF SENESCENT CELLS, AND THEY CONTRIBUTE TOATHEROMA DEVELOPMENT. MACROPHAGE ELASTASE MEDIATES DEVELOPMENT OF EMPHYSEMA FROM CIGARETTE SMOKING.

DISORDERS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

MANY DISORDERS OF NEUTROPHILS EXTEND TO MONONUCLEAR PHAGOCYTES. Thus, drugs that suppress neutrophil production in the bone marrow can cause monocytopenia. Transient monocytopenia occurs after stress or glucocorticoid administration. Monocytosis is associated with tuberculosis, brucellosis, subacute bacterial endocarditis, Rocky Mountain spotted fever, malaria, and visceral leishmaniasis (Kala Azar). Monocytosis also occurs with malignancies, leukemias, myeloproliferative syndromes, hemolytic anemias, chronic idiopathic neutropenias, and granulomatous diseases such as sarcoidosis, regional enteritis, and some collagen vascular diseases. Patients with LAD, hyperimmunoglobulin E-recurrent infection (Job’s) syndrome, CHS, and CGD all have defects in the mononuclear phagocyte system. Monocyte cytokine production or response is impaired in some patients with disseminated nontuberculous mycobacterial infection who are not infected with HIV. Genetic defects in the pathways regulated by IFN-* and IL-12 lead to impaired killing of intracellular bacteria, mycobacteria, salmonellae, and certain viruses (FIG.61-10). Certain viral infections impair mononuclear phagocyte function. For example, influenza virus infection causes abnormal monocyte chemotaxis. Mononuclear phagocytes can be infected by HIV using CCR5, the chemokine receptor that acts as a co-receptor with CD4 for HIV. T lymphocytes produce IFN-* which induces FcR expression and phagocytosis and stimulates hydrogen peroxide production by mononuclear phagocytes and neutrophils. In certain diseases, such as AIDS, IFN-* production may be deficient. While in other diseases, such as T cell lymphomas, excessive release of IFN-* may be associated with erythrophagocytosis by splenic macrophages. Autoinflammatory diseases are characterized by abnormal cytokine regulation leading to excess inflammation in the absence of infection. These diseases can mimic infectious or immunodeficient syndromes. Gain-of-function mutations in the TNF-* receptor cause TNF-* receptor-associated periodic syndrome (TRAPS), which is characterized by recurrent fever in the absence of infection, due to persistent stimulation.
OF THE TNF-\* RECEPTOR (CHAP. 323). DISEASES WITH ABNORMAL IL-1 REGULA-
TION LEADING TO FEVER INCLUDE FAMILIAL MEDITERRANEAN FEVER DUE TO
MUTA-
tIONS IN PYRIN. MUTATIONS IN COLD-INDUCED AUTOINFLAMMATORY SYNDROME 1
LEAD TO NEONATAL ONSET MULTISYSTEM AUTOINFLAMMATORY DISEASE,
FAMILIAL

383 CHAPTER 61 DISORDERS OF GRANULOCYTES AND MONOCYTES

FIGURE 61-10 LYMPHOCYTE-MACROPHAGE INTERACTIONS UNDERLYING
RESISTANCE TO MYCOBACTERIA AND OTHER INTRACELLULAR PARASITES SUCH
AS
SALMONELLA. MYCOBACTERIA INFECT MACROPHAGES, LEADING TO THE PRODUC-
TION OF IL-12, WHICH ACTIVATES T OR NK CELLS THROUGH ITS RECEPTOR,
LEADING
TO PRODUCTION OF IL-2 AND IFN-\*.* IFN-\* ACTS THROUGH ITS RECEPTOR ON
MACROPHAGES TO UPREGULATE TNF-\* AND IL-12 AND KILL INTRACELLULAR PARA-
SITES. MUTANT FORMS OF THE CYTOKINES AND RECEPTORS SHOWN IN LARGE
TYPE
HAVE BEEN FOUND IN SEVERE CASES OF NONTUBERCULOUS MYCOBACTERIAL IN-
FECTION AND SALMONELLOSIS.

COLD URTICARIA, AND MUCKLE-WELLS SYNDROME. PYODERMA GANGRENOSUM,
ACNE, AND STERILE PYOGENIC ARTHRITIS IS CAUSED BY MUTATIONS IN CD2BP1.
IN CONTRAST TO THESE SYNDROMES OF OVEREXPRESSION OF PROINFLAMMATORY
CYTOKINES, BLOCKADE OF TNF-\* BY THE ANTAGONISTS INFLIXIMAB, ETANERCEPT,
AND ADALIMUMAB HAS BEEN ASSOCIATED WITH SEVERE INFECTIONS DUE TO TU-
BERCULOSIS, NONTUBERCULOUS MYCOBACTERIA, AND FUNGI (CHAP. 323).
MONOCYTOPENIA OCCURS WITH ACUTE INFECTIONS, WITH STRESS, AND AFTER
TREATMENT WITH GLUCOCORTICOIDS. MONOCYTOPENIA ALSO OCCURS IN
APLASTIC
ANEMIA, HAIRY CELL LEUKEMIA, ACUTE MYELOID LEUKEMIA, AND AS A DIRECT
RESULT OF MYELOTOXIC DRUGS.

EOSINOPHILS

EOSINOPHILS AND NEUTROPHILS SHARE SIMILAR MORPHOLOGY, MANY LYSOSO-
MAL CONSTITUENTS, PHAGOCYTIC CAPACITY, AND OXIDATIVE METABOLISM. EO-
SINOPHILS EXPRESS A SPECIFIC CHEMOATTRACTANT RECEPTOR AND RESPOND TO
A
SPECIFIC CHEMKINE, EOTAXIN. LITTLE IS KNOWN ABOUT THE ROLE OF EOSINO-
PHILS. EOSINOPHILS ARE MUCH LONGER LIVED THAN NEUTROPHILS, AND UNLIKE
NEUTROPHILS, TISSUE EOSINOPHILS CAN RECIRCULATE. DURING MOST INFEC-
TIONS, EOSINOPHILS ARE NOT IMPORTANT. HOWEVER, IN INVASIVE HELMINTHIC
INFECTIONS, SUCH AS HOOKWORM, SCHISTOSOMIASIS, STRONGYLOIDIASIS, TOXO-
CARIASIS, TRICHINOSIS, FILERIASIS, ECHINOCOCCOSIS, AND CYSTICERCOSIS, THE
EO-
SINOPHIL PLAYS A CENTRAL ROLE IN HOST DEFENSE. EOSINOPHILS ARE
ASSOCIATED
WITH BRONCHIAL ASTHMA, CUTANEOUS ALLERGIC REACTIONS, AND OTHER HYPER-
SENSITIVITY STATES.

THE DISTINCTIVE FEATURE OF THE RED-STAINING (WRIGHT’S STAIN) EOSINOPHIL GRANULE IS ITS CRYSTALLINE CORE CONSISTING OF AN ARGinine-RICH PROTEIN (MAJOR BASIC PROTEIN) WITH HISTAMINASE ACTIVITY, IMPORTANT IN HOST DEFENSE AGAINST PARASITES. EOSINOPHIL GRANULES ALSO CONTAIN A UNIQUE EOSINOPHIL PEROXIDASE THAT CATALYZES THE OXIDATION OF MANY SUBSTANCES BY HYDROGEN PEROXIDE AND MAY FACILITATE KILLING OF MICROORGANISMS. EOSINOPHIL PEROXIDASE, IN THE PRESENCE OF HYDROGEN PEROXIDE AND HALIDE, INITIATES MAST CELL SECRETION IN VITRO AND THEREBY PROMOTES INFLAMMATION. EOSINOPHILS CONTAIN CATIONIC PROTEINS, SOME OF WHICH BIND TO HEPARIN AND REDUCE ITS ANTICOAGULANT ACTIVITY. EOSINOPHIL-DErived NEUROTOXIN AND EOSINOPHIL CATIONIC PROTEIN ARE RIBONUCLEASES THAT CAN KILL RESPIRATORY SYNCYTIAL VIRUS. EOSINOPHIL CYTOPLASM CONTAINS CHARCOT-LEYDEN CRYSTAL PROTEIN, A HEXAGONAL BIPYRAMIDAL CRYSTAL FIRST OBSERVED IN A PATIENT WITH LEUKEMIA AND THEN IN SPUTUM OF PATIENTS WITH ASTHMA; THIS PROTEIN IS LYSOPHOSPHOLIPASE AND MAY FUNCTION TO DETOXIFY CERTAIN LYSOPHOSPHOLIPIDS. SEVERAL FACTORS ENHANCE THE EOSINOPHIL’S FUNCTION IN HOST DEFENSE. T CELL-DErIVED FACTORS ENHANCE THE ABILITY OF EOSINOPHILS TO KILL PARASITES. MAST CELL-DErived EOSINOPHIL CHEMOTACTIC FACTOR OF ANAPHYLAXIS (ECFA) INCREASES THE NUMBER OF EOSINOPHIL COMPLEMENT RECEPTORS AND ENHANCES EOSINOPHIL KILLING OF PARASITES. EOSINOPHIL CSFS (E.G., IL-5) PRODUCED BY MACROPHAGES INCREASE EOSINOPHIL PRODUCTION IN THE BONE MARROW AND ACTIVATE EOSINOPHILS TO KILL PARASITES.

EOSINOPHILIA

EOSINOPHILIA IS THE PRESENCE OF >500 EOSINOPHILS PER *L OF BLOOD AND IS COMMON IN MANY SETTINGS BESIDES PARASITE INFECTION. SIGNIFICANT TISSUE EOSINOPHILIA CAN OCCUR WITHOUT AN ELEVATED BLOOD COUNT. A COMMON CAUSE OF EOSINOPHILIA IS ALLERGIC REACTION TO DRUGS (IODIDES, ASPIRIN, SUL-FONAMIDES, NITROFURANTOIN, PENICILLINS, AND CEPAHLOSPORINS). ALLERGIES SUCH AS HAY FEVER, ASTHMA, ECZEMA, SERUM SICKNESS, ALLERGIC VASCULITIS, AND PEMPHIGUS ARE ASSOCIATED WITH EOSINOPHILIA. EOSINOPHILIA ALSO OCCURS IN COLLAGEN VASCULAR DISEASES (E.G., RHEUMATOID ARTHRITIS, EOSINOPHILIC FASCITIS, ALLERGIC ANGIITIS, AND PERIARTERITIS NODOSA) AND MALIGNANCIES (E.G., HODGKIN’S DISEASE; MYCOSIS FUNGOIDES; CHRONIC MYELOID LEUKEMIA; AND CANCER OF THE LUNG, STOMACH, PANCREAS, OVARY, OR UTERUS), AS WELL AS IN JOB’S SYNDROME AND CGD. EOSINOPHILIA IS COMMONLY PRESENT IN THE HEL-MINTHIC INFECTIONS. IL-5 IS THE DOMINANT EOSINOPHIL GROWTH FACTOR. THERAPEUTIC ADMINISTRATION OF THE CYTOKINES IL-2 AND GM-CSF FREQUENTLY LEADS TO TRANSIENT EOSINOPHILIA. THE MOST DRAMATIC HYPEREOSINOPHILIC SYNDROMES ARE LOEFFLer’S SYNDROME, TROPICAL PULMONARY EOSINOPHILIA, LOEFFLer’S ENDOCARDITIS, EOSINOPHILIC LEUKEMIA, AND IDIOPATHIC HYPEREOSINO-HILIC SYNDROME (50,000-100,000/*L). THE IDIOPATHIC HYPEREOSINOPHILIC SYNDROME REPRESENTS A HETEROGE-
NEOUS GROUP OF DISORDERS WITH THE COMMON FEATURE OF PROLONGED EOSINO-
PHILIA OF UNKNOWN CAUSE AND ORGAN SYSTEM DYSFUNCTION, INCLUDING THE
HEART, CENTRAL NERVOUS SYSTEM, KIDNEYS, LUNGS, GASTROINTESTINAL TRACT,
AND SKIN. THE BONE MARROW IS INVOLVED IN ALL AFFECTED INDIVIDUALS, BUT THE
MOST SEVERE COMPLICATIONS INVOLVE THE HEART AND CENTRAL NERVOUS SYS-
TEM. CLINICAL MANIFESTATIONS AND ORGAN DYSFUNCTION ARE HIGHLY
VARIABLE.

EOSINOPHILS ARE FOUND IN THE INVOLVED TISSUES AND LIKELY CAUSE TISSUE
DAMAGE BY LOCAL DEPOSITION OF TOXIC EOSINOPHIL PROTEINS SUCH AS EOSINO-
PHIL CATIONIC PROTEIN AND MAJOR BASIC PROTEIN. IN THE HEART, THE PATHO-
LOGIC CHANGES LEAD TO THROMBOSIS, ENDOCARDIAL FIBROSIS, AND
RESTRICTIVE ENDOMYOCARDIOPATHY. THE DAMAGE TO TISSUES IN OTHER ORGAN SYSTEMS IS
SIMILAR. SOME CASES ARE DUE TO MUTATIONS INVOLVING THE PLATELET-
DERIVED GROWTH FACTOR RECEPTOR, AND THESE ARE EXTREMELY SENSITIVE TO THE
TYROSINE KINASE INHIBITOR IMATINIB. GLUCOCORTICOIDS, HYDROXYUREA, AND IFN-*
EACH HAVE BEEN USED SUCCESSFULLY, AS HAVE THERAPEUTIC ANTIBODIES AGAINST
IL-5. CARDIOVASCULAR COMPLICATIONS ARE MANAGED AGGRESSIVELY.

THE EOSINOPHILIA-MYALGIA SYNDROME IS A MULTISYSTEM DISEASE, WITH
PROMINENT CUTANEOUS, HEMATOLOGIC, AND VISCERAL MANIFESTATIONS, THAT
FREQUENTLY EVOLVES INTO A CHRONIC COURSE AND CAN OCCASIONALLY BE
FATAL.

THE SYNDROME IS CHARACTERIZED BY EOSINOPHILIA (EOSINOPHIL COUNT
>1000/*L) AND GENERALIZED DISABLING MYALGIAS WITHOUT OTHER RECOG-
NIZED CAUSES. EOSINOPHILIC FASCIITIS, PNEUMONITIS, AND MYOCARDITIS;
NEUROPATHY CULMINATING IN RESPIRATORY FAILURE; AND ENCEPHALOPATHY
MAY OCCUR. THE DISEASE IS CAUSED BY INGESTING CONTAMINANTS IN L-TRYP-
TOPHAN-CONTAINING PRODUCTS. EOSINOPHILS, LYMPHOCYTES, MACROPHAGES,
AND FIBROBLASTS ACCUMULATE IN THE AFFECTED TISSUES, BUT THEIR ROLE IN
PATHOGENESIS IS UNCLEAR. ACTIVATION OF EOSINOPHILS AND FIBROBLASTS
AND THE DEPOSITION OF EOSINOPHIL-DERIVED TOXIC PROTEINS IN AFFECTED TISSUES
MAY CONTRIBUTE. IL-5 AND TRANSFORMING GROWTH FACTOR * HAVE BEEN IM-
PPLICATED AS POTENTIAL MEDIATORS. TREATMENT IS WITHDRAWAL OF PRODUCTS
CONTAINING L-TRYPTOPHAN AND THE ADMINISTRATION OF GLUCOCORTICOIDS.
MOST PATIENTS RECOVER FULLY, REMAIN STABLE, OR SHOW SLOW RECOVERY,
BUT THE DISEASE CAN BE FATAL IN UP TO 5% OF PATIENTS.

EOSINOPENIA

EOSINOPENIA OCCURS WITH STRESS, SUCH AS ACUTE BACTERIAL INFECTION, AND
AFTER TREATMENT WITH GLUCOCORTICOIDS. THE MECHANISM OF EOSINOPENIA

PAGE NO. 30

384 PART 2 CARDINAL MANIFESTATIONS AND PRESENTATION OF
DISEASES

OF ACUTE BACTERIAL INFECTION IS UNKNOWN BUT IS INDEPENDENT OF ENDOGENOUS GLUCOCORTICOIDS, SINCE IT OCCURS IN ANIMALS AFTER TOTAL ADRENALECTOMY. THERE IS NO KNOWN ADVERSE EFFECT OF EOSINOPENIA.

HYPERIMMUNOGLOBULIN E-RECURRENT INFECTION SYNDROME

THE HYPERIMMUNOGLOBULIN E-RECURRENT INFECTION SYNDROME, OR JOB’S SYNDROME, IS A RARE MULTISYSTEM DISEASE IN WHICH THE IMMUNE SYSTEM, BONE, TEETH, LUNG, AND SKIN ARE AFFECTED. ABNORMAL CHEMOTAXIS IS A VARIABLE FEATURE. THE MOLECULAR BASIS FOR THIS SYNDROME IS STILL NOT KNOWN, BUT SOME CASES SHOW CLEAR Autosomal Dominant Transmission With Linkage to 4Q. PATIENTS WITH THIS SYNDROME HAVE CHARACTERISTIC FEATURES WITH BROAD NOSE, KYPHOSCOLIOSIS AND OSTEOPOROSIS, AND ECZEMA. THE PRIMARY TEETH Erupt Normally BUT DO NOT DECIDUATE, OFTEN REQUIRING EXTRACTION. PATIENTS DEVELOP RECURRENT SINOPULMONARY AND CUTANEOUS INFECTIONS THAT TEND TO BE MUCH LESS INFLAMED THAN APPROPRIATE FOR THE DEGREE OF INFECTION AND HAVE BEEN REFERRED TO AS “COLD ABSCESSES.” A HIGH DEGREE OF SUSPICION IS REQUIRED TO DIAGNOSE INFECTIONS IN THESE PATIENTS, WHO MAY APPEAR WELL DESPITE EXTENSIVE DISEASE. THE COLD ABSCESSES HAVE BEEN CONSIDERED A REFLECTION OF TOO FEW PHAGOCYTES ARRIVING TOO LATE, PERHAPS DUE TO A LYMPHOCYTE FACTOR INHIBITING CHEMOTAXIS. HOWEVER, THE CHEMOTACTIC DEFECT IN THESE PATIENTS IS VARIABLE, AND THE FUNDAMENTAL BASIS FOR THE IMPAIRED DEFENSES IS COMPLEX AND POORLY DEFINED.

LABORATORY DIAGNOSIS AND MANAGEMENT

INITIAL STUDIES OF WBC AND DIFFERENTIAL AND OFTEN A BONE MARROW EXAMINATION MAY BE FOLLOWED BY ASSESSMENT OF BONE MARROW RESERVES (STEROID CHALLENGE TEST), MARGINATED CIRCULATING POOL OF CELLS (EPINEPHRINE CHALLENGE TEST), AND MARGINATING ABILITY (ENDOTOXIN CHALLENGE TEST) (FIG. 61-7). IN VIVO ASSESSMENT OF INFLAMMATION IS POSSIBLE WITH A REBUCK SKIN WINDOW TEST OR AN IN VIVO SKIN BLISTER ASSAY, WHICH MEASURES THE ABILITY OF LEUKOCYTES AND INFLAMMATORY MEDIATORS TO ACCUMULATE LOCALLY IN THE SKIN. IN VITRO TESTS OF PHAGOCYTE AGGREGATION, ADHERENCE, CHEMOTAXIS, PHAGOCYTOSIS, DEGRANULATION, AND MICROBICIDAL ACTIVITY (FOR S. AUREUS) MAY HELP PINPOINT CELLULAR OR HUMORAL LESIONS. DEFICIENCIES OF OXIDATIVE METABOLISM ARE DETECTED WITH EITHER THE NITROBLUE TETRAZOLIUM (NBT) DYE TEST OR THE DIHYDRORHODAMINE (DHR) OXIDATION TEST. THESE TESTS ARE BASED ON THE ABILITY OF PRODUCTS OF OXIDATIVE METABOLISM TO ALTER THE OXIDATION STATES OF REPORTER MOLECULES SO THAT
THEY CAN BE DETECTED MICROSCOPICALLY (NBT) OR BY FLOW CYTOMETRY (DHR). QUALITATIVE STUDIES OF SUPEROXIDE AND HYDROGEN PEROXIDE PRODUCTION MAY FURTHER DEFINE NEUTROPHIL OXIDATIVE FUNCTION. PATIENTS WITH LEUKOPENIAS OR LEUKOCYTE DYSFUNCTION OFTEN HAVE DELAYED INFLAMMATORY RESPONSES. THEREFORE, CLINICAL MANIFESTATIONS MAY BE MINIMAL DESPITE OVERWHELMING INFECTION, AND UNUSUAL INFECTIONS MUST ALWAYS BE SUSPECTED. EARLY SIGNS OF INFECTION DEMAND PROMPT, AGGRESSIVE CULTURING FOR MICROORGANISMS, USE OF ANTIBIOTICS, AND SURGICAL DRAINAGE OF ABSCESSES. PROLONGED COURSES OF ANTIBIOTICS ARE OFTEN REQUIRED. IN PATIENTS WITH CGD, PROPHYLACTIC ANTIBIOTICS (TRIMETHOPRIM-SULFAMETHOXAZOLE) AND ANTIFUNGALS (ITRACONAZOLE) MARKedly DIMinish THE FREQUENCY OF LIFE-THREATENING INFECTIONS. SHORT COURSES OF GLUCOCORTICOIDs MAY RELIEVE GASTROINTESTINAL OR GENITOURINARY TRACT OBSTRUCTION BY GRANULOMAS IN PATIENTS WITH CGD. RECOMBINANT HUMAN IFN-*, WHICH NONSPECIFICALLY STIMULATES PHAGOCYTIC CELL FUNCTION, REDUCES THE FREQUENCY OF INFECTIONS IN PATIENTS WITH CGD BY 70% AND REDUCES THE SEVERITY OF INFECTION. THIS EFFECT OF IFN-* IN CGD IS ADDITIVE TO THE EFFECT OF PROPHYLACTIC ANTIBIOTICS. THE RECOMMENDED DOSE IS 50 *G/M###2 SUBCUTANEOUSLY THREE TIMES WEEKLY. IFN-* HAS ALSO BEEN USED SUCCESSFULLY IN THE TREATMENT OF LEPROSY, NONTUBERCULOUS MYCOBACTERIA, AND VISCERAL LEISHMANIASIS. RIGOROUS ORAL HYGIENE REDuces BUT DOES NOT ELIMINATE THE DISCOMFORT OF GINGIVITIS, PERIODONTAL DISEASE, AND APHTHOUS ULCERS; CHLORHEXIDINE MOUTHWASH AND TOOTH BRUSHING WITH A HYDROGEN PEROXIDE-SODIUM BICARBONATE PASTE HELPS MANY PATIENTS. ORAL ANTIFUNGAL AGENTS (FLUCONAZOLE OR ITRACONAZOLE) HAVE REDUCED MUCOCUTANEOUS CANDIDIASIS IN PATIENTS WITH JOB’S SYNDROME. ANDROGENS, GLUCOCORTICOIDs, LITHIUM, AND IMMUNOSUPPRESSIVE THERAPY HAVE BEEN USED TO RESTORE MYELOPOIESIS IN PATIENTS WITH NEUTROPENIA DUE TO IMPAIRED PRODUCTION. RECOMBINANT G-CSF IS USEFUL IN THE MANAGEMENT OF CERTAIN FORMS OF NEUTROPENIA DUE TO DEPRESSED NEUTROPHIL PRODUCTION, ESPECIALLY THOSE RELATED TO CANCER CHEMOTHERAPY. PATIENTS WITH CHRONIC NEUTROPENIA WITH EVIDENCE OF A GOOD BONE MARROW RESERVE NEED NOT RECEIVE PROPHYLACTIC ANTIBIOTICS. PATIENTS WITH CHRONIC OR CYCLIC NEUTROPHIL COUNTS < 500/*L MAY BENEFIT FROM PROPHYLACTIC ANTIBIOTICS AND G-CSF DURING PERIODS OF NEUTROPENIA. ORAL TRIMETHOPRIM-SULFAMETHOXAZOLE (160/800 MG) TWICE DAILY CAN PREVENT INFECTION. INCREASED NUMBERS OF FUNGAL INFECTIONS ARE NOT SEEN IN PATIENTS WITH CGD ON THIS REGIMEN. ORAL QUINOLONES SUCH AS LEVOFLOXACIN AND CIPROFLOXACIN ARE ALTERNATIVES.
IN THE SETTING OF CYTOTOXIC CHEMOTHERAPY WITH SEVERE, PERSISTENT NEUTROPENIA, TRIMETHOPRIM-SULFAMETHOXAZOLE PREVENTS PNEUMOCYSTIS JIROVECI PNEUMONIA. THESE PATIENTS, AND PATIENTS WITH PHAGOCYTIC CELL DYSFUNCTION, SHOULD AVOID HEAVY EXPOSURE TO AIRBORNE SOIL, DUST, OR DECAYING MATTER (MULCH, MANURE), WHICH ARE OFTEN RICH IN NOCARDIA AND THE SPORES OF ASPERGILLUS AND OTHER FUNGI. RESTRICTION OF ACTIVITIES OR SOCIAL CONTACT HAS NO PROVEN ROLE IN REDUCING RISK OF INFECTION. CURE OF SOME CONGENITAL PHAGOCYTE DEFECTS IS POSSIBLE BY BONE MARROW TRANSPLANTATION (CHAP. 108). HOWEVER, COMPLICATIONS OF BONE MARROW TRANSPLANTATION ARE STILL SERIOUS, AND WITH RIGOROUS MEDICAL CARE MANY PATIENTS WITH PHAGOCYTIC DISORDERS CAN GO FOR YEARS WITHOUT A LIFE-THREATENING INFECTION. THE IDENTIFICATION OF SPECIFIC GENE DEFECTS IN PATIENTS WITH LAD 1, CGD, AND OTHER IMMUNODEFICIENCIES HAS LED TO GENE THERAPY TRIALS IN A NUMBER OF GENETIC WHITE CELL DISORDERS.

FURTHER READINGS

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INFORMATIVE EXERCISES A PHYSICIAN CAN PERFORM. WHILE ADVANCES IN AUTOMATED TECHNOLOGY HAVE MADE THE EXAMINATION OF THE PERIPHERAL BLOOD SMEAR BY THE PHYSICIAN SEEM LESS IMPORTANT, THE TECHNOLOGY IS NOT A COMPLETELY SATISFACTORY REPLACEMENT FOR BLOOD SMEAR INTERPRETATION BY A TRAINED MEDICAL PROFESSIONAL WHO ALSO KNOWS THE PATIENT’S CLINICAL HISTORY, FAMILY HISTORY, SOCIAL HISTORY, AND PHYSICAL FINDINGS. IT IS USEFUL TO ASK THE LABORATORY TO GENERATE A Wright’s-stained peripheral blood smear and to examine it.

The best place to examine blood cell morphology is the feathered edge of the blood smear where red cells lie in a single layer, side by side, just barely touching each other but not overlapping. My own approach is to look at the smallest cellular elements first, the platelets, and work my way up in size to red cells and then white cells. Using an oil immersion lens that magnifies the cells 100-fold, one first counts the platelets in five to six fields, averages the number per field, and multiplies by 20,000 to get a rough estimate of the platelet count. The platelets are usually 1-2 μm in diameter and have a blue granulated appearance. There is usually 1 platelet for every 20 or so red cells. Of course, the automated counter is much more accurate, but gross disparities between the automated and manual counts should be assessed. Large platelets may be a sign of rapid platelet turnover, as young platelets are often larger than old platelets; alternatively, certain rare inherited syndromes can produce large platelets. Platelet clumping visible on the smear can be associated with falsely low automated platelet counts. Similarly, neutrophil fragmentation can be a source of falsely elevated automated platelet counts.

Next one examines the red blood cells. One can gauge their size by comparing the red cell to the nucleus of a small lymphocyte. Both are normally about 8 μm wide. Red cells that are smaller than the small lymphocyte nucleus may be microcytic; those larger than the small lymphocyte nucleus may be macrocytic. The automated mean corpuscular volume (MCV) can assist in making a classification. However, some patients may have both iron and vitamin B12 deficiency, which will produce an MCV in the normal range but wide variation in red cell size. When the red cells vary greatly in size, anisocytosis is said to be present. When the red cells vary greatly in shape, poikilocytosis is said to be present.

After red cell size is assessed, one examines the hemoglobin content of the cells. They are either normal in color (normochromic) or they are pale in color (hypochromic). They are never “hyperchromic.” If more than the normal amount of hemoglobin is made, the cells get larger—they do not become darker. In addition to hemoglobin content, the red cells are examined for inclusions. Red cell inclusions are
THE FOLLOWING:

1. **Basophilic Stippling** - Diffuse fine or coarse blue dots in the red cell representing usually RNA residue-especially common in lead poisoning
2. **Howell-Jolly Bodies** - Dense blue circular inclusions that represent nuclear remnants-their presence implies defective splenic function
3. **Nuclei** - Red cells may be released or pushed out of the marrow prematurely before nuclear extrusion-often implies a myelophthisic process
4. **Parasites** - Red cell parasites include malaria and babesia (Chap. El8)

5. **Polydromatophilia** - The red cell cytoplasm has a bluish hue, reflecting the persistence of ribosomes still actively making hemoglobin in a young red cell

VITAL STAINS ARE NECESSARY TO SEE PRECIPITATED HEMOGLOBIN CALLED HEINZ BODIES.

RED CELLS CAN TAKE ON A VARIETY OF DIFFERENT SHAPES. ALL ABNORMALLY SHAPED RED CELLS ARE **POIKILOCYTES**. SMALL RED CELLS WITHOUT THE CENTRAL PAL-LOR ARE **SPHEROCYTES**; THEY CAN BE SEEN IN HEREDITARY SPHEROCYTOSIS, HEMOLYTIC ANEMIAS OF OTHER CAUSES, AND CLOSTROIDAL SEPSIS. **DACROCYTES** ARE TEARDROP-SHAPED CELLS THAT CAN BE SEEN IN HEMOLYTIC ANEMIAS, SEVERE IRON DEFICIENCY, THALASSEMIA, MYELOFIBROSIS, AND MYELODYSPLASTIC SYNDROMES. **SCHISTOCYTES** ARE HELMET-SHAPED CELLS THAT REFLECT MICROANGIOPATHIC HEMOLYTIC ANEMIA OR FRAGMENTATION ON AN ARTIFICIAL HEART VALVE.

**ECHINOCYTES** ARE SPICULATED RED CELLS WITH THE SPIKES EVENLY SPACED; THEY CAN REPRESENT AN ARTIFACT OF ABNORMAL DRYING OF THE BLOOD SMEAR OR REFLECT CHANGES IN STORED BLOOD. THEY CAN ALSO BE SEEN IN RENAL FAILURE AND MALNUTRITION AND ARE OFTEN REVERSIBLE. **ACANTHOCYTES** ARE SPICULATED RED CELLS WITH THE SPIKES IRREGULARLY DISTRIBUTED. THIS PROCESS TENDS TO BE IR-REVERSIBLE AND REFLECTS UNDERLYING RENAL DISEASE, ABETALIPOPROTEINEMIA, OR SPLENECTOMY. **ELLIPTOCYTES** ARE ELLIPTICAL-SHAPED RED CELLS THAT CAN REFLECT AN INHERITED DEFECT IN THE RED CELL MEMBRANE, BUT THEY ARE ALSO SEEN IN IRON DEFICIENCY, MYELODYSPLASTIC SYNDROME, MEGALOBLASTIC ANEMIA, AND THALASSEMIA. **STOMATOCYTES** ARE RED CELLS IN WHICH THE AREA OF CENTRAL PALLOM TAKES ON THE MORPHOLOGY OF A SLIT INSTEAD OF THE USUAL ROUND SHAPE. STOMATOCYTES CAN INDICATE AN INHERITED RED CELL MEMBRANE DEFECT AND CAN ALSO BE SEEN IN ALCOHOLISM. **TARGET CELLS** HAVE AN AREA OF CENTRAL PALLOM THAT CONTAINS A DENSE CENTER, OR BULL’S EYE. THESE CELLS ARE SEEN CLASSICALLY IN THALASSEMIA, BUT THEY ARE ALSO PRESENT IN IRON DEFICIENCY, CHOLESTATIC LIVER DISEASE, AND SOME HEMOGLOBINOPATHIES. THEY CAN ALSO BE GENERATED ARTIFACTUALLY BY IMPROPER SLIDE MAKING.
ONE LAST FEATURE OF THE RED CELLS TO ASSESS BEFORE MOVING TO THE WHITE BLOOD CELLS IS THE DISTRIBUTION OF THE RED CELLS ON THE SMEAR. IN MOST INDIVIDUALS, THE CELLS LIE SIDE BY SIDE IN A SINGLE LAYER. SOME PATIENTS HAVE RED CELL CLUMPING (CALLED AGGLUTINATION) IN WHICH THE RED CELLS PILE UPON ONE ANOTHER; IT IS SEEN IN CERTAIN PARAPROTEINEMIAS AND AUTOIMMUNE HEMOLYTIC ANEMIAS. ANOTHER ABNORMAL DISTRIBUTION INVOLVES RED CELLS LYING IN SINGLE CELL ROWS ON TOP OF ONE ANOTHER LIKE STACKS OF COINS. THIS IS CALLED ROULEAUX FORMATION AND REFLECTS ABNORMAL SERUM PROTEIN LEVELS.

FINALLY, ONE EXAMINES THE WHITE BLOOD CELLS. THREE TYPES OF GRANULOCYTES ARE USUALLY PRESENT; NEUTROPHILS, EOSINOPHILS, AND BASOPHILS, IN DECREASING FREQUENCY. NEUTROPHILS ARE GENERALLY THE MOST ABUNDANT WHITE CELL. THEY ARE ROUND, 10-14 *M WIDE, AND CONTAIN A LOBULATED NUCLEUS WITH TWO TO FIVE LOBES CONNECTED BY A THIN CHROMATIN THREAD. BANDS ARE IMMATURE NEUTROPHILS THAT HAVE NOT YET COMPLETED NUCLEAR CONDENSATION AND HAVE A U-SHAPED NUCLEUS. BANDS REFLECT A LEFT SHIFT IN NEUTROPHIL MATURATION IN AN EFFORT TO MAKE MORE CELLS MORE RAPIDLY. NEUTROPHILS CAN PROVIDE CLUES TO A VARIETY OF CONDITIONS. VACUOLATED NEUTROPHILS MAY BE A SIGN OF BACTERIAL SEPSIS. THE PRESENCE OF 1- TO 2-*M BLUE CYTOPLASMIC INCLUSIONS, CALLED DOHLE BODIES, CAN REFLECT INFECTIONS, BURNS, OR OTHER INFLAMMATORY STATES. IF THE NEUTROPHIL GRANULES ARE LARGER THAN NORMAL AND STAIN A DARKER BLUE, “TOXIC GRANULATIONS” ARE SAID TO BE PRESENT, AND THEY ALSO SUGGEST A SYSTEMIC INFLAMMATION. THE PRESENCE OF NEUTROPHILS WITH MORE THAN FIVE NUCLEAR LOBES SUGGESTS MEGALOBLASTIC ANEMIA. LARGE MISSHAPEN GRANULES MAY REFLECT THE INHERITED CHEDIAK-HIGASHI SYNDROME.

EOSINOPHILS ARE SLIGHTLY LARGER THAN NEUTROPHILS, HAVE BILOBED NUCLEI, AND CONTAIN LARGE RED GRANULES. DISEASES OF EOSINOPHILS ARE ASSOCIATED WITH TOO MANY OF THEM RATHER THAN ANY MORPHOLOGIC OR QUALITATIVE CHANGE. THEY NORMALLY TOTAL LESS THAN ONE-THIRTIETH THE NUMBER OF NEUTROPHILS. BASOPHILS ARE EVEN MORE RARE THAN EOSINOPHILS IN THE BLOOD. THEY HAVE LARGE DARK-BLUE GRANULES AND MAY BE INCREASED AS PART OF CHRONIC MYELOID LEUKEMIA. LYMPHOCYTES CAN BE PRESENT IN SEVERAL MORPHOLOGIC FORMS. MOST COMMON IN HEALTHY INDIVIDUALS ARE THE SMALL LYMPHOCYTES WITH A SMALL DARK NUCLEUS AND SCARCE CYTOPLASM. IN THE PRESENCE OF VIRAL INFECTIONS, MORE OF THE LYMPHOCYTES ARE LARGER, ABOUT THE SIZE OF NEUTROPHILS, WITH ABUNDANT CYTOPLASM AND A LESS CONDENSED NUCLEAR CHROMATIN. THESE
PART 3: GENETICS AND DISEASE

62 PRINCIPLES OF HUMAN GENETICS
J. LARRY JAMESON, PETER KOPP

IMPACT OF GENETICS ON MEDICAL PRACTICE

The beginning of the new millennium was marked by the announcement that the vast majority of the human genome had been sequenced. This milestone in the exploration of the human genome was preceded by numerous conceptual and technological advances. They include, among others, the elucidation of the DNA double-helix structure, the discovery of restriction enzymes and the polymerase chain reaction (PCR), the development and automatization of DNA sequencing, and the generation of genetic and physical maps by the human genome project (HGP). The consequences of this wealth of knowledge for the practice of medicine are profound. To date, the most significant impact of genetics has been to enhance our understanding of disease etiology and pathogenesis. However, genetics is rapidly playing a more prominent role in the diagnosis, prevention, and treatment of disease (Chap. 64). Genetic approaches have proven invaluable for the detection of infectious pathogens and are used clinically to identify agents that are difficult to culture such as mycobacteria, viruses, and parasites. In many cases, molecular genetics has improved the feasibility and accuracy of diagnostic testing and is beginning to open new avenues for therapy, including gene and cellular therapy (Chaps. 65 and 67). Molecular genetics has already significantly changed the treatment of human disease. Peptide hormones, growth factors, cytokines, and vaccines can now be produced in large amounts using recombinant DNA technology. Targeted modification of these peptides provides the practitioner with improved therapeutic tools, as illustrated by genetically modified insulin analogues with more favorable kinetics. There is hope that a better understanding of the genetic basis of human disease will also have an increasing impact on disease prevention.

Genetics has traditionally been viewed through the window of relatively rare single-gene diseases. Taken together, these disorders account for ~10% of pediatric admissions and childhood mortality. It is, however, increasingly apparent that virtually every medical condition, maybe with the exception of simple trauma, has a genetic component. As is often evident from a patient's family history, many common disorders such as hypertension, heart disease, asthma, diabetes mellitus, and mental illnesses are significantly influenced by the genetic background. These polygenic or multifactorial (complex) disorders

...
INVOLVE
THE CONTRIBUTIONS OF MANY DIFFERENT GENES, AS WELL AS ENVIRONMENTAL
FACTORS, THAT CAN MODIFY DISEASE RISK (CHAP. 64). A MAJOR CURRENT CHAL-
LENGE IS TO ELUCIDATE THE GENETIC COMPONENTS THAT CONTRIBUTE TO THE
PATHOGENESIS OF COMPLEX DISORDERS. THE RECENT PUBLICATION OF A COM-
PREHENSIVE CATALOGUE OF HUMAN SINGLE-NUCLEOTIDE POLYMORPHISM
(SNP) HAPLOTYPES, THE HAPMAP PROJECT, PROVIDES AN ESSENTIAL RESOURCE
FOR GENOME-WIDE ASSOCIATION STUDIES (SEE BELOW).
CANCER HAS A GENETIC BASIS SINCE IT RESULTS FROM ACQUIRED SOMATIC
MUTATIONS IN GENES CONTROLLING GROWTH, APOPTOSIS, AND CELLULAR DIFFER-
ENTIATION (CHAP. 79). IN ADDITION, THE DEVELOPMENT OF MANY CANCERS IS
ASSOCIATED WITH A HEREDITARY PREDISPOSITION. THE PREVALENCE OF GENETIC
DISEASES, COMBINED WITH THEIR SEVERITY AND CHRONIC NATURE, IMPOSES A
GREAT FINANCIAL, SOCIAL, AND EMOTIONAL BURDEN ON SOCIETY.
GENETICS HAS HISTORICALLY FOCUSED ON CHROMOSOMAL AND METABOLIC DIS-
ORDERS, REFLECTING THE LONG-STANDING AVAILABILITY OF TECHNIQUES TO
DIAG-
NOSE THESE CONDITIONS. FOR EXAMPLE, CONDITIONS SUCH AS TRISOMY 21
(DOWN
SYNDROME) OR MONOSOMY X (TURNER SYNDROME) CAN BE DIAGNOSED
USING CYTOGENETICS (CHAP. 63). LIKewise, MANY METABOLIC DISORDERS
(E.G., PHENYLKETONURIA, FAMILIAL HYPERCHOLESTEROLEMIA) ARE DIAGNOSED US-
ING BIOCHEMICAL ANALYSES. RECENT ADVANCES IN DNA DIAGNOSTICS HAVE EX-
TENDED THE FIELD OF GENETICS TO INCLUDE VIRTUALLY ALL MEDICAL
SPECIALTIES. IN
CARDIOLOGY, FOR EXAMPLE, THE MOLECULAR BASIS OF INHERITED
CARDIOMYOPA-
THIES AND ION CHANNEL DEFECTS THAT PREDISPOSE TO ARRHYTHMIAS IS BEING DE-
FINED (CHAPS. 226 AND 231). IN NEUROLOGY, GENETICS HAS UNMASKED THE
PATHOPHYSIOLOGY OF A STARTLING NUMBER OF NEURODEGENERATIVE
DISORDERS
(CHAP. 360). HEMATOLOGY HAS EVOLVED DRAMATICALLY, FROM ITS INCipient
GENETIC DESCRIPTIONS OF HEMOGLOBINOPATHIES TO THE CURRENT UNDERSTAND-
ING OF THE MOLECULAR BASIS OF RED CELL MEMBRANE DEFECTS, ClOTTING
DISOR-
DERS, AND THROMBOTIC DISORDERS (CHAPS. 99 AND 110).
NEW CONCEPTS DERIVED FROM GENETIC STUDIES CAN SOMETIMES CLARIFY
THE PATHOGENESIS OF DISORDERS THAT WERE PREVIOUSLY OPAQUE. FOR EXAM-
PLE, ALTHOUGH MANY DIFFERENT GENETIC DEFECTS CAN CAUSE PERIPHERAL
NEU-
ROPATHIES, DISRUPTION OF THE NORMAL FOLDING OF THE MYELIN SHEATHS IS
FREQUENTLY A COMMON FINAL PATHWAY (CHAP. 379). SEVERAL GENETIC CAUS-
ES OF OBESITY APPEAR TO CONVERGE ON A PHYSIOLOGIC PATHWAY THAT INVOLVES
PRODUCTS OF THE PRO OPIOMELANOCORTIN POLYPEPTIDE AND THE MC4R RE-
CEPTOR, THUS IDENTIFYING A KEY MECHANISM FOR APPETITE CONTROL (CHAP.
74). A SIMILAR PHENOMENON IS EMERGING FOR GENETICALLY DISTINCT FORMS
OF ALZHEIMER’S DISEASE, SEVERAL OF WHICH LEAD TO THE FORMATION OF NEU-
ROFIBRILLARY TANGLES (CHAP. 365). THE IDENTIFICATION OF DEFECTIVE GENES
OFTEN LEADS TO THE DETECTION OF CELLULAR PATHWAYS INVOLVED IN KEY
PHYSI-
OLOGIC PROCESSES. EXAMPLES INCLUDE IDENTIFICATION OF THE CYSTIC FIBROSIS CONDUCTANCE REGULATOR (CFTR) GENE; THE DUCHENNE MUSCULAR DYSTROPHY (DMD) GENE, WHICH ENCODES DYSTROPHIN; AND THE FIBROBLAST GROWTH FACTOR RECEPTOR-3 (FGFR3) GENE, WHICH IS RESPONSIBLE FOR ACHONDROPLASTIC DWARFISM. SIMILARLY, TRANSGENIC (OVER) EXPRESSION, AND TARGETED GENE “KNOCK-OUT” AND “KNOCK-IN” MODELS HELP TO UNRAVEL THE PHYSIOLOGIC FUNCTION OF GENES. THE ASTOUNDING RATE AT WHICH NEW GENETIC INFORMATION IS BEING GENERATED CREATES A MAJOR CHALLENGE FOR PHYSICIANS, HEALTH CARE PROVIDERS, AND BASIC INVESTIGATORS. THE TERMINOLOGY AND TECHNIQUES USED FOR DISCOVERY EVOLVE CONTINUOUSLY. MUCH GENETIC INFORMATION PRESENTLY RESIDES IN COMPUTER DATABASES OR IS BEING PUBLISHED IN BASIC SCIENCE JOURNALS. DATABASES PROVIDE EASY ACCESS TO THE EXPANDING INFORMATION ABOUT THE HUMAN GENOME, GENETIC DISEASE, AND GENETIC TESTING (TABLE 62-1). FOR EXAMPLE, SEVERAL THOUSAND MONOGENIC DISORDERS ARE SUMMARIZED IN A LARGE, CONTINUOUSLY EVOLVING COMPRENDIUM, REFERRED TO AS THE ONLINE MENDELIAN INHERITANCE IN MAN (OMIM) CATALOGUE (TABLE 62-1). THE ONGOING REFINEMENT OF BIOINFORMATICS IS SIMPLIFYING THE ACCESS TO THIS SEEMINGLY DAUNTING ONSLAUGHT OF NEW INFORMATION.

CHROMOSOMES AND DNA REPLICATION
ORGANIZATION OF DNA INTO CHROMOSOMES ∗ SIZE OF THE HUMAN GENOME

THE HUMAN GENOME IS DIVIDED INTO 23 DIFFERENT CHROMOSOMES, INCLUDING 22 AUTOSOMES (NUMBERED 1-22) AND THE X AND Y SEX CHROMOSOMES. ADULT CELLS ARE DIPLOID, MEANING THEY CONTAIN TWO HOMOLOGOUS SETS OF 22 AUTOSOMES AND A PAIR OF SEX CHROMOSOMES. FEMALES HAVE TWO X CHROMOSOMES (XX), WHEREAS MALES HAVE ONE X AND ONE Y CHROMOSOME (XY). AS A CONSEQUENCE OF MEIOSIS, GERM CELLS (SPERM OR OOCYTES) ARE HAPLOID AND CONTAIN ONE SET OF 22 AUTOSOMES AND ONE OF THE SEX CHROMOSOMES. AT THE TIME OF FERTILIZATION, THE DIPLOID GENOME IS RECONSTITUTED BY PAIRING OF THE HOMOLOGOUS CHROMOSOMES FROM THE MOTHER AND FATHER. WITH EACH CELL DIVISION (MITOSIS), CHROMOSOMES ARE REPLICATED, PAIRED, SEGREGATED, AND DIVIDED INTO TWO DAUGHTER CELLS (CHAP. 63). THE HUMAN GENOME IS ESTIMATED TO CONTAIN ~30,000-40,000 GENES, A SMALLER NUMBER THAN INITIALLY PREDICTED, THAT ARE DIVIDED AMONG THE 23 CHROMOSOMES. A GENE IS A FUNCTIONAL UNIT THAT IS REGULATED BY TRANSCRIPTION (SEE BELOW) AND ENCODES A RNA PRODUCT, WHICH IS MOST COMMONLY, BUT NOT ALWAYS, TRANSLATED INTO A PROTEIN THAT EXERTS ACTIVITY WITHIN OR OUTSIDE THE CELL. HISTORICALLY, GENES WERE IDENTIFIED BECAUSE THEY CONFERRED SPECIFIC TRAITS THAT ARE TRANSMITTED FROM ONE GENERATION TO THE NEXT. INCREASINGLY, THEY ARE CHARACTERIZED BASED ON EXPRESSION IN
# Part 3: Genetics and Disease

## Table 62-1 Selected Databases Relevant for Genomics and Genetic Disorders

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>National Center for Biotechnology Information (NCBI)</td>
<td></td>
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<tr>
<td>National Human Genome Research Institute</td>
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<tr>
<td>Ensembl Genome Browser</td>
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<tr>
<td>Online Mendelian Inheritance in Man</td>
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<tr>
<td>Office of Biotechnology Activities National Institutes of Health</td>
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<tr>
<td>American College of Medical Genetics</td>
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<tr>
<td>Cancer Genome Anatomy Project (CGAP)</td>
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<td>GenLink</td>
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<td>Genetests</td>
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<tr>
<td>Genomes Online Database (GOLD)</td>
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<tr>
<td>HUGO Gene Nomenclature</td>
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<tr>
<td>MITOMAP, A Human Mitochondrial Genome Database</td>
<td></td>
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<tr>
<td>Mitochondrial Disorders DNA Repeat Sequences &amp; Disease</td>
<td></td>
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<tr>
<td>Online Mendelian Inheritance in Animals (OMIA)</td>
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<tr>
<td>The Jackson Laboratory</td>
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<tr>
<td>International Hapmap Project</td>
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</table>
NUCLEAR RECEPTOR SIGNALING ATLAS
DOLAN DNA LEARNING CENTER, COLD SPRING HARBOR LABORATORIES
THE ONLINE METABOLIC AND MOLECULAR BASES OF INHERITED DISEASE (OMMBID)

URL

HTTP://WWW.NCBI.NLM.NIH.GOV/
HTTP://WWW.GENOME.GOV/
HTTP://WWW.ENSEMBL.ORG/
HTTP://WWW.NCBI.NLM.NIH.GOV/OMIM/
WWW4.OD.NIH.GOV/OBA/
HTTP://WWW.ACMG.NET/
HTTP://CGAP.NCI.NIH.GOV/
HTTP://WWW.GENIINK.WUSTL.EDU
HTTP://WWW.GENETESTS.ORG/
HTTP://WWW.GENOMESONLINE.ORG/
HTTP://WWW.GENE.UCL.AC.UK/NOMENCLATURE
HTTP://WWW.MITOMAP.ORG/
HTTP://WWW.NEURO.WUSTL.EDU/NEUROMUSCULAR/MITOSYN.HTML
HTTP://WWW.NEURO.WUSTL.EDU/NEUROMUSCULAR/MOTHER/DNAREP.HTM
HTTP://OMIA.ANGIS.ORG.AU/
HTTP://WWW.JAX.ORG/
HTTP://WWW.HAPMAP.ORG/
HTTP://NURSA.ORG
HTTP://WWW.DNAIC.ORG/
HTTP://GENETICS.ACCESSMEDICINE.COM

COMMENT
MOLECULAR BIOLOGY INFORMATION, PUBLIC DATABASES, COMPUTATIONAL BIOLOGY.
SOFTWARE FOR ANALYZING GENOME DATA.
EXTENSIVE LINKS TO OTHER DATABASES,
GENOME RESOURCES, AND TUTORIALS
WEB LINKS PROVIDING INFORMATION ABOUT
THE HUMAN GENOME SEQUENCE,
GENOMES OF OTHER ORGANISMS, AND
GENOMIC RESEARCH
MAPS AND SEQUENCE INFORMATION OF
EUCHARYOTIC GENOMES
ONLINE COMPENDIUM OF MENDELIAN DISORDERS AND HUMAN GENES CAUSING
GENETIC DISORDERS
INFORMATION ABOUT RECOMBINANT DNA
AND GENE TRANSFER
MEDICAL, ETHICAL, LEGAL, AND SOCIAL ISSUES
RAISED BY GENETIC TESTING
MEDICAL, ETHICAL, LEGAL, AND SOCIAL ISSUES
RAISED BY XENOTRANSPLANTATION
EXTENSIVE LINKS TO OTHER DATABASES RELEVANT FOR THE DIAGNOSIS, TREATMENT,
AND PREVENTION OF GENETIC DISEASE
INFORMATION ABOUT GENE EXPRESSION
PROFILES OF NORMAL, PRECANCER, AND
CANCER CELLS
MULTIMEDIA DATABASE RESOURCE FOR
HUMAN GENETICS AND TELOMERE
RESEARCH
INTERNATIONAL DIRECTORY OF GENETIC TESTING LABORATORIES AND PRENATAL
DIAGNOSIS CLINICS
REVIEWS AND EDUCATIONAL MATERIALS
INFORMATION ON PUBLISHED AND
UNPUBLISHED GENOMES
GENE NAMES AND SYMBOLS

A COMPENDIUM OF POLYMORPHISMS
AND MUTATIONS OF THE HUMAN
MITOCHONDRIAL DNA
OVERVIEW ON CLINICAL SYNDROMES
ASSOCIATED WITH MTDNA MUTATIONS
OVERVIEW ON CLINICAL SYNDROMES
ASSOCIATED WITH DNA REPEATS

ONLINE COMPENDIUM OF MENDELIAN DISORDERS IN ANIMALS
INFORMATION ABOUT MURINE MODELS AND THE MOUSE GENOME
CATALOGUE OF HAPLOTYPES IN DIFFERENT ETHNIC GROUPS RELEVANT FOR ASSOCIATION STUDIES AND PHARMACOGENOMICS
ATLAS OF NUCLEAR RECEPTORS, COREGULATORS, AND LIGANDS
EDUCATIONAL MATERIAL ABOUT SELECTED GENETIC DISORDERS, DNA, EUGENICS, AND GENETIC ORIGIN

ONLINE VERSION OF THE COMPREHENSIVE TEXT ON THE METABOLIC AND MOLECULAR BASES OF INHERITED DISEASE, 8E

**NOTE:** DATABASES ARE EVOLVING CONSTANTLY. PERTINENT INFORMATION MAY BE FOUND BY USING LINKS LISTED IN THE FEW SELECTED DATABASES. INSTRUCTIONS FOR THE USE OF GENOME-RELATED DATABASES HAVE BEEN PUBLISHED [NAT GENET 32(SUPPL): 1-79, 2002].

VARIOUS TISSUES. THE NUMBER OF GENES GREATLY UNDERESTIMATES THE COMPLEXITY OF GENETIC EXPRESSION, AS SINGLE GENES CAN GENERATE MULTIPLE SPliced MRNA PRODUCTS, WHICH ARE TRANSLATED INTO PROTEINS THAT ARE SUBJECT TO COMPLEX POSTTRANSLATIONAL MODIFICATION, SUCH AS PHOSPHORYLATION. **PROTEOMICS,** THE STUDY OF THE PROTEOME USING TECHNOLOGIES OF LARGE-SCALE PROTEIN SEPARATION AND IDENTIFICATION, IS AN EMERGING FIELD FOCUSED ON PROTEIN VARIATION AND FUNCTION. SIMILARLY, THE FIELD OF **METABOLOMICS** AIMS AT DETERMINING THE COMPOSITION AND MODIFICATIONS OF THE **METABOLOME,** THE COMPLEMENT OF LOW-MOLECULAR-WEIGHT MOLECULES, MANY OF WHICH PARTICIPATE IN VARIOUS METABOLIC FUNCTIONS. THESE ANALYSES, WHICH ARE HEAVILY DEPENDENT ON BIOINFORMATICS, REVEAL THAT PHYSIOLOGIC OR PATHOLOGIC ALTERATIONS HAVE MYRIAD EFFECTS ON THE PROTEOME AND THE METABOLOME AND EMPHASIZE THAT THESE PROCESSES INVOLVE **MODULAR NETWORKS** RATHER THAN **LINEAR PATHWAYS.**

HUMAN DNA CONSISTS OF ~3 BILLION BASE PAIRS (BP) OF DNA PER HAPLOID GENOME. DNA LENGTH IS NORMALLY MEASURED IN UNITS OF 1000 BP (KILOBASES, KB) OR 1,000,000 BP (MEGABASES, MB). NOT ALL DNA ENCODES GENES. IN FACT, GENES ACCOUNT FOR ONLY ~10-15% OF DNA. MUCH OF THE REMAINING DNA CONSISTS OF HIGHLY REPETITIVE SEQUENCES, THE FUNCTION OF WHICH IS POORLY UNDERSTOOD. THESE REPETITIVE DNA REGIONS, ALONG WITH NONREPEITIVE SEQUENCES THAT DO NOT ENCODE GENES, MAY SERVE A STRUCTURAL ROLE IN THE PACKAGING OF DNA INTO CHROMATIN, I.E., DNA BOUND TO HISTONE PROTEINS, AND CHROMOSOMES (**FIG. 62-1**). IF ONLY 10% OF DNA IS EXPRESSED AND THERE ARE 30,000 GENES, THE AVERAGE GENE WOULD BE ~10 KB IN LENGTH. ALTHOUGH MANY GENES ARE ABOUT THIS SIZE, THE RANGE IS QUITE BROAD. FOR EXAMPLE, SOME GENES ARE ONLY A FEW HUNDRED BP, WHEREAS OTHERS, SUCH AS THE **DMD** GENE, ARE EXTRAORDINARILY LARGE (2 MB).
**STRUCTURE OF DNA**  
Each gene is composed of a linear polymer of DNA. DNA is a double-stranded helix composed of four different bases: adenine (A), thymidine (T), guanine (G), and cytosine (C). Adenine is paired to thymidine, and guanine is paired to cytosine, by hydrogen bond interactions that span the double helix. DNA has several remarkable features that make it ideal for the transmission of genetic information. It is relatively stable, at least in comparison to RNA or proteins. The double-stranded nature of DNA and its feature of strict base-pair complementarity permit faithful replication during cell division. As described below, complementarity also allows the transmission of genetic information from DNA → protein (Fig. 62-2). Messenger RNA (mRNA) is encoded by the so-called sense or coding strand of the DNA double helix and is translated into proteins by ribosomes. The presence of four different bases provides surprising genetic diversity. In the protein-coding regions of genes, the DNA bases are arranged into codons, a triplet of bases that specifies a particular amino acid. It is possible to arrange the four bases into 64 different triplet codons (4###3). Each codon specifies 1 of the 20 different amino acids, or a regulatory signal, such as initiation and stop of translation. Because there are more codons than amino acids, the genetic code is degenerate; that is, most amino acids can be specified by several different codons. By arranging the codons in different combinations and in various lengths, it is possible to generate the tremendous diversity of primary protein structure.

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**387 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS**

**FIGURE 62-1 STRUCTURE OF CHROMATIN AND CHROMOSOMES.** Chromatin is composed of double-strand DNA that is wrapped around histone and nonhistone proteins forming nucleosomes. The nucleosomes are further organized into solenoid structures. Chromosomes assume their characteristic structure, with short (P) and long (Q) arms at the metaphase stage of the cell cycle.

**REPLICATION OF DNA AND MITOSES**  
Genetic information in DNA is transmitted to daughter cells under two different circumstances: (1) somat-
IC CELLS DIVIDE BY MITOSIS, ALLOWING THE DIPLOID \((2N)\) GENOME TO REPLICATE.

**FIGURE 62-2 FLOW OF GENETIC INFORMATION.**

MULTIPLE EXTRACELLULAR SIGNALS ACTIVATE INTRACELLULAR SIGNAL CASCADES THAT RESULT IN ALTERED REGULATION OF GENE EXPRESSION THROUGH THE INTERACTION OF TRANSCRIPTION FACTORS WITH REGULATORY REGIONS OF GENES. RNA POLYMERASE TRANSCRIPTS DNA INTO RNA THAT IS PROCESSED TO MRNA BY EXCISION OF INTRONIC SEQUENCES. THE MRNA IS TRANSLATED INTO A POLYPEPTIDE CHAIN TO FORM THE MATURE PROTEIN AFTER UNDERGOING POSTTRANSLATIONAL PROCESSING. HAT, HISTONE ACETYL TRANSFERASE; CBP, CREB-BINDING PROTEIN; CREB, CYCLIC AMP RESPONSE ELEMENT-BINDING PROTEIN; CRE, CYCLIC AMP RESPONSIVE ELEMENT; COA, CO ACTIVATOR; TAF, TBP-ASSOCIATED FACTORS; GTF, GENERAL TRANSCRIPTION FACTORS, TBP, TATA-BINDING PROTEIN. TATA, TATA BOX; RE, RESPONSE ELEMENT; NH###2. AMINOTERMINUS; COOH, CA RBOXYTERM INUS.

ITSELF COMPLETELY IN CONJUNCTION WITH CELL DIVISION; AND (2) GERM CELLS (SPERM AND OVA) UNDERGO MEIOSIS, A PROCESS THAT ENABLES THE REDUCTION OF THE DIPLOID \((2N)\) SET OF CHROMOSOMES TO THE HAPLOID STATE \((1N)\) (CHAP. 63).

PRIOR TO MITOSIS, CELLS EXIT THE RESTING, OR G###0 STATE, AND ENTER THE CELL CYCLE (CHAP. 80). AFTER TRAVERSING A CRITICAL CHECKPOINT IN G###1, CELLS UNDERGO DNA SYNTHESIS (S PHASE), DURING WHICH THE DNA IN EACH CHROMOSOME IS REPLICATED, YIELDING TWO PAIRS OF SISTER CHROMATIDS \((2N * 4N)\). THE PROCESS OF DNA SYNTHESIS REQUIRES STRINGENT FIDELITY IN ORDER TO AVOID TRANSMITTING ERRORS TO SUBSEQUENT GENERATIONS OF CELLS. GENETIC ABNORMALITIES OF DNA MISMATCH/REPAIR INCLUDE XERODERMA PIGMENTOSUM, BLOOM SYNDROME, ATAXIA TELANGIECTASIA, AND HEREDITARY NONPOLYPOSIS COLON CANCER (HNPPC), AMONG OTHERS. MANY OF THESE DISORDERS STRONGLY PREDISPOSE TO NEOPLASIA BECAUSE OF THE RAPID ACQUISITION OF ADDITIONAL MUTATIONS (CHAP. 79). AFTER COMPLETION OF DNA SYNTHESIS, CELLS ENTER G###2 AND PROGRESS THROUGH A SECOND CHECKPOINT BEFORE ENTERING MITOSIS. AT THIS STAGE, THE CHROMOSOMES CONDENSE AND ARE ALIGNED ALONG THE EQUATORIAL PLATE AT METAPHASE. THE TWO IDENTICAL SISTER CHROMATIDS, HELD TOGETHER AT THE CENTROMERE, DIVIDE AND MIGRATE TO OPPOSITE POLES OF THE CELL (SEE FIG. 63-3). AFTER FORMATION OF A NUCLEAR MEMBRANE AROUND THE TWO SEPARATED SETS OF CHROMATIDS, THE CELL DIVIDES AND TWO DAUGHTER CELLS ARE FORMED, THUS RESTORING THE DIPLOID \((2N)\) STATE.

**ASSORTMENT AND SEGREGATION OF GENES DURING MEIOSIS** MEIOSIS OCCURS ONLY IN GERM CELLS OF THE GONADS. IT SHARES CERTAIN FEATURES WITH MITOSIS BUT INVOLVES TWO DISTINCT STEPS OF CELL DIVISION THAT REDUCE THE
CHROMO-
SOME NUMBER TO THE HAPLOID STATE. IN ADDITION, THERE IS ACTIVE RECOMBI-
NATION THAT GENERATES GENETIC DIVERSITY. DURING THE FIRST CELL 
DIVISION, 
TWO SISTER CHROMATIDS (2N * 4N) ARE FORMED FOR EACH CHROMOSOME 
PAIR AND THERE IS AN EXCHANGE OF DNA BETWEEN HOMOLOGOUS PATERNAL 
AND MATERNAL CHROMOSOMES. THIS PROCESS INVOLVES THE FORMATION OF 
CHIASMATA, STRUCTURES THAT CORRESPOND TO THE DNA SEGMENTS THAT CROSS 
OVER BETWEEN THE MATERNAL AND PATERNAL HOMOLOGUES (FIG. 62-3). USU-
ALLY THERE IS AT LEAST ONE CROSSOVER ON EACH CHROMOSOMAL ARM;
RECOMB-
INATION OCCURS MORE FREQUENTLY IN FEMALE MEIOSIS THAN IN MALE MEIOSIS. 
SUBSEQUENTLY, THE CHROMOSOMES SEGREGATE RANDOMLY. BECAUSE THERE ARE 
23 CHROMOSOMES, THERE EXIST 2###23 (>8 MILLION) POSSIBLE COMBINATIONS 
OF CHROMOSOMES. TOGETHER WITH THE GENETIC EXCHANGES THAT OCCUR DUR-
ING RECOMBINATION, CHROMOSOMAL SEGREGATION GENERATES TREMENDOUS 
DIVERSITY, AND EACH GAMETE IS GENETICALLY UNIQUE. THE PROCESS OF 
RECOM-

388 PART 3: GENETICS AND DISEASE

FIGURE 62-3 CROSSING-OVER AND GENETIC RECOMBINATION. DURING 
CHIASMA FORMATION, EITHER OF THE TWO SISTER CHROMATIDS ON ONE CHRO-
MOSOME PAIRS WITH ONE OF THE CHROMATIDS OF THE HOMOLOGOUS CHRO-
MOSOME GENETIC RECOMBINATION OCCURS THROUGH CROSSING-OVER AND 
RESULTS IN RECOMBINANT AND NONRECOMBINANT CHROMOSOME SEGMENTS 
IN THE GAMETES. TOGETHER WITH THE RANDOM SEGREGATION OF THE MATER-
NAL AND PATERNAL CHROMOSOMES, RECOMBINATION CONTRIBUTES TO GENETIC 
DIVERSITY AND FORMS THE BASIS OF THE CONCEPT OF LINKAGE.

BINATION, AND THE INDEPENDENT SEGREGATION OF CHROMOSOMES, PROVIDE 
THE FOUNDATION FOR PERFORMING LINKAGE ANALYSES, WHEREBY ONE 
ATTEMPTS 
TO CORRELATE THE INHERITANCE OF CERTAIN CHROMOSOMAL REGIONS (OR 
LINKED 
GENES) WITH THE PRESENCE OF A DISEASE OR GENETIC TRAIT (SEE BELOW). 
AFTER THE FIRST MEIOTIC DIVISION, WHICH RESULTS IN TWO DAUGHTER CELLS 
(2N), THE TWO CHROMATIDS OF EACH CHROMOSOME SEPARATE DURING A SEC-
OND MEIOTIC DIVISION TO YIELD FOUR GAMETES WITH A HAPLOID STATE (1N). 
WHEN THE EGG IS FERTILIZED BY SPERM, THE TWO HAPLOID SETS ARE COM-
BINED, THEREBY RESTORING THE DIPLOID STATE (2N) IN THE ZYGOTE.

REGULATION OF GENE EXPRESSION

MECHANISMS THAT REGULATE GENE EXPRESSION PLAY A CRITICAL ROLE IN THE 
FUNCTION OF GENES. THE TRANSCRIPTION OF GENES IS CONTROLLED PRIMARILY 
BY TRANSCRIPTION FACTORS THAT BIND TO DNA SEQUENCES IN THE REGULATORY 
REGIONS OF GENES. AS DESCRIBED BELOW, MUTATIONS IN TRANSCRIPTION FAC-
TORS CAUSE A SIGNIFICANT NUMBER OF GENETIC DISORDERS. GENE EXPRESSION
IS ALSO INFLUENCED BY EPIGENETIC EVENTS, SUCH AS X-INACTIVATION AND IMPRINTING, PROCESSES IN WHICH DNA METHYLATION OR HISTONE MODIFICATIONS ARE ASSOCIATED WITH GENE SILENCING. SEVERAL GENETIC DISORDERS, SUCH AS PRADER-WILLI SYNDROME (NEONATAL HYPOTONIA, DEVELOPMENTAL DELAY, OBESITY, SHORT STATURE, AND HYPOGONADISM) AND ALBRIGHT HEREDITARY OSTEODYSTROPHY (RESISTANCE TO PARATHYROID HORMONE, SHORT STATURE, BRACHYDACTYLY, RESISTANCE TO OTHER HORMONES IN CERTAIN SUBTYPES), EXHIBIT THE CONSEQUENCES OF GENOMIC IMPRINTING. MOST STUDIES OF GENE EXPRESSION HAVE FOCUSED ON THE REGULATORY DNA ELEMENTS OF GENES THAT CONTROL TRANSCRIPTION. HOWEVER, IT SHOULD BE EMPHASIZED THAT GENE EXPRESSION REQUIRES A SERIES OF STEPS, INCLUDING MRNA PROCESSING, PROTEIN TRANSLATION, AND POSTTRANSLATIONAL MODIFICATIONS, ALL OF WHICH ARE ACTIVELY REGULATED (FIG. 62-2).

THE NEW FIELD OF FUNCTIONAL GENOMICS IS BASED ON THE CONCEPT THAT UNDERSTANDING ALTERATIONS OF GENE EXPRESSION UNDER VARIOUS PHYSIOLOGIC AND PATHOLOGIC CONDITIONS PROVIDES INSIGHT INTO THE UNDERLYING PROCESSES, AND BY REVEALING CERTAIN GENE EXPRESSION PROFILES, THIS KNOWLEDGE MAY BE OF DIAGNOSTIC AND THERAPEUTIC RELEVANCE. THE LARGE-SCALE STUDY OF EXPRESSION PROFILES, WHICH TAKES ADVANTAGE OF MICROARRAY TECHNOLOGIES, IS ALSO REFERRED TO AS TRANSCRIPTOMICS BECAUSE THE COMPLEMENT OF MRNAS TRANSCRIBED BY THE CELLULAR GENOME IS CALLED THE TRANSCRIPTOME.

STRUCTURE OF GENES A GENE PRODUCT IS USUALLY A PROTEIN BUT CAN OCCASIONALLY CONSIST OF RNA THAT IS NOT TRANSLATED (E.G., MICRORNAS). EXONS REFER TO THE PORTION OF GENES THAT ARE EVENTUALLY SPLICED TOGETHER TO FORM MRNA. INTRONS REFER TO THE SPACING REGIONS BETWEEN THE EXONS THAT ARE SPLICED OUT OF PRECURSOR RNAS DURING RNA PROCESSING (FIG. 62-2). THE GENE LOCUS ALSO INCLUDES REGIONS THAT ARE NECESSARY TO CONTROL ITS EXPRESSION. THE REGULATORY REGIONS MOST COMMONLY INVOLVE SEQUENCES UPSTREAM (5') OF THE TRANSCRIPTION START SITE, ALTHOUGH THERE ARE ALSO EXAMPLES OF CONTROL ELEMENTS WITHIN INTRONS OR DOWNSTREAM OF THE CODING REGIONS OF A GENE. THE UPSTREAM REGULATORY REGIONS ARE ALSO REFERRED TO AS THE PROMOTER. THE MINIMAL PROMOTER USUALLY CONSISTS OF A TATA BOX (WHICH BINDS TATA-BINDING PROTEIN, TBP) AND INITIATOR SEQUENCES THAT ENHANCE THE FORMATION OF AN ACTIVE TRANSCRIPTION COMPLEX. A GENE MAY GENERATE VARIOUS TRANSCRIPTS THROUGH THE USE OF ALTERNATIVE PROMOTERS AND/OR ALTERNATIVE SPLICING OF EXONS, MECHANISMS THAT CONTRIBUTE TO THE ENORMOUS DIVERSITY OF PROTEINS AND THEIR FUNCTIONS. TRANSCRIPTIONAL TERMINATION SIGNALS RESIDE DOWNSTREAM, OR 3', OF A GENE. SPECIFIC SEQUENCES, SUCH AS THE AAUAAA SEQUENCE AT THE 3' END OF THE MRNA, DESIGNATE THE SITE FOR POLYADENYLATION (POLY-A TAIL), A PROCESS THAT INFLUENCES MRNA TRANSPORT TO THE CYTOPLASM, STABILITY, AND TRANSLATION EFFICIENCY.
A rigorous test of the regulatory region boundaries involves expressing a gene in a transgenic animal to determine whether the isolated DNA flanking sequences are sufficient to recapitulate the normal developmental, tissue-specific, and signal-responsive features of the endogenous gene. This has been accomplished for only a few genes; there are many examples in which large genomic fragments only partially reconstruct normal gene regulation in vivo, implying the presence of distant regulatory sequences. Genome-wide analyses of selected transcription factor binding sites, such as for the estrogen receptor, reveal that the majority of regulatory sites are very distant from the transcription start sites of genes. A detailed understanding of mechanisms that regulate genes is also relevant for gene therapy strategies that require normal gene regulation (Chap. 65).

The number of DNA sequences and transcription factors that regulate transcription is much greater than originally anticipated. Most genes contain at least 15-20 discrete regulatory elements within 300 bp of the transcription start site. This densely packed promoter region often contains binding sites for ubiquitous transcription factors such as C/EBP, CREB, SP-1, or AP-1. However, factors involved in cell-specific expression may also bind to these sequences. For example, basic helix-loop-helix (bHLH) proteins bind to E-boxes in the promoters of myogenic genes, and steriodogenic factor 1 (SF-1) binds to a specific recognition site in the regulatory region of multiple steriodogenic enzyme genes. Key regulatory elements may also reside at a large distance from the proximal promoter. The globin and the immunoglobulin genes, for example, contain locus control regions that are several kilobases away from the structural sequences of the gene. Specific groups of transcription factors that bind to these promoter and enhancer sequences provide a combinatorial code for regulating transcription. In this manner, relatively ubiquitous factors interact with more restricted factors to allow each gene to be expressed and regulated in a unique manner that is dependent on developmental state, cell type, and numerous extracellular stimuli. As described below, the transcription factors that bind to DNA actually represent only the first level of regulatory control. Other proteins—coactivators and co-repressors—interact with the DNA-binding transcription factors to generate large regulatory complexes. These complexes are subject to control by numerous cell-signaling pathways, including phosphorylation, acetylation, sumoylation, and ubiquitinylation. Ultimately, the recruited transcription factors interact with, and stabilize, components of the

TRANSCRIPTIONAL ACTIVATION AND REPRESSION EVERY GENE IS CONTROLLED UNIQUELY, WHETHER IN ITS SPATIAL OR TEMPORAL PATTERN OF EXPRESSION OR IN ITS RESPONSE TO EXTRACELLULAR SIGNALS. IT IS ESTIMATED THAT TRANSCRIPTION FACTORS ACCOUNT FOR ~30% OF EXPRESSED GENES. A GROWING NUMBER OF IDENTIFIED GENETIC DISEASES INVOLVE TRANSCRIPTION FACTORS (TABLE 62-2). THE MODY (MATURITY-ON-SET DIABETES OF THE YOUNG) DISORDERS ARE REPRESENTATIVE OF THIS GROUP OF DISEASES; MUTATIONS IN SEVERAL DIFFERENT ISLET CELL-SPECIFIC TRANSCRIPTION FACTORS CAUSE VARIOUS FORMS OF MODY (CHAP. 338). TRANSCRIPTIONAL ACTIVATION CAN BE DI-
VIDED INTO THREE MAIN MECHANISMS:

1. EVENTS THAT ALTER CHROMATIN STRUCTURE CAN ENHANCE THE ACCESS OF TRANSCRIPTION FACTORS TO DNA. FOR EXAMPLE, HISTONE ACETYLATION GENERALLY OPENS CHROMATIN STRUCTURE AND IS CORRELATED WITH TRANSCRIPTIONAL ACTIVATION.

2. POSTTRANSLATIONAL MODIFICATIONS OF TRANSCRIPTION FACTORS, SUCH AS PHOSPHORYLATION, CAN INDUCE THE ASSEMBLY OF ACTIVE TRANSCRIPTION COMPLEXES. AS AN EXAMPLE, PHOSPHORYLATION OF CREB PROTEIN ON SERINE 133 INDUCES A CONFORMATIONAL CHANGE THAT ALLOWS THE RECRUITMENT OF CREB-BINDING PROTEIN (CBP), A FACTOR THAT INTEGRATES THE ACTIONS OF MANY TRANSCRIPTION FACTORS, INCLUDING PROTEINS, WITH HISTONE ACETYLTRANSFERASE ACTIVITY.

3. TRANSCRIPTIONAL ACTIVATORS CAN DISPLACE A REPRESSOR PROTEIN. THIS MECHANISM IS PARTICULARLY COMMON DURING DEVELOPMENT WHEN THE PATTERN OF TRANSCRIPTION FACTOR EXPRESSION CHANGES DYNAMICALLY.

OF COURSE, THESE MECHANISMS ARE NOT MUTUALLY EXCLUSIVE, AND MOST GENES ARE ACTIVATED BY SOME COMBINATION OF THESE EVENTS. SUPPRESSION OF GENE EXPRESSION IS AS IMPORTANT AS GENE ACTIVATION IN THE CONTROL OF CELL DIFFERENTIATION AND FUNCTION. SOME MECHANISMS OF REPRESSION ARE THE COROLLARY OF ACTIVATION. FOR EXAMPLE, REPRESSION IS OFTEN ASSOCIATED WITH HISTONE DEACETYLATION OR PROTEIN DEPHOSPHORYLATION. FOR NUCLEAR HORMONE RECEPTORS, TRANSCRIPTIONAL SILENCING INVOLVES THE RECRUITMENT OF REPRESSION COMPLEXES THAT CONTAIN HISTONE DEACETYLASE ACTIVITY. ABERRANT EXPRESSION OF REPRESSOR PROTEINS IS SOMETIMES ASSOCIATED WITH NEOPLASIA. THE T(15;17) CHROMOSOMAL TRANSLOCATION THAT OCCURS IN PROMYELOCYTIC LEUKEMIA FUSES THE PML GENE TO A PORTION OF THE RETINOIC ACID RECEPTOR * (RAR *) GENE (TABLE 62-2). THIS EVENT CAUSES UNREGULATED TRANSCRIPTIONAL REPRESSION IN A MANNER THAT PRECLUDES NORMAL CELLULAR DIFFERENTIATION. THE ADDITION OF THE RAR LIGAND, RETINOIC ACID, ACTIVATES THE RECEPTOR, THEREBY RELIEVING REPRESSION AND ALLOWING CELLS TO DIFFERENTIATE AND ULTIMATELY UNDERGO APOPTOSIS. THIS MECHANISM HAS THERAPEUTIC IMPORTANCE AS THE ADDITION OF RETINOIC ACID TO TREATMENT REGIMENS INDUCES A HIGHER REMISSION RATE IN PATIENTS WITH PROMYELOCYTIC LEUKEMIA (CHAP. 104). METHYLATION OF PROMOTER REGIONS IS FREQUENTLY FOUND IN NEOPLASMS AND SILENCES GENE EXPRESSION.

CLONING AND SEQUENCING DNA
A DESCRIPTION OF RECOMBINANT DNA TECHNIQUES, THE METHODOLOGY USED FOR THE MANIPULATION, ANALYSIS, AND CHARACTERIZATION OF DNA SEGMENTS, IS BEYOND THE SCOPE OF THIS CHAPTER. AS THESE METHODS ARE WIDE-

**FIGURE 62-5 A.** EXAMPLES OF MUTATIONS. THE CODING STRAND IS SHOWN WITH THE ENCODED AMINO ACID SEQUENCE. **B.** CHROMATOGRAMS OF SEQUENCE ANALYSES AFTER AMPLIFICATION OF GENOMIC DNA BY POLYMERASE CHAIN REACTION.

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**390 PART 3: GENETICS AND DISEASE**

**TABLE 62-2 SELECTED EXAMPLES OF DISEASES CAUSED BY MUTATIONS AND REARRANGEMENTS IN TRANSCRIPTION FACTOR CLASSES**

<table>
<thead>
<tr>
<th>TRANSCRIPTION FACTOR CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUCLEAR RECEPTORS</td>
</tr>
<tr>
<td>ZINC FINGER PROTEINS</td>
</tr>
<tr>
<td>BASIC HELIX-LOOP-HELIX</td>
</tr>
<tr>
<td>HOMEobox</td>
</tr>
<tr>
<td>LEUCINE ZIPPER</td>
</tr>
<tr>
<td>HIGH MOBILITY GROUP (HMG) PROTEINS</td>
</tr>
<tr>
<td>FORKHEAD</td>
</tr>
<tr>
<td>PAIRED BOX</td>
</tr>
<tr>
<td>T-BOX</td>
</tr>
<tr>
<td>CELL CYCLE CONTROL PROTEINS</td>
</tr>
<tr>
<td>COACTIVATORS</td>
</tr>
<tr>
<td>GENERAL TRANSCRIPTION FACTORS</td>
</tr>
<tr>
<td>TRANSCRIPTION ELONGATION FACTOR</td>
</tr>
<tr>
<td>RUNT</td>
</tr>
<tr>
<td>CHIMERIC PROTEINS DUE TO TRANSLOCATIONS</td>
</tr>
</tbody>
</table>

**EXAMPLE**
ANDROGEN RECEPTOR

WT1

MITF

IPF1

RETINA LEUCINE ZIPPER (NRL)

SRY

HNF4*, HNF1*;

HNF1*

PAX 3

TBX5

P53

CREB BINDING PROTEIN (CBP)

TATA-BINDING PROTEIN (TBP)

VHL

CBFA2

PML-RAR

ASSOCIATED DISORDER

COMPLETE OR PARTIAL ANDROGEN INSENSITIVITY (RECESSIVE MISSENSE MUTATIONS)

SPINOBULBAR MUSCULAR ATROPHY (CAG REPEAT EXPANSION)

WAGR SYNDROME: WILM’S TUMOR, ANIRIDIA, GENI-TOURINARY MALFORMATIONS, MENTAL RETARDATION

WAARDENBURG SYNDROME TYPE 2A

MATURITY ONSET OF DIABETES MELLITUS TYPE 4 (HETEROZYGOUS MUTATION/HAPLOINSUFFICIENCY)

PANCREATIC AGENESIS (HOMOZYGOUS MUTATION)

AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA

SEX-REVERSAL

MATURITY-ONSET OF DIABETES MELLITUS TYPES 1, 3, 5

WAARDENBURG SYNDROME TYPES 1 AND 3

HOLT-ORAM SYNDROME (THUMB ANOMALIES, ATRIAL OR VENTRICULAR SEPTUM DEFECTS, PHOCOMELIA)

LI-FRAUMENI SYNDROME, OTHER CANCERS

RUBINSTEIN-TAYBI SYNDROME

SPINOCEREBELLAR ATAXIA 17 (CAG EXPANSION)
VON HIPPEL-LINDAU SYNDROME (RENAL CELL CARCINOMA, PHEOCHROMOCYTOMA, Pancreatic TUMORS, HEMANGIOBLASTOMAS) AUTOSOMATIC DOMINANT INHERITANCE, SOMATIC INACTIVATION OF SECOND ALLELE (KNUDSON TWO-HIT MODEL) FAMILIAL THROMBOCYTOPENIA WITH PROPENSITY TO ACUTE MYELOGENOUS LEUKEMIA ACUTE PROMYELOCYTIC LEUKEMIA T(15;17)(Q22;Q11.2-Q12) TRANSLOCATION

**NOTE:** SELECTED ABBREVIATIONS INCLUDE: SRY, SEX DETERMINING REGION Y; HNF, HEPATOCYTE NUCLEAR FACTOR; CREB(CAMP RESPONSIVE ELEMENT BINDING) BINDING PROTEIN; VHL VON HIPPEL-LINDAU, PML, PIOMYELOCYTIC LEUKEMIA; RAR, RETINOIC ACID RECEPTOR.

LY USED IN GENETICS AND MOLECULAR DIAGNOSTICS, HOWEVER, IT IS USEFUL TO REVIEW BRIEFLY SOME OF THE FUNDAMENTAL PRINCIPLES OF CLONING AND DNA SEQUENCING.

**CLONING OF GENES** CLONING REFERS TO THE CREATION OF A RECOMBINANT DNA MOLECULE THAT CAN BE PROPAGATED INDEFINITELY. THE ABILITY TO CLONE GENES AND CDNAS THEREFORE PROVIDES A PERMANENT AND RENEWABLE SOURCE OF THESE REAGENTS. CLONING IS ESSENTIAL FOR DNA SEQUENCING, NUCLEIC ACID HYBRIDIZATION STUDIES, EXPRESSION OF RECOMBINANT PROTEINS, AND OTHER RECOMBINANT DNA PROCEDURES.

THE CLONING OF DNA INVOLVES THE INSERTION OF A DNA FRAGMENT INTO A CLONING VECTOR, FOLLOWED BY THE PROPAGATION OF THE RECOMBINANT DNA IN A HOST CELL. THE MOST STRAIGHTFORWARD CLONING STRATEGY INVOLVES INSERTING A DNA FRAGMENT INTO BACTERIAL PLASMIDS. PLASMIDS ARE SMALL, AUTONOMOUSLY REPLICATING, CIRCULAR DNA MOLECULES THAT PROPAGATE SEPARATELY FROM THE CHROMOSOME IN BACTERIAL CELLS. THE PROCESS OF DNA INSERTION RELIES HEAVILY ON THE USE OF RESTRICTION ENZYMES, WHICH CLEAVE DNA AT HIGHLY SPECIFIC SEQUENCES (USUALLY 4-6 BP IN LENGTH). RESTRICTION ENZYMES GENERATE COMPLEMENTARY, COHESIVE SEQUENCES AT THE ENDS OF THE DNA FRAGMENT, WHICH ALLOW THEM TO BE EFFICIENTLY LIGATED TO THE PLASMID VECTOR. BECAUSE PLASMIDS CONTAIN GENES THAT CONFER RESISTANCE TO ANTIBIOTICS, THEIR PRESENCE IN THE HOST CELL CAN BE USED FOR SELECTION AND DNA AMPLIFICATION.

A VARIETY OF VECTORS (E.G., PLASMIDS, PHAGE, BACTERIAL, OR YEAST ARTIFICIAL CHROMOSOMES) ARE USED FOR CLONING. MANY OF THESE ARE USED FOR CREATING LIBRARIES, A TERM THAT REFERS TO A COLLECTION OF DNA CLONES. A GENOMIC LIBRARY REPRESENTS AN ARRAY OF CLONES DERIVED FROM GENOMIC DNA. THESE OVERLAPPING DNA FRAGMENTS REPRESENT THE ENTIRE GENOME AND CAN ULTIMATELY BE ARRANGED ACCORDING TO THEIR LINEAR ORDER. CDNA LIBRARIES REFLECT CLONES DERIVED FROM MRNA, TYPICALLY FROM A PARTICULAR TISSUE SOURCE. THUS, A CDNA LIBRARY FROM THE HEART CONTAINS COPIES OF MRNA EXPRESSED SPECIFICALLY IN CARDIAC MYOCYTES, IN AD-
DITION TO THOSE THAT ARE EXPRESSED UBQUITOUSLY. FOR THIS REASON, A HEART CDNA LIBRARY WILL BE ENRICHED WITH CARDIAC-SPECIFIC GENE PRODUCTS AND WILL DIFFER FROM CDNA LIBRARIES GENERATED FROM LIVER OR PITUITARY MRNAS. AS AN EXAMPLE OF THE COMPLEXITY OF A GENOMIC LIBRARY, CONSIDER THAT THE HUMAN GENOME CONTAINS \(3 \times 10^{9}\) BP AND THE AVERAGE GENOMIC INSERT IN A PHAGE LIBRARY IS \(~10^{4}\) BP. THEREFORE, IT REQUIRES AT LEAST \(3 \times 10^{5}\) CLONES TO REPRESENT ALL GENOMIC DNA. SPECIFIC CLONES ARE ISOLATED FROM THE SEVERAL HUNDRED THOUSAND CLONES BY USING DNA HYBRIDIZATION. WITH COMPLETION OF THE HGP, ALL HUMAN GENES HAVE BEEN CLONED AND SEQUENCED. AS A RESULT, MANY OF THESE CLONING PROCEDURES ARE NOW UNNECESSARY OR GREATLY FACILITATED BY THE EXTENSIVE INFORMATION CONCERNING DNA MARKERS AND THE SEQUENCE OF DNA (SEE BELOW).

**NUCLEIC ACID HYBRIDIZATION** NUCLEIC ACID HYBRIDIZATION IS A FUNDAMENTAL PRINCIPLE IN MOLECULAR BIOLOGY THAT TAKES ADVANTAGE OF THE FACT THAT THE TWO COMPLEMENTARY STRANDS OF NUCLEIC ACIDS BIND, OR HYBRIDIZE, TO ONE ANOTHER WITH VERY HIGH SPECIFICITY. THE GOAL OF HYBRIDIZATION IS TO DETECT SPECIFIC NUCLEIC ACID (DNA OR RNA) SEQUENCES IN A COMPLEX BACKGROUND OF OTHER SEQUENCES. THIS TECHNIQUE IS USED FOR SOUTHERN BLOTTING, NORTHERN BLOTTING, AND FOR SCREENING LIBRARIES (SEE ABOVE). FURTHER ADAPTATION OF HYBRIDIZATION TECHNIQUES HAS LED TO THE DEVELOPMENT OF MICROARRAY DNA CHIPS.

**SOUTHERN BLOT** SOUTHERN BLOTTING IS USED TO ANALYZE WHETHER GENES HAVE BEEN DELETED OR REARRANGED. IT IS ALSO USED TO DETECT RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPS). GENOMIC DNA IS DIGESTED WITH RESTRICTION ENDONUCLEASES AND SEPARATED BY GEL ELECTROPHORESIS. INDIVIDUAL FRAGMENTS CAN THEN BE TRANSFERRED TO A MEMBRANE AND DETECTED AFTER HYBRIDIZATION WITH SPECIFIC RADIOACTIVE DNA PROBES. BECAUSE SINGLE BASE-PAIR MISMATCHES CAN DISRUPT THE HYBRIDIZATION OF SHORT DNA PROBES (OLIGONUCLEOTIDES), A VARIATION OF THE SOUTHERN BLOT, TERMED OLGONUCLEOTIDE-SPECIFIC HYBRIDIZATION (OSH), USES SHORT OLIGONUCLEOTIDES TO DISTINGUISH NORMAL FROM MUTANT GENES.

**NORTHERN BLOT** NORTHERN BLOTS ARE USED TO ANALYZE PATTERNS AND LEVELS OF GENE EXPRESSION IN DIFFERENT TISSUES. IN A NORTHERN BLOT, MRNA IS SEPARATED ON A GEL AND TRANSFERRED TO A MEMBRANE, AND SPECIFIC TRANSCRIPTS ARE DETECTED USING RADIOLABELED DNA AS A PROBE. THIS TECHNIQUE HAS BEEN LARGELY SUPPLANTED BY MORE SENSITIVE AND COMPREHENSIVE METHODS SUCH AS REVERSE TRANSCRIPTASE (RT)-PCR AND GENE EXPRESSION ARRAYS ON DNA CHIPS (SEE BELOW).

**MICROARRAY TECHNOLOGY** A COMPREHENSIVE APPROACH TO GENOME-SCALE STUDIES CONSISTS OF MICROARRAYS, OR DNA CHIPS. THESE MICROARRAYS CONSIST
OF THOUSANDS OF SYNTHETIC NUCLEIC ACID SEQUENCES ALIGNED ON THIN GLASS OR SILICON SURFACES. FLUORESCENTLY LABELED TEST SAMPLE DNA OR RNA IS HYBRIDIZED TO THE CHIP, AND A COMPUTERIZED SCANNER DETECTS SEQUENCE MATCHES. MICROARRAYS ALLOW THE DETECTION OF VARIATIONS IN DNA SEQUENCE AND ARE USED FOR MUTATIONAL ANALYSIS AND GENOTYPING. ALTERNATIVELY, THE EXPRESSION PATTERN OF LARGE NUMBERS OF MRNA TRANSCRIPTS CAN BE DETERMINED BY HYBRIDIZATION OF RNA SAMPLES TO CDNA OR GENOMIC MICROAR-

PAGE NO. 38

391 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS

RAYS. THIS METHOD HAS TREMENDOUS POTENTIAL IN THE ERA OF FUNCTIONAL GENOMICS AND PERMITS COMPREHENSIVE ANALYSES OF GENE EXPRESSION PROFILES. AS ONE EXAMPLE, MICROARRAYS CAN BE USED TO DEVELOP GENETIC FINGERPRINTS OF DIFFERENT TYPES OF MALIGNANCIES, PROVIDING INFORMATION USEFUL FOR CLASSIFICATION, PATHOPHYSIOLOGY, PROGNOSIS, AND TREATMENT.

THE POLYMERASE CHAIN REACTION THE PCR, INTRODUCED IN 1985, HAS REVOLUTIONIZED THE WAY DNA ANALYSES ARE PERFORMED AND HAS BECOME A CORNERSTONE OF MOLECULAR BIOLOGY AND GENETIC ANALYSIS. IN ESSENCE, PCR PROVIDES A RAPID WAY OF AMPLIFYING SPECIFIC DNA FRAGMENTS IN VITRO. EXQUISITE SPECIFICITY IS CONFERRED BY THE USE OF PCR PRIMERS, WHICH ARE DESIGNED FOR A GIVEN DNA SEQUENCE. THE GEOMETRIC AMPLIFICATION OF THE DNA AFTER MULTIPLE CYCLES YIELDS REMARKABLE SENSITIVITY. AS A RESULT, PCR CAN BE USED TO AMPLIFY DNA FROM VERY SMALL SAMPLES, INCLUDING SINGLE CELLS. THESE PROPERTIES ALSO ALLOW DNA AMPLIFICATION FROM A VARIETY OF TISSUE SOURCES INCLUDING BLOOD SAMPLES, BIOPSIES, SURGICAL OR AUTOPSY SPECIMENS, OR CELLS FROM HAIR OR SALIVA. PCR CAN ALSO BE USED TO STUDY MRNA. IN THIS CASE, THE ENZYME RT IS FIRST USED TO CONVERT THE RNA TO DNA, WHICH CAN THEN BE AMPLIFIED BY PCR. THIS PROCEDURE, COMMONLY KNOWN AS RT-PCR, IS USEFUL AS A QUANTITATIVE MEASURE OF GENE EXPRESSION. PCR PROVIDES A KEY COMPONENT OF MOLECULAR DIAGNOSTICS. IT PROVIDES A STRATEGY FOR THE RAPID AMPLIFICATION OF DNA (OR MRNA) TO SEARCH FOR MUTATIONS BY A WIDE ARRAY OF TECHNIQUES, INCLUDING DNA SEQUENCING. PCR IS ALSO USED FOR THE AMPLIFICATION OF HIGHLY POLYMORPHIC DI- OR TRINUCLEOTIDE REPEAT SEQUENCES OR THE GENOTYPING OF SNPS, WHICH ALLOW VARIOUS POLYMORPHIC ALLELES TO BE TRACED IN GENETIC LINKAGE OR ASSOCIATION STUDIES. PCR IS INCREASINGLY USED TO DIAGNOSE VARIOUS MICROBIAL PATHOGENS.

DNA SEQUENCING DNA SEQUENCING IS NOW AN AUTOMATED PROCEDURE. ALTHOUGH MANY PROTOCOLS EXIST, THE MOST COMMONLY USED STRATEGY CURRENTLY USES THE CAPIL-
LARY ELECTROPHORESIS-BASED SANGER METHOD IN WHICH DIDEOXYNUDEOTIDES ARE USED TO RANDOMLY TERMINATE DNA POLYMERIZATION AT EACH OF THE FOUR BASES (A,G,T,C). AFTER SEPARATING THE ARRAY OF TERMINATED DNA FRAGMENTS USING HIGH-RESOLUTION GEL OR CAPILLARY ELECTROPHORESIS, IT IS POSSIBLE TO DEDUCE THE DNA SEQUENCE BY EXAMINING THE PROGRESSION OF FRAGMENT LENGTHS GENERATED IN EACH OF THE FOUR NUCLEOTIDE REACTIONS. THE USE OF FLUORESCENTLY LABELED DIDEOXYNUDEOTIDES ALLOWS AUTOMATED DETECTION OF THE DIFFERENT BASES AND DIRECT COMPUTER ANALYSIS OF THE DNA SEQUENCE (FIG. 62-5). SIGNIFICANT EFFORTS ARE UNDERWAY TO DEVELOP FASTER, MORE COST-EFFECTIVE DNA SEQUENCING TECHNOLOGIES. THESE INCLUDE THE USE OF PYROSEQUENCING CHEMISTRIES; WHOLE-GENOME SEQUENCING USING SOLID-PHASE SEQUENCING; MASS SPECTROMETRY; DETECTION OF FLUORESCENTLY LABELED BASES IN FLOW CYTOMETRY; DIRECT READING OF THE DNA SEQUENCE BY SCANNING, TUNNELING, OR ATOMIC FORCE MICROSCOPY; AND SEQUENCE ANALYSIS USING DNA CHIPS.

TRANSGENIC MICE AS MODELS OF GENETIC DISEASE

SEVERAL ORGANISMS HAVE BEEN STUDIED EXTENSIVELY AS GENETIC MODELS, INCLUDING MUS MUSCULUS (MOUSE), DROSOPHILA MELANOGASTER (FRUIT FLY), CAENORHABDITIS ELEGANS (NEMATODE), SACCHAROMYCES CEREVISIAE (BAKER’S YEAST), AND ESCHERICHIA COLI (COLONIC BACTERIUM). THE ABILITY TO USE THESE EVOLUTIONARILY DISTANT ORGANISMS AS GENETIC MODELS THAT ARE RELEVANT TO HUMAN PHYSIOLOGY REFLECTS A SURPRISING CONSERVATION OF GENETIC PATHWAYS AND GENE FUNCTION. TRANSGENIC MOUSE MODELS HAVE BEEN PARTICULARLY VALUABLE, BECAUSE MANY HUMAN AND MOUSE GENES EXHIBIT SIMILAR STRUCTURE AND FUNCTION, AND BECAUSE MANIPULATION OF THE MOUSE GENOME IS RELATIVELY STRAIGHTFORWARD COMPARED TO THOSE OF OTHER MAMMALIAN SPECIES. TRANSGENIC STRATEGIES IN MICE CAN BE DIVIDED INTO TWO MAIN APPROACHES: (1) EXPRESSION OF A GENE BY RANDOM INSERTION INTO THE GENOME, AND (2) DELETION OR TARGETED MUTAGENESIS OF A GENE BY HOMOLOGOUS RECOMBINATION WITH THE NATIVE ENDOGENOUS GENE (KNOCK-OUT, KNOCK-IN) (FIG. 62-6; TABLE 62-3). TRANSGENIC MICE ARE GENERATED BY PRONUCLEAR INJECTION OF FOREIGN DNA INTO FERTILIZED MOUSE OOCYTES AND SUBSEQUENT TRANSFER INTO THE OVIDUCT OF PSEUDOPREGNANT FOSTER MOTHERS. TRANSGENIC EXPRESSION OF GENES CAN BE USEFUL FOR STUDYING DISORDERS THAT ARE SENSITIVE TO GENE DOSAGE. OVEREXPRESSION OF PMP22, FOR EXAMPLE, MIMICS A COMMON DUPLICATION OF THIS GENE IN TYPE IA CHARCOT-MARIE-TOOTH DISEASE (CHAP. 379). DUPLICATION OF THE PMP22 GENE RESULTS IN HIGH LEVELS OF EXPRESSION OF PERIPHERAL MYELIN PROTEIN 22, AND THIS DOSAGE EFFECT IS RESPONSIBLE FOR THE DEMYELINATING NEUROPATHY. EX-
PRESSION OF THE Y CHROMOSOME-SPECIFIC GENE, SRY, IN XX FEMALES DEMONSTRATES THAT SRY IS SUFFICIENT TO INDUCE THE FORMATION OF TESTES. THIS FINDING CONFIRMS THE PATHOGENIC ROLE OF SRY TRANSLOCATIONS TO THE X CHROMOSOME IN SEX-REVERSED XX FEMALES. HUNTINGTON DISEASE IS AN AUTOSOMAL DOMINANT DISORDER CAUSED BY EXPANSION OF A CAG TRINUCLEOTIDE REPEAT THAT ENCODES A POLYGLUTAMINE TRACT. TARGETED DELETION OF THE HUNTINGTON DISEASE (HD) GENE DOES NOT INDUCE THE NEUROLOGIC DISORDER. ON THE OTHER HAND, TRANSGENIC EXPRESSION OF THE ENTIRE GENE OR OF THE FIRST EXON CONTAINING THE SEQUENCE ENCODING THE EXPANDED POLYGLUTAMINE REPEAT IS SUFFICIENT TO CAUSE MANY FEATURES OF THE NEUROLOGIC DISORDER, INDICATING A GAIN-OF-FUNCTION PROPERTY FOR THE EXPANDED POLYGLUTAMINE-CONTAINING PROTEIN. TRANSGENIC STRATEGIES CAN ALSO BE USED AS A PRECURSOR TO GENE THERAPY. EXPRESSION OF DYSTROPHIN, THE PRO-

**FIGURE 62-6 TRANSGENIC MOUSE MODELS.** LEFT: TRANSGENIC MICE ARE GENERATED BY PRONUCLEAR INJECTION OF FOREIGN DNA INTO FERTILIZED MOUSE OOCYTES AND SUBSEQUENT TRANSFER INTO THE OVIDUCT OF PSEUDOPREGNANT FOSTER MOTHERS. RIGHT: FOR TARGETED MUTAGENESIS (GENE KNOCK-OUT/KNOCK-IN), EMBRYONIC STEM (ES) CELLS ARE TRANSFECTED WITH THE TARGETED (MUTAGENIZED) TRANSGENE. THE TRANSGENE UNDERGOES HOMOLOGOUS RECOMBINATION WITH THE WILD-TYPE GENE. AFTER SELECTION, POSITIVE ES CELLS ARE INTRODUCED INTO BLASTOCYSTS AND IMPLANTED INTO FOSTER MOTHERS. CHIMERIC MICE CAN BE IDENTIFIED BASED ON THE MIXED COAT COLOR OF THE OFFSPRING. HETEROZYGOUS MICE ARE BRED TO OBTAIN MICE HOMOZYGOUS FOR THE MUTANT ALLELE.

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**392 PART 3: GENETICS AND DISEASE**

**TABLE 62-3 GENETICALLY MODIFIED ANIMALS**

**COMMONLY USED DESCRIPTION**

TRANSGENIC

(TARGETED) KNOCK-OUT

(TARGETED) KNOCK-IN

FORWARD GENETICS
CONGENIC STRAINS

CLONING

TECHNICAL PRINCIPLE

PRONUCLEAR INJECTION OF TRANSGENE

SUBSTITUTION OF FUNCTIONAL GENE WITH INACTIVE GENE BY HOMOLOGOUS RECOMBINATION IN EMBRYONIC STEM CELLS
INTRODUCTION OF SUBTLE MUTATION(S) INTO GENE BY SUBSTITUTION OF ENDOGENOUS GENE WITH GENE CARRYING A SPECIFIC MUTATION.
HOMOLOGOUS RECOMBINATION IN EMBRYONIC STEM CELLS
MUTATIONS CREATED RANDOMLY BY EMU (N-ETHYL-N-NITROUREA)

MATING OF AN INBRED DONOR STRAIN WITH A DISEASE PHENOTYPE WITH AN INBRED RECIPIENT STRAIN IN ORDER TO DEFINE THE GENOMIC REGION RESPONSIBLE FOR THE DISORDER
INTRODUCTION OF NUCLEUS INTO ENU-CLEATED EGGS (NUCLEAR TRANSFER)

REMARKS

COMMONLY USED
GENOMIC DMA OR CDMA CONSTRUCTS
RANDOM INTEGRATION OF TRANSGENE
VARIABLE COPY NUMBERS OF TRANSGENE
VARIABLE EXPRESSION IN EACH INDIVIDUAL FOUNDER
GAIN-OF-FUNCTION MODELS DUE TO OVEREXPRESSION USING TISSUE-SPECIFIC PROMOTERS
LOSS-OF-FUNCTION MODELS USING ANTI-SENSE AND DOMINANT NEGATIVE TRANSGENES
INDUCIBLE EXPRESSION POSSIBLE (TETRACYCLINE, ECDYSONE)
APPLICABLE TO SEVERAL SPECIES
PREDOMINANTLY USED IN MICE
TISSUE-SPECIFIC KNOCK-OUT POSSIBLE (CRE/LOX)
ABSENCE OF PHENOTYPE POSSIBLE DUE TO REDUNDANCY
PREDOMINANTLY USED IN MICE
CAN ACCURATELY MODEL HUMAN DISEASE

SELECTION OF PHENOTYPE FOLLOWED BY GENETIC CHARACTERIZATION
USEFUL FOR IDENTIFYING NOVEL GENES
USEFUL FOR MAPPING DISEASE-CAUSING GENES
SUCCESSFUL IN SEVERAL MAMMALIAN SPECIES INCLUDING SHEEP (DOLLY), MICE, COWS, MONKEYS
CLONING OF GENETICALLY IDENTICAL INDIVIDUALS MAY AFFECT LIFE-SPAN
ETHICAL CONCERNS

TEIN THAT IS DELETED IN DUCHENNE MUSCULAR DYSTROPHY, PARTIALLY CORRECTS THE DISORDER IN A MOUSE MODEL OF DUCHENNE’S. TARGETED EXPRESSION OF ONCOGENES HAS BEEN VALUABLE TO STUDY MECHANISMS OF NEOPLASIA AND TO GENERATE IMMORTALIZED CELL LINES. FOR EXAMPLE, EXPRESSION OF THE SIMIAN VIRUS 40 (SV40) LARGE T ANTIGEN UNDER THE DIRECTION OF THE INSULIN PROMOTER INDUCES THE FORMATION OF ISLET CELL TUMORS. THE CREATION OF GENE KNOCK-OUT AND KNOCK-IN MODELS TAKES ADVANTAGE OF THE FACT THAT A SEGMENT OF DNA CAN BE SUBSTITUTED BY ANOTHER THAT IS IDENTICAL (HOMOLOGOUS), OR NEARLY IDENTICAL, BY RECOMBINATION. THIS PERMITS INTEGRATION OF DELETIONS THAT DISRUPT THE GENE (KNOCK-OUT) OR SELECTED MUTATIONS (KNOCK-IN) INTO THE TARGET GENE OF CHOICE. THE TRANSGENE IS INTRODUCED INTO EMBRYONIC STEM (ES) CELLS BY TRANSFECTION AND, AFTER SELECTION OF CELLS WITH AN INTEGRATED TRANSGENE, THE POSITIVE ES CELLS ARE INTRODUCED INTO BLASTOCYSTS AND IMPLANTED INTO FOSTER MOTHERS. CHIMERIC MICE CAN BE IDENTIFIED BASED ON THE MIXED COAT COLOR OF THE OFFSPRING. HETEROZYGOUS MICE ARE BRED TO OBTAIN MICE HOMOZYGOUS FOR THE MUTANT ALLELE. THIS IS PARTICULARLY USEFUL FOR GENES THAT WOULD BE LE-THAL IF DELETED UNIVERSALLY OR DURING EARLY DEVELOPMENT. THE LIST OF GENES THAT HAVE BEEN MODIFIED BY THIS APPROACH IS VERY LARGE. MANY OF THESE KNOCK-OUTS DO NOT HAVE AN APPARENT PHENOTYPE, EITHER BECAUSE OF REDUNDANT FUNCTIONS OF THE OTHER GENES OR BECAUSE THE PHENOTYPE IS SUBTLE. FOR EXAMPLE, DELETION OF THE HYPOXANTHINE PHOSPHORIBOSYLMUTASERASE (HPRT) GENE (HPRT) DOES NOT CAUSE CHARACTERISTIC FEATURES OF LESCH-NYHAN SYNDROME IN MICE BECAUSE OF THEIR RELIANCE ON ADENINE PHOSPHORIBOSYLMUTASERASE (APRT) IN THE PURINE SALVAGE PATHWAY. DELETION OF THE RETINOBLASTOMA (RB) GENE ENCODING P105 DOES NOT LEAD TO RETINOBLASTOMA OR OTHER TUMORS THAT CHARACTERIZE THE HUMAN SYNDROME. HOWEVER, MICE WITH COMBINATORIAL DELETION OF SEVERAL RB-RELATED PROTEINS EXHIBIT FEATURES SIMILAR TO THE HUMAN DISORDER. THESE EXAMPLES UN-DERSCORE THE FACT THAT THE FUNCTIONS OF GENES, AND THEIR INTERACTIONS WITH GENETIC BACKGROUND AND THE ENVIRONMENT, ARE NOT NECESSARILY IDENTICAL IN MICE AND HUMANS. ON THE OTHER HAND, THE DELETION OF MANY GENES PROVIDES A REMARKABLY FAITHFUL MODEL OF HU-
MAN DISORDERS. IN ADDITION TO CLARIFYING PATHOPHYSIOLOGY, THESE MODELS FACILITATE THE DEVELOPMENT OF THERAPIES, BOTH GENETIC AND PHARMACEUTICAL. MANY VARIATIONS OF THESE BASIC APPROACHES NOW EXIST THAT ALLOW GENES TO BE EXPRESSED OR DELETED IN SPECIFIC CELL TYPES, AT DIFFERENT TIMES DURING DEVELOPMENT, OR AT VARYING LEVELS. CONSEQUENTLY, TRANSGENIC TECHNOLOGY HAS EMERGED AS A POWERFUL STRATEGY FOR DEFINING THE PHYSIOLOGIC EFFECTS OF DELETING OR OVEREXpressING A GENE, AS WELL AS PROVIDING UNIQUE GENETIC MODELS FOR DISSECTING PATHOPHYSIOLOGY OR TESTING THERAPIES. IN ADDITION TO TRANSGENIC ANIMAL MODELS, NATURALLY OCCURRING MUTATIONS IN MICE AND OTHER SPECIES CONTINUE TO PROVIDE FUNDAMENTAL INSIGHTS INTO HUMAN DISEASE. A COMPENDIUM OF NATURAL AND TRANSGENIC ANIMAL MODELS IS PROVIDED IN CONTINUOUSLY EVOLVING DATABASES (TABLE 62-1).

IMPLICATIONS OF THE HUMAN GENOME PROJECT

THE HGP WAS INITIATED IN THE MID-1980S AS AN AMBITIOUS EFFORT TO CHARACTERIZE THE HUMAN GENOME, CULMINATING IN A COMPLETE DNA SEQUENCE. THE INITIAL MAIN GOALS WERE (1) CREATION OF GENETIC MAPS, (2) DEVELOPMENT OF PHYSICAL MAPS, AND (3) DETERMINATION OF THE COMPLETE HUMAN DNA SEQUENCE. SOME ANALOGIES HELP IN APPRECIATING THE SCOPE OF THE HGP. THE 23 PAIRS OF HUMAN CHROMOSOMES ENCODE ~30,000-40,000 GENES. THE TOTAL LENGTH OF DNA IS ~3 BILLION BP, WHICH IS NEARLY 1000-FOLD GREATER THAN THAT OF THE E. COLI GENOME. IF THE HUMAN DNA SEQUENCE WERE PRINTED OUT, IT WOULD CORRESPOND TO ABOUT 120 VOLUMES OF HARRISON'S PRINCIPLES OF INTERNAL MEDICINE.

THE GENETIC MAP  GIVEN THE SIZE AND COMPLEXITY OF THE HUMAN GENOME, INITIAL EFFORTS AIMED AT DEVELOPING GENETIC MAPS TO PROVIDE ORIENTATION AND TO DELIMIT WHERE A GENE OF INTEREST MAY BE LOCATED. A GENETIC MAP DESCRIBES THE ORDER OF GENES AND DEFINES THE POSITION OF A GENE RELATIVE TO OTHER LOCI ON THE SAME CHROMOSOME. IT IS CONSTRUCTED BY ASSESSING HOW FREQUENTLY TWO MARKERS ARE INHERITED TOGETHER (I.E., LINKED) BY ASSOCIATION STUDIES. DISTANCES OF THE GENETIC MAP ARE EXPRESSED IN RECOMBINATION UNITS, OR CENTIMORGANS (CM). ONE CM CORRESPONDS TO A RECOMBINATION FREQUENCY OF 1% BETWEEN TWO POLYMORPHIC MARKERS; 1 CM CORRESPONDS TO ~1 MB OF DNA (FIG. 62-3). ANY POLYMORPHIC SEQUENCE VARIATION CAN BE USEFUL FOR MAPPING PURPOSES. EXAMPLES OF POLYMORPHIC MARKERS INCLUDE VARIABLE NUMBER OF TANDEM REPEATS (VNTRS), RFLPS, MICROSATELLITE REPEATS, AND SNPS; THE LATTER
TWO METHODS ARE NOW USED PREDOMINANTLY BECAUSE OF THE HIGH DENSITY OF MARKERS AND BECAUSE THEY ARE AMENABLE TO AUTOMATED PROCEDURES.

THE PHYSICAL MAP  CYTOGENETICS AND CHROMOSOMAL BANDING TECHNIQUES PROVIDE A RELATIVELY LOW-RESOLUTION MICROSCOPIC VIEW OF GENETIC LOCI. PHYSICAL MAPS INDICATE THE POSITION OF A LOCUS OR GENE IN ABSOLUTE VALUES. SEQUENCE-TAGGED SITES (STSS) ARE USED AS A STANDARD UNIT FOR PHYSICAL MAPPING AND SERVE AS SEQUENCE-SPECIFIC LANDMARKS FOR ARRANGING OVERLAPPING CLONED FRAGMENTS IN THE SAME ORDER AS THEY OCCUR IN THE GENOME. THESE OVERLAPPING CLONES ALLOW THE CHARACTERIZATION OF CONTIGUOUS DNA SEQUENCES, COMMONLY REFERRED TO AS CONTIGS. THIS APPROACH LED TO HIGH-RESOLUTION PHYSICAL MAPS BY CLONING THE WHOLE GENOME INTO OVERLAPPING FRAGMENTS AND HAS BEEN ESSENTIAL FOR THE IDENTIFICATION OF DISEASE-CAUSING GENES BY POSITIONAL CLONING.

PAGE NO. 40

393 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS

FIGURE 62-7 CHROMOSOME 7 IS SHOWN WITH THE DENSITY OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) AND GENES ABOVE. A 200-KB REGION IN 7q31.2 CONTAINING THE CFTR GENE IS SHOWN BELOW. THE CFTR GENE CONTAINS 27 EXONS. MORE THAN 1420 MUTATIONS IN THIS GENE HAVE BEEN FOUND IN PATIENTS WITH CYSTIC FIBROSIS. A 20-KB REGION ENCOMPASSING EXONS 4-9 IS SHOWN IN FURTHER AMPLIFIED IN ORDER TO ILLUSTRATE THE SNPS IN THIS REGION.

RECENT INSIGHTS INTO THE STRUCTURE OF THE NORMAL HUMAN GENOME SHOW THAT CERTAIN BLOCKS OF DNA SEQUENCES, OFTEN CONTAINING NUMEROUS GENES, CAN BE DUPLICATED ONE OR SEVERAL TIMES. THIS COPY NUMBER VARIATION (CNV), WHICH TENDS TO VARY IN A SPECIFIC MANNER AMONG DIFFERENT POPULATIONS, IS ASSOCIATED WITH HOT SPOTS OF CHROMOSOMAL REARRANGEMENTS AND IS THOUGHT TO PLAY AN IMPORTANT ROLE IN NORMAL HUMAN VARIATION AND IN GENETIC DISEASE. THE IDENTIFICATION OF THE ~10 MILLION SNPS ESTIMATED TO OCCUR IN THE HUMAN GENOME HAS GENERATED A CATALOGUE OF COMMON GENETIC VARIANTS THAT OCCUR IN HUMAN BEINGS FROM DISTINCT ETHNIC BACKGROUNDS (FIG. 62-7). SNPS THAT ARE IN CLOSE PROXIMITY ARE INHERITED TOGETHER, I.E., THEY ARE LINKED, AND ARE REFERRED TO AS HAPLOTYPES, HENCE THE NAME HAPMAP (FIG. 62-8). THE HAPMAP DESCRIBES THE NATURE AND LOCATION OF THESE SNP HAPLOTYPES AND HOW THEY ARE DISTRIBUTED AMONG INDIVIDUALS WITHIN AND AMONG POPULATIONS. THE HAPMAP INFORMATION IS GREATLY FACILITATING GENOME-WIDE ASSOCIATION STUDIES DESIGNED TO ELUCIDATE THE COMPLEX INTERACTIONS AMONG MULTIPLE GENES AND LIFESTYLE FACTORS IN MULTIFACTORIAL DISORDERS (SEE BELOW). HOWEVER, HAPLOTYPING ANALYSES MAY BECOME USEFUL TO ASSESS VARIATIONS IN RESPONSES TO MEDICATIONS (PHARMACOGENOMICS) AND ENVIRONMENTAL FACTORS, AS WELL AS THE PREDICTION OF DISEASE PREDISPOSITION.
THE HUMAN DNA SEQUENCE. THE COMPLETE DNA SEQUENCE OF EACH CHROMOSOME PROVIDES THE HIGHEST RESOLUTION PHYSICAL MAP. THE PRIMARY FOCUS OF THE HGP WAS TO OBTAIN DNA SEQUENCE FOR THE ENTIRE HUMAN GENOME AS WELL AS MODEL ORGANISMS. ALTHOUGH THE PROSPECT OF FIGURE 62-8 THE ORIGIN OF HAPLOTYPES IS DUE TO REPEATED RECOMBINATION EVENTS OCCURRING IN MULTIPLE GENERATIONS. OVER TIME, THIS LEADS TO DISTINCT HAPLOTYPES. THESE HAPLOTYPE BLOCKS CAN OFTEN BE CHARACTERIZED BY GENOTYPING SELECTED TAG SINGLE NUCLEOTIDE POLYMORPHISMS, AN APPROACH THAT NOW FACILITATES PERFORMING GENOME-WIDE ASSOCIATION STUDIES.

PAGE NO. 41

394 PART 3: GENETICS AND DISEASE


THE CURRENT DIRECTIONS ARISING FROM THE HGP INCLUDE, AMONG OTHERS, (1) THE COMPARISON OF ENTIRE GENOMES (COMPARATIVE GENOMICS), (2) THE STUDY OF LARGE-SCALE EXPRESSION OF RNAS (FUNCTIONAL GENOMICS) AND PROTEINS (PROTEOMICS) IN ORDER TO DETECT DIFFERENCES BETWEEN VARIOUS TISSUES IN HEALTH AND DISEASE, (3) THE CHARACTERIZATION OF THE VARIATION AMONG INDIVIDUALS BY ESTABLISHING CATALOGUES OF SEQUENCE VARIATIONS AND SNPS (HAPMAP PROJECT), AND (4) THE IDENTIFICATION OF GENES THAT PLAY CRITICAL ROLES IN THE DEVELOPMENT OF POLYGENIC AND MULTIFACTORIAL DISORDERS.

ETHICAL ISSUES IMPLICIT IN THE HGP IS THE IDEA AND HOPE THAT IDENTIFYING DISEASE-CAUSING GENES CAN LEAD TO IMPROVEMENTS IN DIAGNOSIS, TREATMENT, AND PREVENTION. IT IS ESTIMATED THAT MOST INDIVIDUALS HARBOUR SEVERAL SERIOUS RECESSIVE GENES. HOWEVER, COMPLETION OF THE HUMAN GENOME SEQUENCE, DETERMINATION OF THE ASSOCIATION OF GENETIC DEFECTS WITH DISEASE, AND STUDIES OF GENETIC VARIATION RAISE MANY NEW ISSUES
WITH IMPLICATIONS FOR THE INDIVIDUAL AND MANKIND. THE CONTROVERSIES CONCERNING THE CLONING OF MAMMALS AND THE ESTABLISHMENT OF HUMAN ES CELLS UNDERSCORE THE RELEVANCE OF THESE QUESTIONS. MOREOVER, THE IN-
FORMATION GLEANED FROM GENOTYPIC RESULTS CAN HAVE QUITE DIFFERENT IM-
PACTS, DEPENDING ON THE AVAILABILITY OF STRATEGIES TO MODIFY THE COURSE
OF DISEASE. FOR EXAMPLE, THE IDENTIFICATION OF MUTATIONS THAT CAUSE
MULTIPLE ENDOCRINE NEOPLASIA (MEN) TYPE 2 OR HEMOCHROMATOSIS AL-
LOWS SPECIFIC INTERVENTIONS FOR AFFECTED FAMILY MEMBERS. ON THE OTHER
HAND, AT PRESENT, THE IDENTIFICATION OF AN ALZHEIMER OR HUNTINGTON DIS-
EASE GENE DOES NOT ALTER THERAPY AND OUTCOMES. IN ADDITION, THE
PROGRESS IN THIS AREA IS UNPREDICTABLE, AS UNDERSCORED BY THE FINDING
THAT ANGIOTENSIN II RECEPTOR BLOCKERS MAY SLOW DISEASE PROGRESSION IN
MARFAN SYNDROME.

GENETIC TEST RESULTS CAN GENERATE ANXIETY IN AFFECTED INDIVIDUALS AND
FAMILY MEMBERS, AND THERE IS THE POSSIBILITY OF DISCRIMINATION ON THE
BASIS OF THE TEST RESULTS. MOST GENETIC DISORDERS ARE LIKELY TO FALL
INTO AN
INTERMEDIATE CATEGORY WHERE THE OPPORTUNITY FOR PREVENTION OR TREAT-
MENT IS SIGNIFICANT BUT LIMITED (CHAP. 64). FOR THESE REASONS, THE SCIENT-
IFIC COMPONENTS OF THE HGP HAVE BEEN PARALLELED BY EFFORTS TO
EXAMINE ETHICAL AND LEGAL IMPLICATIONS AS NEW ISSUES ARISE. ABOUT 5%
OF THE HGP BUDGET HAS BEEN ALLOCATED TO STUDIES ADDRESSING THE
ETHICAL,
LEGAL, AND SOCIAL IMPLICATIONS ASSOCIATED WITH THE INCREASING
KNOWLEDGE
ABOUT THE HUMAN GENOME AND THE GENETIC BASIS OF DISEASE.

MANY ISSUES RAISED BY THE GENOME PROJECT ARE FAMILIAR, IN PRINCIPLE,
TO MEDICAL PRACTITIONERS. FOR EXAMPLE, AN ASYMPTOMATIC PATIENT WITH
INCREASED LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL, HIGH BLOOD PRES-
SURE, OR A STRONG FAMILY HISTORY OF EARLY MYOCARDIAL INFARCTION IS
KNOWN
TO BE AT INCREASED RISK OF CORONARY HEART DISEASE. IN SUCH CASES, IT IS
CLEAR THAT THE IDENTIFICATION OF RISK FACTORS AND AN APPROPRIATE INTER-
VENTION ARE BENEFICIAL. LIKewise, PATIENTS WITH PHENYLKETONURIA,
CYSTIC
FIBROSIS, OR SICKLE CELL ANEMIA ARE OFTEN IDENTIFIED AS HAVING A GENETIC
DISEASE EARLY IN LIFE. THESE PRECEDENTS CAN BE HELPFUL FOR ADAPTING
POLI-
CIES THAT RELATE TO GENETIC INFORMATION. WE CAN ANTICIPATE SIMILAR EF-
FORTS, WHETHER BASED ON GENOTYPES OR OTHER MARKERS OF GENETIC
PREDISPOSITION, TO BE APPLIED TO MANY DISORDERS. ONE CONFOUNING AS-
PECT OF THE RAPID EXPANSION OF INFORMATION IS THAT OUR ABILITY TO MAKE
CLINICAL DECISIONS OFTEN LAGS BEHIND INITIAL INSIGHTS INTO GENETIC
MECHA-
NISMS OF DISEASE. FOR EXAMPLE, WHEN GENES THAT PREDISPOSE TO BREAST
CANCER, SUCH AS BRCA1, ARE DESCRIBED, THEY GENERATE TREMENDOUS PUB-
LIC INTEREST IN THE POTENTIAL TO PREDICT DISEASE, BUT MANY YEARS OF
CLINICAL
RESEARCH ARE STILL REQUIRED TO RIGOROUSLY ESTABLISH GENOTYPE AND
PHENO-
TYPE CORRELATIONS.

WHETHER RELATED TO INFORMED CONSENT, PARTICIPATION IN RESEARCH, OR
THE MANAGEMENT OF A GENETIC DISORDER THAT AFFECTS AN INDIVIDUAL OR
THEIR FAMILIES, THERE IS A GREAT NEED FOR MORE INFORMATION ABOUT FUNDAMENTAL
KNOWLEDGE.
MENTAL PRINCIPLES OF GENETICS. THE PERVASIVE NATURE OF THE ROLE OF GENETICS IN MEDICINE MAKES IT IMPERATIVE FOR PHYSICIANS AND OTHER HEALTH CARE PROFESSIONALS TO BECOME MORE INFORMED ABOUT GENETICS AND TO PROVIDE ADVICE AND COUNSELING IN CONJUNCTION WITH TRAINED GENETIC COUNSELORS (CHAP. 64). THE APPLICATION OF SCREENING AND PREVENTION STRATEGIES WILL THEREFORE REQUIRE INTENSIVE PATIENT AND PHYSICIAN EDUCATION, CHANGES IN HEALTH CARE FINANCING, AND LEGISLATION TO PROTECT PATIENT’S RIGHTS.

TRANSMISSION OF GENETIC DISEASE

ORIGINS AND TYPES OF MUTATIONS A MUTATION CAN BE DEFINED AS ANY CHANGE IN THE PRIMARY NUCLEOTIDE SEQUENCE OF DNA REGARDLESS OF ITS FUNCTIONAL CONSEQUENCES. SOME MUTATIONS MAY BE LETHAL, OTHERS ARE LESS DELETERIOUS, AND SOME MAY CONFER AN EVOLUTIONARY ADVANTAGE. MUTATIONS CAN OCCUR IN THE GERMLINE (SPERM OR OOCYTES); THESE CAN BE TRANSMITTED TO PROGENY. ALTERNATIVELY, MUTATIONS CAN OCCUR DURING EMBRYOGENESIS OR IN SOMATIC TISSUES. MUTATIONS THAT OCCUR DURING DEVELOPMENT LEAD TO MOSAICISM, A SITUATION IN WHICH TISSUES ARE COMPOSED OF CELLS WITH DIFFERENT GENETIC CONSTITUTIONS. IF THE GERMLINE IS MOSAIC, A MUTATION CAN BE TRANSMITTED TO SOME PROGENY BUT NOT OTHERS, WHICH SOMETIMES LEADS TO CONFUSION IN ASSESSING THE PATTERN OF INHERITANCE. SOMATIC MUTATIONS THAT DO NOT AFFECT CELL SURVIVAL CAN SOMETIMES BE DETECTED BECAUSE OF VARIABLE PHENOTYPIC EFFECTS IN TISSUES (E.G., PIGMENTED LESIONS IN MCCUNE-ALBRIGHT SYNDROME). OTHER SOMATIC MUTATIONS ARE ASSOCIATED WITH NEOPLASIA BECAUSE THEY CONFER A GROWTH ADVANTAGE TO CELLS. EPIGENETIC EVENTS, HERITABLE CHANGES THAT DO NOT INVOLVE CHANGES IN GENE SEQUENCE (E.G., ALTERED DNA METHYLATION), MAY INFLUENCE GENE EXPRESSION OR FACILITATE GENETIC DAMAGE. WITH THE EXCEPTION OF TRIPLET NUCLEOTIDE REPEATS, WHICH CAN EXPAND (SEE BELOW), MUTATIONS ARE USUALLY STABLE. MUTATIONS ARE STRUCTURALLY DIVERSE-THEY CAN INVOLVE THE ENTIRE GENOME, AS IN TRIPLOIDY (ONE EXTRA SET OF CHROMOSOMES), OR GROSS NUMERICAL OR STRUCTURAL ALTERATIONS IN CHROMOSOMES OR INDIVIDUAL GENES (CHAP. 63). LARGE DELETIONS MAY AFFECT A PORTION OF A GENE OR AN ENTIRE GENE, OR, IF SEVERAL GENES ARE INVOLVED, THEY MAY LEAD TO A CONTIGUOUS GENE SYNDROME. UNEQUAL CROSSING-OVER BETWEEN HOMOLOGOUS GENES CAN RESULT IN FUSION GENE MUTATIONS, AS ILLUSTRATED BY COLOR BLINDNESS (CHAP. 29). MUTATIONS INVOLVING SINGLE NUCLEOTIDES ARE REFERRED TO AS POINT MUTATIONS (FIG. 62-5). SUBSTITUTIONS ARE CALLED TRANSITIONS IF A PURINE IS REPLACED BY ANOTHER PURINE BASE (A G) OR IF A PYRIMIDINE IS REPLACED BY ANOTHER PYRIMIDINE (C T). CHANGES FROM A PURINE TO A PYRIMIDINE, OR VICE VERSA, ARE REFERRED TO AS TRANSVERSIONS. IF THE DNA SEQUENCE CHANGE OCCURS IN A CODING REGION AND ALTERS AN AMINO ACID, IT IS CALLED A MISSENSE MUTATION. DEPENDING ON THE FUNCTIONAL CONSEQUENCES OF SUCH A MISSENSE MUTATION, AMINO ACID SUBSTITUTIONS IN DIFFERENT REGIONS OF THE PROTEIN CAN LEAD TO DISTINCT PHENOTYPES. POLYMORPHISMS ARE SEQUENCE VARIATIONS THAT HAVE A FREQUENCY OF AT LEAST...
Usually, they do not result in a perceptible phenotype. Often they consist of single base-pair substitutions that do not alter the protein coding sequence because of the degenerate nature of the genetic code (synonymous polymorphism), although it is possible that some might alter mRNA stability, translation, or the amino acid sequence (non-synonymous polymorphism) (Fig. 62-7). These types of base substitutions are encountered frequently during genetic testing and must be distinguished from true mutations that alter protein expression or function. Small nucleotide deletions or insertions cause a shift of the codon reading frame (frameshift). Most commonly, reading frame alterations result in an abnormal protein segment of variable length before termination of translation occurs at a stop codon (nonsense mutation) (Fig. 62-5). Mutations in intronic sequences or in exon junctions may destroy or create splice donor or splice acceptor sites. Mutations may also be found in the regulatory sequences of genes, resulting in reduced gene transcription.

**Mutation Rates**  As noted before, mutations represent an important cause of genetic diversity as well as disease. Mutation rates are difficult to determine in humans because many mutations are silent and because testing is often not adequate to detect the phenotypic consequences. Mutation rates vary in different genes but are estimated to occur at a rate of ~10^-10^-10/BP per cell division. Germline mutation rates (as opposed to somatic mutations) are relevant in the transmission of genetic disease. Because the population of oocytes is established very early in development, only ~20 cell divisions are required for completed oogenesis, whereas spermatogenesis involves ~30 divisions by the time of puberty and 20 cell divisions each year thereafter. Consequently, the probability of acquiring new point mutations is much greater in the male germline than the female germline, in which rates of aneuploidy are increased (Chap. 63). Thus, the incidence of new point mutations in spermatogonia increases with paternal age (e.g., achondrodysplasia, marfan syndrome, neurofibromatosis). It is estimated that about 1 in 10 sperm carries a new deleterious mutation. The rates for new mutations are calculated most readily for autosomal dominant and X-linked disorders and are ~10^-5-10^-6/LOCUS PER GENERATION. Because most monogenic diseases are relatively rare, new mutations account for a significant fraction of cases. This is important in the context of genetic counseling, as a new mutation can be transmitted to the affected individual but does not necessarily imply that the parents are at risk to transmit the disease to other children. An exception to this is when the new mutation occurs early in germ-line development, leading to gonadal mosaicism.
UNEQUAL CROSSING-OVER  NORMALLY, DNA RECOMBINATION IN GERM CELLS OCCURS WITH REMARKABLE FIDELITY TO MAINTAIN THE PRECISE JUNCTION SITES FOR THE EXCHANGED DNA SEQUENCES (FIG. 62-3). HOWEVER, MISPAIRING OF HOMOLOGOUS SEQUENCES LEADS TO UNEQUAL CROSSOVER, WITH GENE DUPLICATION ON ONE OF THE CHROMOSOMES AND GENE DELETION ON THE OTHER CHROMOSOME. A SIGNIFICANT FRACTION OF GROWTH HORMONE (GH) GENE DELETIONS, FOR EXAMPLE, INVOLVE UNEQUAL CROSSING-OVER (CHAP. 333). THE GH GENE IS A MEMBER OF A LARGE GENE CLUSTER THAT INCLUDES A GROWTH HORMONE VARIANT GENE AS WELL AS SEVERAL STRUCTURALLY RELATED CHORIONIC SOMATOMAMMOTROPIN GENES AND PSEUDOGENES (HIGHLY HOMOLOGOUS BUT FUNCTIONALLY INACTIVE RELATIVES OF A NORMAL GENE). BECAUSE SUCH GENE CLUSTERS CONTAIN MULTIPLE HOMOLOGOUS DNA SEQUENCES ARRANGED IN TANDEM, THEY ARE PARTICULARLY PRONE TO UNDERGO RECOMBINATION AND, CONSEQUENTLY, GENE DUPLICATION OR DELETION. ON THE OTHER HAND, DUPLICATION OF THE PMP22 GENE BECAUSE OF UNEQUAL CROSSING-OVER RESULTS IN INCREASED GENE DOSAGE AND TYPE IA CHARCOT-MARIE-TOOTH DISEASE. UNEQUAL CROSSING-OVER RESULTING IN DELETION OF PMP22 CAUSES A DISTINCT NEUROPATHY CALLED HEREDITARY LIABILITY TO PRESSURE PALSY (CHAP. 379). GLUCOCORTICOID-REMEDIAL ALDOSTERONISM (GRA) IS CAUSED BY A REARRANGEMENT INVOLVING THE GENES THAT ENCODE ALDOSTERONE SYNTHASE (CYP11B2) AND STEROID 11*-HYDROXYLASE (CYP11B1), NORMALLY ARRANGED IN TANDEM ON CHROMOSOME 8Q. THESE TWO GENES ARE 95% IDENTICAL, PREDISPOSING TO GENE DUPLICATION AND DELETION BY UNEQUAL CROSSING-OVER. THE REARRANGED GENE PRODUCT CONTAINS THE REGULATORY REGIONS OF 11*-HYDROXYLASE FUSED TO THE CODING SEQUENCE OF ALDOSTERONE SYNTHETASE. CONSEQUENTLY, THE LATTER ENZYME IS EXPRESSED IN THE ADRENOCORTICOTROPIC HORMONE (ACTH)-DEPENDENT ZONA FASCICULATA OF THE ADRENAL GLAND, RESULTING IN OVERPRODUCTION OF MINERALOCORTICOIDS AND HYPERTENSION (CHAP. 336).

GENE CONVERSION REFERS TO A NONRECIPROCAL EXCHANGE OF HOMOLOGOUS GENETIC INFORMATION; IT IS PROBABLY MORE COMMON THAN GENERALLY RECOGNIZED. IN HUMAN GENETICS, GENE CONVERSION HAS BEEN USED TO EXPLAIN HOW AN INTERNAL PORTION OF A GENE IS REPLACED BY A HOMOLOGOUS SEGMENT COPIED FROM ANOTHER ALLELE OR LOCUS; THESE GENETIC ALTERATIONS MAY RANGE FROM A FEW NUCLEOTIDES TO A FEW THOUSAND NUCLEOTIDES. AS A RESULT OF GENE CONVERSION, IT IS POSSIBLE FOR SHORT DNA SEGMENTS OF TWO CHROMOSOMES TO BE IDENTICAL, EVEN THOUGH THESE SEQUENCES ARE DISTINCT IN THE PARENTS. A PRACTICAL CONSEQUENCE OF THIS PHENOMENON IS THAT NUCLEOTIDE SUBSTITUTIONS CAN OCCUR DURING GENE CONVERSION BETWEEN RELATED GENES, OFTEN ALTERING THE FUNCTION OF THE GENE. IN DISEASE STATES, GENE CONVERSION OFTEN INVOLVES INTERGENIC EXCHANGE OF DNA BETWEEN A GENE AND A RELATED PSEUDOGENE. FOR EXAMPLE, THE 21-HYDROXYLASE GENE (CYP21A2) IS ADJACENT TO A NONFUNCTIONAL PSEUDOGENE (CYP21A1P). MANY OF THE NUCLEOTIDE SUBSTITUTIONS THAT ARE FOUND IN THE CYP21A2 GENE IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA CORRESPOND TO SEQUENCES THAT ARE PRESENT IN THE CYP21A1P PSEUDOGENE, SUGGESTING GENE CONVERSION AS A MECHANISM OF MUTAGENESIS. IN ADDITION, MITOTIC GENE CONVERSION HAS BEEN SUGGESTED AS A MECHANISM TO EXPLAIN REVERTANT MOSAICISM IN WHICH AN INHERITED MUTATION IS “CORRECTED” IN CERTAIN CELLS. FOR EXAMPLE, PATIENTS WITH AUTOSOMAL RECESSIVE
GENERALIZED ATROPHIC BENIGN EPIDERMOLYSIS BULLOSA HAVE ACQUIRED REVERSE MUTA-
TIONS IN ONE OF THE TWO MUTATED COL17A1 ALLELES, LEADING TO CLINICALLY
UNAFFECTED PATCHES OF SKIN.

**INSERTIONS AND DELETIONS** THOUGH MANY INSTANCES OF INSERTIONS AND
DELETIONS OCCUR AS A CONSEQUENCE OF UNEQUAL CROSSING-OVER, THERE IS
ALSO EVIDENCE FOR INTERNAL DUPLICATION, INVERSION, OR DELETION OF DNA
SEQUENCES. THE FACT THAT CERTAIN DELETIONS OR INSERTIONS APPEAR TO
REPEATEDLY OCCUR REPEATEDLY AS INDEPENDENT EVENTS SUGGESTS THAT SPECIFIC REGIONS WITHIN
THE DNA SEQUENCE PREDISPOSE TO THESE ERRORS. FOR EXAMPLE, CERTAIN RE-
GIONS OF THE DMD GENE APPEAR TO BE HOT SPOTS FOR DELETIONS. SOME RE-
GIONS WITHIN THE HUMAN GENOME ARE REARRANGEMENT HOT SPOTS AND LEAD
TO CNVS (SEE ABOVE).

**ERRORS IN DNA REPAIR** BECAUSE MUTATIONS CAUSED BY DEFECTS IN DNA
REPAIR ACCUMULATE AS SOMATIC CELLS DIVIDE, THESE TYPES OF MUTATIONS ARE
PARTICULARLY IMPORTANT IN THE CONTEXT OF NEOPLASTIC DISORDERS (CHAP,
80). SEVERAL GENETIC DISORDERS INVOLVING DNA REPAIR ENZYMES UNDER-
SCORE THEIR IMPORTANCE. PATIENTS WITH XERODERMA PIGMENTOSUM HAVE
DEFECTS IN DNA DAMAGE RECOGNITION OR IN THE NUCLEOTIDE EXCISION AND
REPAIR PATHWAY (CHAP. 83). EXPOSED SKIN IS DRY AND PIGMENTED AND IS
EXTRAORDINARILY SENSITIVE TO THE MUTAGENIC EFFECTS OF ULTRAVIOLET
IRRADIA-
TION. MORE THAN 10 DIFFERENT GENES HAVE BEEN SHOWN TO CAUSE THE DI-
FERENT FORMS OF XERODERMA PIGMENTOSUM. THIS FINDING IS CONSISTENT
WITH THE EARLIER CLASSIFICATION OF THIS DISEASE INTO DIFFERENT
COMPLEmen-
TATION GROUPS IN WHICH NORMAL FUNCTION IS RESCUED BY THE FUSION OF
CELLS DERIVED FROM TWO DIFFERENT FORMS OF XERODERMA PIGMENTOSUM.
ATAXIA TELANGIECTASIA CAUSES LARGE TELANGIECTATIC LESIONS OF THE FACE,
CEREBELLAR ATAXIA, IMMUNOLOGIC DEFECTS, AND HYPERSENSITIVITY TO
IONIZING
RADIATION (CHAP. 368). THE DISCOVERY OF THE ATAXIA TELANGIECTASIA
MUTAT-
ED (ATM) GENE REVEALS THAT IT IS HOMOLOGOUS TO GENES INVOLVED IN DNA
REPAIR AND CONTROL OF CELL CYCLE CHECKPOINTS. MUTATIONS IN THE ATM
GENE
GIVE RISE TO DEFECTS IN MEIOSIS AS WELL AS INCREASING SUSCEPTIBILITY TO
DAM-
AGE FROM IONIZING RADIATION. FANCONI’S ANEMIA IS ALSO ASSOCIATED WITH AN
INCREASED RISK OF MULTIPLE ACQUIRED GENETIC ABNORMALITIES. IT IS
CHARAC-
TERIZED BY DIVERSE CONGENITAL ANOMALIES AND A STRONG PREDISPOSITION TO
DEVELOP APLASTIC ANEMIA AND ACUTE MYELOGENOUS LEUKEMIA (CHAP. 104).
CELLS FROM THESE PATIENTS ARE SUSCEPTIBLE TO CHROMOSOMAL BREAKS
CAUSED
BY A DEFECT IN GENETIC RECOMBINATION. AT LEAST EIGHT DIFFERENT COMPLE-
MENTATION GROUPS HAVE BEEN IDENTIFIED, AND SEVERAL LOCI AND GENES
ASSO-
CIATED WITH FANCONI’S ANEMIA HAVE BEEN MAPPED OR CLONED. HNPCC
(LYNCH SYNDROME) IS CHARACTERIZED BY AUTOSOMAL DOMINANT TRANSMIS-
SION OF COLON CANCER, YOUNG AGE (<50 YEARS) OF PRESENTATION, PREDISPOSITION TO LESIONS IN THE PROXIMAL LARGE BOWEL, AND ASSOCIATED MALIGNANCIES SUCH AS UTERINE CANCER AND OVARIAN CANCER. HNPCC IS CAUSED BY MUTATIONS IN ONE OF SEVERAL DIFFERENT MISMATCH REPAIR (MMR) GENES INCLUDING MUTS HOMOLOGUE 2 (MSH2), MUTL HOMOLOGUE 1 (MLH1), AND MSH6 (CHAP. 87). THESE ENZYMES ARE INVOLVED IN THE DETECTION OF NUCLEOTIDE MISMATCHES AND IN THE RECOGNITION OF SLIPPED-STRAND TRINUCLEOTIDE REPEATS. GERMINE MUTATIONS IN THESE GENES LEAD TO MICROSATELLITE INSTABILITY AND A HIGH MUTATION RATE IN COLON CANCER. GENETIC SCREENING TESTS FOR THIS DISORDER ARE NOW BEING USED FOR FAMILIES CONSIDERED TO BE AT RISK (CHAP. 64). RECOGNITION OF HNPCC ALLOWS EARLY SCREENING WITH COLONOSCOPY AND THE IMPLEMENTATION OF PREVENTION STRATEGIES USING NONSTEROIDAL ANTI-INFLAMMATORY DRUGS.

PAGE NO. 43

396 PART 3: GENETICS AND DISEASE

DIPYRIMIDINE AND CPG SEQUENCES CERTAIN DNA SEQUENCES ARE PARTICULARLY SUSCEPTIBLE TO MUTAGENESIS. SUCCESSIVE PYRIMIDINE RESIDUES (E.G., T-T OR C-C) ARE SUBJECT TO THE FORMATION OF ULTRAVIOLET LIGHT-INDUCED PHOTOADDUCTS. IF THESE PYRIMIDINE DIMERS ARE NOT REPAIRED BY THE NUCLEOTIDE EXCISION REPAIR PATHWAY, MUTATIONS WILL BE INTRODUCED AFTER DNA SYNTHESIS. THE DINUCLEOTIDE C-G, OR CPG, IS ALSO A HOT SPOT FOR A SPECIFIC TYPE OF MUTATION. IN THIS CASE, METHYLATION OF THE CYTOSINE IS ASSOCIATED WITH AN ENHANCED RATE OF DEAMINATION TO URACIL, WHICH IS THEN REPLACED WITH THYMINE. THIS C *T TRANSITION (OR G *A ON THE OPPOSITE STRAND) ACCOUNTS FOR AT LEAST ONE-THIRD OF POINT MUTATIONS ASSOCIATED WITH POLYMORPHISMS AND MUTATIONS. MANY OF THE MSH2 MUTATIONS IN HNPCC, FOR EXAMPLE, INVOLVE CPG SEQUENCES. IN ADDITION TO THE FACT THAT CERTAIN TYPES OF MUTATIONS (C *T OR G * A) ARE RELATIVELY COMMON, THE NATURE OF THE GENETIC CODE ALSO RESULTS IN OVERREPRESENTATION OF CERTAIN AMINO ACID SUBSTITUTIONS.

UNSTABLE DNA SEQUENCES TRINUCLEOTIDE REPEATS MAY BE UNSTABLE AND EXPAND BEYOND A CRITICAL NUMBER. MECHANISTICALLY, THE EXPANSION IS THOUGHT TO BE CAUSED BY UNEQUAL RECOMBINATION AND SLIPPED MISPAIRING. A PREMUTATION REPRESENTS A SMALL INCREASE IN TRINUCLEOTIDE COPY NUMBER. IN SUBSEQUENT GENERATIONS, THE EXPANDED REPEAT MAY INCREASE FURTHER IN LENGTH AND RESULT IN AN INCREASINGLY SEVERE PHENOTYPE, A PROCESS CALLED DYNAMIC MUTATION (SEE BELOW FOR DISCUSSION OF ANTICIPATION). TRINUCLEOTIDE EXPANSION WAS FIRST RECOGNIZED AS A CAUSE OF THE FRAGILE X SYNDROME, ONE OF THE MOST COMMON CAUSES OF MENTAL RETARDATION. OTHER DISORDERS ARISING FROM A SIMILAR MECHANISM INCLUDE HUNTINGTON DISEASE (CHAP. 365), X-LINKED SPINOBULBAR MUSCULAR ATROPHY (CHAP. 369), AND MYOTONIC DYSTROPHY (CHAP. 382). MALIGNANT CELLS ARE ALSO CHARACTERIZED BY GENETIC INSTABILITY, INDICATING A
BREAK-DOWN IN MECHANISMS THAT REGULATE DNA REPAIR AND THE CELL CYCLE.

FUNCTIONAL CONSEQUENCES OF MUTATIONS FUNCTIONALLY, MUTATIONS CAN BE BROADLY CLASSIFIED AS GAIN-OF-FUNCTION AND LOSS-OF-FUNCTION MUTATIONS. GAIN-OF-FUNCTION MUTATIONS ARE TYPICALLY DOMINANT, I.E., THEY RESULT IN PHENOTYPIC ALTERATIONS WHEN A SINGLE ALLELE IS AFFECTED. INACTIVATING MUTATIONS ARE USUALLY RECESSIVE, AND AN AFFECTED INDIVIDUAL IS HOMOZYGOUS OR COMPOUND HETEROZYGOUS (E.G., CARRYING TWO DIFFERENT MUTANT ALLELES OF THE SAME GENE) FOR THE DISEASE-CAUSING MUTATIONS. ALTERNATIVELY, MUTATION IN A SINGLE ALLELE CAN RESULT IN HAPLOINSUFFICIENCY, A SITUATION IN WHICH ONE NORMAL ALLELE IS NOT SUFFICIENT TO MAINTAIN A NORMAL PHENOTYPE. HAPLOINSUFFICIENCY IS A COMMONLY OBSERVED MECHANISM IN DISEASES ASSOCIATED WITH MUTATIONS IN TRANSCRIPTION FACTORS (TABLE 62-2). REMARKABLY, THE CLINICAL FEATURES AMONG PATIENTS WITH AN IDENTICAL MUTATION IN A TRANSCRIPTION FACTOR OFTEN VARY SIGNIFICANTLY. ONE MECHANISM UNDERLYING THIS VARIABILITY CONSISTS IN THE INFLUENCE OF MODIFYING GENES. HAPLOINSUFFICIENCY CAN ALSO AFFECT THE EXPRESSION OF RATE-LIMITING ENZYMES. FOR EXAMPLE, HAPLOINSUFFICIENCY IN ENZYMES INVOLVED IN HEME SYNTHESIS CAN CAUSE PORPHYRIAS (CHAP. 352). AN INCREASE IN DOSAGE OF A GENE PRODUCT MAY ALSO RESULT IN DISEASE, AS ILLUSTRATED BY THE DUPLICATION OF THE DAX1 GENE IN DOSAGE-SENSITIVE SEX-REVERSAL (CHAP. 343). MUTATION IN A SINGLE ALLELE CAN ALSO RESULT IN LOSS OF FUNCTION DUE TO A DOMINANT-NEGATIVE EFFECT. IN THIS CASE, THE MUTATED ALLELE INTERFERES WITH THE FUNCTION OF THE NORMAL GENE PRODUCT BY ONE OF SEVERAL DIFFERENT MECHANISMS: (1) A MUTANT PROTEIN MAY INTERFERE WITH THE FUNCTION OF A MULTIMERIC PROTEIN COMPLEX, AS ILLUSTRATED BY MUTATIONS IN TYPE 1 COLLAGEN (COL1A1, COL1A2) GENES IN OSTEOGENESIS IMPERFECTA (CHAP. 357); (2) A MUTANT PROTEIN MAY OCCUPY BINDING SITES ON PROTEINS OR PROMOTER RESPONSE ELEMENTS, AS ILLUSTRATED BY THYROID HORMONE RESISTANCE, A DISORDER IN WHICH INACTIVATED THYROID HORMONE RECEPTOR BINDS TO TARGET GENES AND FUNCTIONS AS AN ANTAGONIST OF NORMAL RECEPTORS (CHAP. 335); OR (3) A MUTANT PROTEIN CAN BE CYTOTOXIC AS IN *###1 ANTITRYPSIN DEFICIENCY (CHAP. 254) OR AUTOSOMAL DOMINANT NEUROHYPOPHYSEAL DIABETES INSIPIDUS (CHAP. 334), IN WHICH THE ABNORMALLY FOLDED PROTEINS ARE TRAPPED WITHIN THE ENDOPLASMIC RETICULUM AND ULTIMATELY CAUSE CELLULAR DAMAGE.

GENOTYPE AND PHENOTYPE * ALLELES, GENOTYPES, AND HAPLOTYPES
AN OBSERVED TRAIT IS REFERRED TO AS A **PHENOTYPE**; THE GENETIC INFORMATION DEFINING THE PHENOTYPE IS CALLED THE **GENOTYPE**. ALTERNATIVE FORMS OF A GENE OR A GENETIC MARKER ARE REFERRED TO AS **ALLELES**. ALLELES MAY BE POLYMORPHIC VARIANTS OF NUCLEIC ACIDS THAT HAVE NO APPARENT EFFECT ON GENE EXPRESSION OR FUNCTION. IN OTHER INSTANCES, THESE VARIANTS MAY HAVE SUBTLE EFFECTS ON GENE EXPRESSION, THEREBY CONFERRING THE ADAPTIVE ADVANTAGES ASSOCIATED WITH GENETIC DIVERSITY. ON THE OTHER HAND, ALLELIC VARIANTS MAY REFLECT MUTATIONS IN A GENE THAT CLEARLY ALTER ITS FUNCTION.

THE COMMON GLU6VAL (E6V) SICKLE CELL MUTATION IN THE *-**GLOBIN** GENE AND THE *F508 DELETION OF PHENYLALANINE (F) IN THE **CFTR** GENE ARE EXAMPLES OF ALLELIC VARIANTS OF THESE GENES THAT RESULT IN DISEASE. BECAUSE EACH INDIVIDUAL HAS TWO COPIES OF EACH CHROMOSOME (ONE INHERITED FROM THE MOTHER AND ONE INHERITED FROM THE FATHER), HE OR SHE CAN HAVE ONLY TWO ALLELES AT A GIVEN LOCUS. HOWEVER, THERE CAN BE MANY DIFFERENT ALLELES IN THE POPULATION. THE NORMAL OR COMMON ALLELE IS USUALLY REFERRED TO AS **WILD TYPE**. WHEN ALLELES AT A GIVEN LOCUS ARE IDENTICAL, THE INDIVIDUAL IS **HOMOZYGOUS**. INHERITING IDENTICAL COPIES OF A MUTANT ALLELE OCCURS IN MANY AUTOSOMAL RECESSIVE DISORDERS, PARTICULARLY IN CIRCUMSTANCES OF CONSANGUINITY. IF THE ALLELES ARE DIFFERENT ON THE MATERNAL AND THE PATERNAL COPY OF THE GENE, THE INDIVIDUAL IS **HETEROZYGOUS** AT THIS LOCUS (FIG. 62-5). IF TWO DIFFERENT MUTANT ALLELES ARE INHERITED AT A GIVEN LOCUS, THE INDIVIDUAL IS SAID TO BE A **COMPOUND HETEROZYGOTE**.

**Hemizygous** is used to describe males with a mutation in an X CHROMOSOMAL GENE OR A FEMALE WITH A LOSS OF ONE X CHROMOSOMAL LOCUS. GENOTYPES DESCRIBE THE SPECIFIC ALLELES AT A PARTICULAR LOCUS. FOR EXAMPLE, THERE ARE THREE COMMON ALLELES (E2, E3, E4) OF THE **APOLIPOPROTEIN E (APOE)** GENE. THE GENOTYPE OF AN INDIVIDUAL CAN THEREFORE BE DESCRIBED AS **APOE3/4** OR **APOE4/4** OR ANY OTHER VARIANT. THESE DESIGNATIONS INDICATE WHICH ALLELES ARE PRESENT ON THE TWO CHROMOSOMES IN THE APOE GENE AT LOCUS 19Q13.2. IN OTHER CASES, THE GENOTYPE MIGHT BE ASSIGNED ARBITRARY NUMBERS (E.G., 1/2) OR LETTERS (E.G., B/B) TO DISTINGUISH DIFFERENT ALLELES. A **HAPLOTYPE** REFERS TO A GROUP OF ALLELES THAT ARE CLOSELY LINKED TOGETHER AT A GENOMIC LOCUS (FIG. 62-8). HAPLOTYPES ARE USEFUL FOR TRACKING THE TRANSMISSION OF GENOMIC SEGMENTS WITHIN FAMILIES AND FOR DETECTING EVIDENCE OF GENETIC RECOMBINATION, IF THE CROSSOVER EVENT OCCURS BETWEEN THE ALLELES (FIG. 62-3). AS AN EXAMPLE, VARIOUS ALLELES AT THE HISTOCOMPATIBILITY LOCUS ANTIGEN (HLA) ON CHROMOSOME 6P ARE USED TO ESTABLISH HAPLOTYPES ASSOCIATED WITH CERTAIN DISEASE STATES. FOR EXAMPLE, 21-HYDROXYLASE DEFICIENCY, COMPLEMENT DEFICIENCY, AND HEMOCHROMATOSIS ARE EACH ASSOCIATED WITH SPECIFIC HLA HAPLOTYPES. IT IS NOW RECOGNIZED THAT THESE GENES LIE IN CLOSE VICINITY TO THE HLA LOCUS, WHICH EXPLAINS WHY HLA ASSOCIATIONS WERE IDENTIFIED EVEN BEFORE THE
DISEASE GENES WERE CLONED AND LOCALIZED. IN OTHER CASES, SPECIFIC HLA ASSOCIATIONS WITH DISEASES SUCH AS ANKYLOSING SPONDYLITIS (HLA-B27) OR TYPE 1 DIABETES MELLITUS (HLA-DR4) REFLECT THE ROLE OF SPECIFIC HLA ALLELIC VARIANTS IN SUSCEPTIBILITY TO THESE AUTOIMMUNE DISEASES. THE RECENT CHARACTERIZATION OF COMMON SNP HAPLOTYPES IN FOUR POPULATIONS FROM DIFFERENT PARTS OF THE WORLD THROUGH THE HAPMAP PROJECT IS PROVIDING A NOVEL TOOL FOR ASSOCIATION STUDIES DESIGNED TO DETECT GENES INVOLVED IN THE PATHOGENESIS OF COMPLEX DISORDERS (TABLE 62-1). THE PRESENCE OR ABSENCE OF CERTAIN HAPLOTYPES MAY ALSO BECOME RELEVANT FOR THE CUSTOMIZED CHOICE OF MEDICAL THERAPIES (PHARMACOGENOMICS) OR FOR PREVENTATIVE STRATEGIES.

ALLELIC HETEROGENEITY ALLELIC HETEROGENEITY REFERS TO THE FACT THAT DIFFERENT MUTATIONS IN THE SAME GENETIC LOCUS CAN CAUSE AN IDENTICAL OR SIMILAR PHENOTYPE. FOR EXAMPLE, MANY DIFFERENT MUTATIONS OF THE *-GLOBIN LOCUS CAN CAUSE *-THALASSEMIA (TABLE 62-4) (FIG. 62-4). IN ESSENCE, ALLELIC HETEROGENEITY REFLECTS THE FACT THAT MANY DIFFERENT MUTATIONS ARE CAPABLE OF ALTERING PROTEIN STRUCTURE AND FUNCTION. FOR THIS REASON, MAPS OF INACTIVATING MUTATIONS IN GENES USUALLY SHOW A NEAR-RANDOM DISTRIBUTION. EXCEPTIONS INCLUDE: (1) A FOUNDER EFFECT, IN WHICH A PARTICULAR MUTATION THAT DOES NOT AFFECT REPRODUCTIVE CAPACITY CAN BE TRACED TO A SINGLE INDIVIDUAL; (2) “HOT SPOTS” FOR MUTATIONS, IN WHICH THE NATURE OF THE DNA SEQUENCE PREDISPOSES TO A RECURRING MUTATION; AND (3) LOCALIZATION OF MUTATIONS TO CERTAIN DOMAINS THAT ARE PARTICULARLY CRITICAL FOR PROTEIN FUNCTION. ALLELIC HETEROGENEITY CREATES A PRACTICAL PROBLEM FOR GENETIC TESTING BECAUSE ONE MUST OFTEN EXAMINE THE ENTIRE GENETIC LOCUS FOR MUTATIONS, AS THESE CAN DIFFER IN EACH PA-

397 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS

<table>
<thead>
<tr>
<th>GENE, PROTEIN</th>
<th>PHENOTYPIC HETEROGENEITY</th>
<th>PHENOTYPIC HETEROGENEITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMNA, LAMIN A/C</td>
<td>EMERY-DREIFUSS MUSCULAR DYSTROPHY (AD)</td>
<td>FAMILIAL PARTIAL LIPODYSTROPHY DUNNIGAN</td>
</tr>
</tbody>
</table>
HUTCHINSON-GILFORD PROGERIA
ATYPICAL WERNER SYNDROME
DILATED CARDIOMYOPATHY
EARLY-ONSET ATRIAL FIBRILLATION
EMERY-DREIFUSS MUSCULAR DYSTROPHY (AR)
LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE IB
CHARCOT-MARIE-TOOTH TYPE2B1
NOONAN SYNDROME
CARDIO-FACIO-CUTANEOUS SYNDROME

INHERITANCE

AD
AD
AD
AD
AD
AR
AR
AR
AD
AD

OMIM

181350
151660
176670
150330
115200
607554
604929
159001
605588
163950
115150

LOCUS HETEROGENEITY

CHROMOSOMAL

PHENOTYPE

FAMILIAL HYPERTROPHIC CARDIOMYOPATHY
GENES ENCODING SARCOMERIC PROTEINS

GENES ENCODING
NONSARCOMERIC PROTEINS

POLYCYSTIC KIDNEY DISEASE
NOONAN SYNDROME

GENE

MYH7
TNNT2
TPM1
MYBPC3
TNN13
MYL2
MYL3
TTN
ACTC
MYH6
MYLK2
CAV3
MTT1
MTTG
PRKAG2

DMPK

FRDA
PKD1
PKD2
PKHD1
PTPN11
KRAS

LOCATION

14Q12
1Q2
15Q22.1
11P11Q
19Q13.4
12Q23-24.3
3P
2Q24.3
15Q11
14Q1
20Q13.3
3P25
MITOCHONDRIAL
MITOCHONDRIAL
7Q35-Q36

19Q13.2-13.3

9Q13
16P13.3-13.12
4Q21 -23
6P21.1-P12
12Q24.1
PROTEIN

MYOSIN HEAVY CHAIN BETA
TROPONIN-T2
TROPOMYOSIN ALPHA
MYOSIN BINDING PROTEIN C
TROPONIN 1
MYOSIN LIGHT CHAIN 2
MYOSIN LIGHT CHAIN 3
CARDIAC TITIN
CARDIAC ALPHA ACTIN
MYOSIN HEAVY CHAIN ALPHA
MYOSIN LIGHT-PEPTIDE KINASE
CAVEOLIN 3
TRNA ISOLEUCINE
TRNA GLYCINE
AMP-ACTIVATED PROTEIN KINASE *2
SUBUNIT
MYOTONIN PROTEIN KINASE
(MYOTONIC DYSTROPHY)
FRATAxin (FRIEDREICH ATAXIA)
POLycystin 1 (AD)
POLycystin 2 (AD)
FIBROCYSTIN (AR)
PROTEIN-TYROSINE PHOSPHATASE 2C
KRAS

NOTE: AD, AUTOSOMAL DOMINANT; AR, AUTOSOMAL RECESSIVE.

TIENT. FOR EXAMPLE, THERE ARE >1400 REPORTED MUTATIONS IN THE CFTR GENE (FIG. 62-7). THE MUTATIONAL ANALYSIS INITIALLY FOCUSES ON A PANEL OF MUTATIONS THAT ARE PARTICULARLY FREQUENT (OFTEN TAKING THE ETHNIC BACK-
GROUND OF THE PATIENT INTO ACCOUNT), BUT A NEGATIVE RESULT DOES NOT EX-
CLUDE THE PRESENCE OF A MUTATION ELSEWHERE IN THE GENE. ONE SHOULD
ALSO BE AWARE THAT MUTATIONAL ANALYSES GENERALLY FOCUS ON THE
CODING REGION OF A GENE WITHOUT CONSIDERING REGULATORY AND INTRONIC REGIONS. BECAUSE DISEASE-CAUSING MUTATIONS MAY BE LOCATED OUTSIDE THE CODING REGIONS, NEGATIVE RESULTS SHOULD BE INTERPRETED WITH CAUTION.

PHENOTYPIC HETEROGENEITY PHENOTYPIC HETEROGENEITY OCCURS WHEN MORE THAN ONE PHENOTYPE IS CAUSED BY ALLELIC MUTATIONS (E.G., DIFFERENT MUTA-
TIONS IN THE SAME GENE) (TABLE 62-4). FOR EXAMPLE, LAMINOPATHIES ARE MONOGENIC MULTISYSTEM DISORDERS THAT RESULT FROM MUTATIONS IN THE LMNA GENE, WHICH ENCODES THE NUCLEAR LAMINS A AND C. TWELVE AUTOSO-
MAL DOMINANT AND FOUR AUTOSOMAL RECESSIVE DISORDERS ARE CAUSED BY MU-
TATIONS IN THE LMNA GENE. THEY INCLUDE SEVERAL FORMS OF LIPODYSTROPHIES,
EMERY-DREIFUSS MUSCULAR DYSTROPHY, PROGERIA SYNDROMES, A FORM OF NEU
RONAL CHARCOT-MARIE-TOOTH DISEASE (TYPE 2B1), AND A GROUP OF OVERLAP-
PING SYNDROMES. REMARKABLY, HIERARCHICAL CLUSTER ANALYSIS HAS REVEALED THAT THE PHENOTYPES VARY DEPENDING ON THE POSITION OF THE MUTATION. SIMILARLY, IDENTICAL MUTATIONS IN THE FGFR2 GENE CAN RESULT IN VERY DISTINCT PHENOTYPES: CROUZON SYNDROME (CRANIOFACIAL SYNOSTOSIS), OR PFEIFFER SYNDROME (ACROCEPHALOPOLYSYNDACTYL).  

LOCUS OR NONALLELIC HETEROGENEITY AND PHENOCOPIES  NONALLELIC OR LOCUS HETEROGENEITY  

REFERS TO THE SITUATION IN WHICH A SIMILAR DISEASE PHENOTYPE RESULTS FROM MUTATIONS AT DIFFERENT GENETIC LOCI. THIS OFTEN OCCURS WHEN MORE THAN ONE GENE PRODUCT PRODUCES DIFFERENT SUBUNITS OF AN INTERACTING COMPLEX OR WHEN DIFFERENT GENES ARE INVOLVED IN THE SAME GENETIC CASCADE OR PHYSIOLOGIC PATHWAY. FOR EXAMPLE, OSTEOGENESIS IMPERFECTA CAN ARISE FROM MUTATIONS IN TWO DIFFERENT PROCOLLAGEN GENES (COL1A1 OR COL1A2) THAT ARE LOCATED ON DIFFERENT CHROMOSOMES (CHAP. 357). THE EFFECTS OF INACTIVATING MUTATIONS IN THESE TWO GENES ARE SIMILAR BECAUSE THE PROTEIN PRODUCTS COMPRISÉ DIFFERENT SUBUNITS OF THE HELICAL COLLAGEN FIBER. SIMILARLY, MUSCULAR DYSTROPHY SYNDROMES CAN BE CAUSED BY MUTATIONS IN VARIOUS GENES, CONSISTENT WITH THE FACT THAT IT CAN BE TRANSMITTED IN AN X-LINKED (DUCHENNE OR BECKER), AUTOSOMAL DOMINANT (LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 1), OR AUTOSOMAL RECESSIVE (LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 2) MANNER (CHAP. 382). MUTATIONS IN THE X-LINKED DMD GENE, WHICH ENCODES DYSTROPHIN, ARE THE MOST COMMON CAUSE OF MUSCULAR DYSTROPHY. THIS FEATURE REFLECTS THE LARGE SIZE OF THE GENE AS WELL AS THE FACT THAT THE PHENOTYPE IS EXPRESSED IN HEMIZYGOUS MALES BECAUSE THEY HAVE ONLY A SINGLE COPY OF THE X CHROMOSOME. DYSTROPHIN IS ASSOCIATED WITH A LARGE PROTEIN COMPLEX LINKED TO THE MEMBRANE-ASSOCIATED CYTOSKELETON IN MUSCLE. MUTATIONS IN SEVERAL DIFFERENT COMPONENTS OF THIS PROTEIN COMPLEX CAN ALSO CAUSE MUSCULAR DYSTROPHY SYNDROMES. ALTHOUGH THE PHENOTYPIC FEATURES OF SOME OF THESE DISORDERS ARE DISTINCT, THE PHENOTYPIC SPECTRUM CAUSED BY MUTATIONS IN DIFFERENT GENES OVERLAPS, THEREBY LEADING TO NONALLELIC HETEROGENEITY. IT SHOULD BE NOTED THAT MUTATIONS IN DYSTROPHIN ALSO CAUSE ALLELIC HETEROGENEITY. FOR EXAMPLE, MUTATIONS IN THE DMD GENE CAN CAUSE EITHER DUCHENNE OR THE LESS SEVERE BECKER MUSCULAR DYSTROPHY, DEPENDING ON THE SEVERITY OF THE PROTEIN DEFECT.  

RECOGNITION OF NONALLELIC HETEROGENEITY IS IMPORTANT FOR SEVERAL REASONS: (1) THE ABILITY TO IDENTIFY DISEASE LOCI IN LINKAGE STUDIES IS REDUCED
BY INCLUDING PATIENTS WITH SIMILAR PHENOTYPES BUT DIFFERENT GENETIC DISORDERS; (2) GENETIC TESTING IS MORE COMPLEX BECAUSE SEVERAL DIFFERENT GENES NEED TO BE CONSIDERED ALONG WITH THE POSSIBILITY OF DIFFERENT MUTATIONS IN EACH OF THE CANDIDATE GENES; AND (3) NOVEL INFORMATION IS GAINED ABOUT HOW GENES OR PROTEINS INTERACT, PROVIDING UNIQUE INSIGHTS INTO MOLECULAR PHYSIOLOGY.

**Phenocopies** refer to circumstances in which nongenetic conditions mimic a genetic disorder. For example, features of toxin- or drug-induced neurologic syndromes can resemble those seen in Huntington disease, and vascular causes of dementia share phenotypic features with familial forms of Alzheimer dementia (Chap. 365). Children born with activating mutations of the thyroid-stimulating hormone receptor (TSH-R) exhibit goiter and thyrotoxicosis similar to that seen in neonatal Graves' disease, which is caused by the transfer of maternal autoantibodies to the fetus (Chap. 335). As in nonallelic heterogeneity, the presence of phenocopies has the potential to confound linkage studies and genetic testing. Patient history and subtle differences in phenotype can often provide clues that distinguish these disorders from related genetic conditions.

**Variable expressivity and incomplete penetrance** the same genetic mutation may be associated with a phenotypic spectrum in different affected individuals, thereby illustrating the phenomenon of variable expressivity. This may include different manifestations of a disorder variably involving different organs (e.g., men), the severity of the disorder (e.g., cystic fibrosis), or the age of disease onset (e.g., Alzheimer dementia). Men-1 illustrates several of these features. Families with this autosomal dominant disorder develop tumors of the parathyroid gland, endocrine pancreas, and the pituitary gland (Chap. 345). However, the pattern of tumors in the different glands, the age at which tumors develop, and the types of hormones produced vary among affected individuals, even within a given family. In this example, the phenotypic variability arises, in part, because of the requirement for a second mutation in the normal copy of the Men1 gene, as well as the large array of different cell types that are susceptible to the effects of Men1 gene mutations. In part, variable expression reflects the influence of modifier genes, or genetic background, on the effects of a particular mutation. Even in identical twins, in whom the genetic constitution is essentially the same, one can occasionally see variable expression of a genetic disease. Interactions with the environment can also influence the course of a disease. For example, the manifestations and severity of hemochromatosis can be influenced by iron intake (Chap. 351), and the course of
PHENYLKETONURIA IS AFFECTED BY EXPOSURE TO PHENYLALANINE IN THE DIET (CHAP. 358). OTHER METABOLIC DISORDERS, SUCH AS HYPERLIPIDEMIAS AND PORPHYRIA, ALSO FALL INTO THIS CATEGORY. MANY MECHANISMS, INCLUDING GENETIC EFFECTS AND ENVIRONMENTAL INFLUENCES, CAN THEREFORE LEAD TO VARIABLE EXPRESSIVITY. IN GENETIC COUNSELING, IT IS PARTICULARLY IMPORTANT TO RECOGNIZE THIS VARIABILITY, AS ONE CANNOT ALWAYS PREDICT THE COURSE OF DISEASE, EVEN WHEN THE MUTATION IS KNOWN.

*Penetrance* refers to the proportion of individuals with a mutant genotype that express the phenotype. If all carriers of a mutant express the phenotype, penetrance is complete, whereas it is said to be incomplete or reduced if some individuals do not have any features of the phenotype. Dominant conditions with incomplete penetrance are characterized by skipping of generations with unaffected carriers transmitting the mutant gene. For example, hypertrophic obstructive cardiomyopathy (HCM) caused by mutations in the *Myosin-Binding Protein C* gene is a dominant disorder with clinical features in only a subset of patients who carry the mutation (CHAP. 231). Patients who have the mutation but no evidence of the disease can still transmit the disorder to subsequent generations. In many conditions with postnatal onset, the proportion of gene carriers who are affected varies with age. Thus, when describing penetrance, one has to specify age. For example, for disorders such as Huntington disease or familial amyotrophic lateral sclerosis, which present late in life, the rate of penetrance is influenced by the age at which the clinical assessment is performed.

*Imprinting* can also modify the penetrance of a disease (see below). For example, in patients with Albright hereditary osteodystrophy, mutations in the Gsa subunit (*GNAS1* gene) are expressed clinically only in individuals who inherit the mutation from their mother (CHAP. 347).

**Sex-influenced phenotypes** Certain mutations affect males and females quite differently. In some instances, this is because the gene resides on the X or Y sex chromosomes (X-linked disorders and Y-linked disorders). As a result, the phenotype of mutated X-linked genes will be expressed fully in males but variably in heterozygous females, depending on the degree of X-inactivation and the function of the gene. For example, most heterozygous female carriers of factor VIII deficiency (hemophilia A) are asymptomatic because sufficient factor VIII is produced to prevent a defect in coagulation (CHAP. 110). On the other hand, some females heterozygous for the X-linked lipid storage defect caused by *-*Galactosidase A deficiency (Fabry disease) experience mild manifestations of painful neuropathy, as well as other features of the disease (CHAP. 355). Because only males have a Y chromosome, mutations in genes such as *SRY*, which causes male-to-female sex-reversal, or *Daz* (deleted in azoospermia), which causes abnormalities of spermatogenesis, are unique to males (CHAP. 343). Other diseases are expressed in a sex-limited manner because of the differential function of the gene product in males and females.

CHROMOSOMAL DISORDERS CHROMOSOMAL OR CYTOGENETIC DISORDERS ARE CAUSED BY NUMERICAL OR STRUCTURAL ABERRATIONS IN CHROMOSOMES. DEVIATIONS IN CHROMOSOME NUMBER ARE COMMON CAUSES OF ABORTIONS, DEVELOPMENTAL DISORDERS, AND MALFORMATIONS. CONTIGUOUS GENE SYNDROMES, I.E., LARGE DELETIONS AFFECTING SEVERAL GENES, HAVE BEEN USEFUL FOR IDENTIFYING THE LOCATION OF NEW DISEASE-CAUSING GENES. BECAUSE OF THE VARIABLE SIZE OF GENE DELETIONS IN DIFFERENT PATIENTS, A SYSTEMATIC COMPARISON OF PHENOTYPES AND LOCATIONS OF DELETION BREAKPOINTS ALLOWS POSITIONS OF PARTICULAR GENES TO BE MAPPED WITHIN THE CRITICAL GENOMIC REGION. FOR DISCUSSION OF DISORDERS OF CHROMOSOME NUMBER AND STRUCTURE, SEE CHAP. 63.

MONOGENIC MENDELIAN DISORDERS MONOGENIC HUMAN DISEASES ARE FREQUENTLY REFERRED TO AS MENDELIAN DISORDERS BECAUSE THEY OBEY THE PRINCIPLES OF GENETIC TRANSMISSION ORIGINALY SET FORTH IN GREGOR MENDEL’S CLASSIC WORK. THE CONTINUOUSLY UPDATED OMIM CATALOGUE LISTS SEVERAL THOUSAND OF THESE DISORDERS AND PROVIDES INFORMATION ABOUT THE CLINICAL PHENOTYPE, MOLECULAR BASIS, ALLELIC VARIANTS, AND PERTINENT ANIMAL MODELS (TABLE 62-1). THE MODE OF INHERITANCE FOR A GIVEN PHENOTYPIC TRAIT OR DISEASE IS DETERMINED BY PEDIGREE ANALYSIS. ALL AFFECTED AND
UN-AFFECTED INDIVIDUALS IN THE FAMILY ARE RECORDED IN A PEDIGREE USING STANDARD SYMBOLS (FIG. 62-9). THE PRINCIPLES OF ALLELIC SEGREGATION, AND THE TRANSMISSION OF ALLELES FROM PARENTS TO CHILDREN, ARE ILLUSTRATED IN FIG. 62-10. ONE DOMINANT (A) ALLELE AND ONE RECESSIVE (a) ALLELE CAN DISPLAY THREE MENDELIAN MODES OF INHERITANCE: AUTOSOMAL DOMINANT, AUTOSOMAL RECESSIVE, AND X-CHROMOSOMAL. ABOUT 65% OF HUMAN MONOGENIC DISORDERS ARE AUTOSOMAL DOMINANT, 25% ARE AUTOSOMAL RECESSIVE, AND 5% ARE X-LINKED. GENETIC TESTING IS NOW AVAILABLE FOR MANY OF THESE DISORDERS AND PLAYS AN INCREASINGLY IMPORTANT ROLE IN CLINICAL MEDICINE (CHAP. 64).

AUTOSOMAL DOMINANT DISORDERS AUTOSOMAL DOMINANT DISORDERS ASSUME PARTICULAR RELEVANCE BECAUSE MUTATIONS IN A SINGLE ALLELE ARE SUFFICIENT TO CAUSE THE DISEASE. IN CONTRAST TO RECESSIVE DISORDERS, IN WHICH DISEASE PATHOGENESIS IS RELATIVELY STRAIGHTFORWARD BECAUSE THERE IS LOSS OF GENE FUNCTION, DOMINANT DISORDERS CAN BE CAUSED BY VARIOUS DISEASE MECHANISMS, MANY OF WHICH ARE UNIQUE TO THE FUNCTION OF THE GENETIC PATHWAY INVOLVED. IN AUTOSOMAL DOMINANT DISORDERS, INDIVIDUALS ARE AFFECTED IN SUCCESSIVE GENERATIONS; THE DISEASE DOES NOT OCCUR IN THE OFFSPRING OF UNAFFECTED INDIVIDUALS. MALES AND FEMALES ARE AFFECTED WITH EQUAL FREQUENCY BECAUSE THE DEFECTIVE GENE RESIDES ON ONE OF THE 22 AUTOSOMES (FIG. 62-11A). AUTOSOMAL DOMINANT MUTATIONS ALTER ONE OF THE TWO ALLELES AT A GIVEN LOCUS. BECAUSE THE ALLELES SEGREGATE RANDOMLY AT MEIOSIS, THE PROBABILITY THAT AN OFFSPRING WILL BE AFFECTED IS 50%. UNLESS THERE IS A NEW GERMLINE MUTATION, AN AFFECTED INDIVIDUAL HAS AN AF-

PAGE NO. 46

400 PART 3: GENETICS AND DISEASE

DIFFERS IN THE TWO SEXES. BECAUSE MALES HAVE ONLY ONE X CHROMOSOME, THEY ARE HEMIZYGOUS FOR THE MUTANT ALLELE; THUS, THEY ARE MORE LIKELY TO DEVELOP THE MUTANT PHENOTYPE, REGARDLESS OF WHETHER THE MUTATION IS DOMINANT OR RECESSIVE. A FEMALE MAY BE EITHER HETEROZYGOUS OR HOMOZYGOUS FOR THE MUTANT ALLELE, WHICH MAY BE DOMINANT OR RECESSIVE. THE TERMS X-LINKED DOMINANT OR X-LINKED RECESSIVE ARE THEREFORE ONLY APPLICABLE TO EXPRESSION OF THE MUTANT PHENOTYPE IN WOMEN. IN ADDITION, THE EXPRESSION OF X-CHROMOSOMAL GENES IS INFLUENCED BY X CHROMOSOME INACTIVATION (SEE BELOW).
**Y-Linked Disorders**

The Y chromosome has a relatively small number of genes. One such gene, the sex-region determining Y factor (SRY), which encodes the testis-determining factor (TDF), is crucial for normal male development. Normally there is infrequent exchange of sequences on the Y chromosome with the X chromosome. The SRY region is adjacent to the pseudoautosomal region, a chromosomal segment on the X and Y chromosomes with a high degree of homology. A crossing-over occasionally involves the SRY region with the distal tip of the X chromosome during meiosis in the male. Translocations can result in XY females with the Y chromosome lacking the SRY gene or XX males harboring the SRY gene on one of the X chromosomes (Chap. 343). Point mutations in the SRY gene may also result in individuals with an XY genotype and an incomplete female phenotype. Most of these mutations occur de novo. Men with oligospermia/azoospermia frequently have microdeletions on the long arm of the Y chromosome that involve one or more of the azoospermia factor (AZF) genes.

**Exceptions to Simple Mendelian Inheritance Patterns**

**Mitochondrial Disorders**

Mendelian inheritance refers to the transmission of genes encoded by DNA contained in the nuclear chromosomes. In addition, each mitochondrion contains several copies of a small circular chromosome. The mitochondrial DNA (mtDNA) is ~ 16.5 kb and encodes transfer and ribosomal RNAs and 13 proteins that are components of the respiratory chain involved in oxidative phosphorylation and ATP generation. The mitochondrial genome does not recombine and is inherited through the maternal line because sperm does not contribute significant cytoplasmic components to the zygote. A noncoding region of the mitochondrial chromosome, referred to as D-loop, is highly polymorphic. This property, together with the absence of mtDNA recombination, makes it a valuable tool for studies tracing human migration and evolution, and it is also used for specific forensic applications.

Inherited mitochondrial disorders are transmitted in a matrilineal fashion; all children from an affected mother will inherit the disease, but it will not be transmitted from an affected father to his children (Fig. 62-11D). Alterations in the mtDNA affecting enzymes required for oxidative phosphorylation lead to reduction of ATP supply, generation of free radicals, and induction of apoptosis. Several syndromic disorders arising from mutations in the mitochondrial genome are known in humans and they affect both protein-coding and tRNA genes (Table 62-1 and Table 62-5). The broad clinical spectrum often involves (cardio) myopathies and encephalopathies because of the high dependence of these tissues on oxidative phosphorylation. The age of onset and the clinical course are highly variable because of the unusual mechanisms of mtDNA transmission, which replicates independently from nuclear DNA. During cell replication, the proportion of wild-type and mutant mitochondria can drift among different cells and tissues. The resulting
HETEROGENEITY IN THE PROPORTION OF MITOCHONDRIA WITH AND WITHOUT A MUTATION IS REFERRED TO AS HETEROPLASMIA AND UNDERLIES THE PHENOTYPIC VARIABILITY THAT IS CHARACTERISTIC OF MITOCHONDRIAL DISEASES. ACQUIRED SOMATIC MUTATIONS IN MITOCHONDRIA ARE THOUGHT TO BE INVOLVED IN SEVERAL AGE-DEPENDENT DEGENERATIVE DISORDERS AFFECTING PREDOMINANTLY MUSCLE AND THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM (E.G., ALZHEIMER’S AND PARKINSON’S DISEASE). ESTABLISHING THAT A MTDNA ALTERATION IS CAUSAL FOR A CLINICAL PHENOTYPE IS CHALLENGING BECAUSE OF THE HIGH DEGREE OF POLYMORPHISM IN MTDNA AND THE PHENOTYPIC VARIABILITY CHARACTERISTIC OF THESE DISORDERS. CERTAIN PHARMACOLOGIC TREATMENTS MAY HAVE AN IMPACT ON MITOCHONDRIA AND/OR THEIR FUNCTION.

**TABLE 62-5 SELECTED MITOCHONDRIAL DISEASES**

<table>
<thead>
<tr>
<th>DISEASE/SYNDROME</th>
<th>OMIM #</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELAS SYNDROME: MITOCHONDRIAL MYOPATHY WITH LACTIC ACIDOSIS</td>
<td>540000</td>
</tr>
<tr>
<td>ENCEPHALOPATHY, LACTACIDOSIS, AND STROKE</td>
<td>535000</td>
</tr>
<tr>
<td>LEBER'S OPTIC ATROPHY: HEREDITARY OPTICAL NEUROPATHY</td>
<td>530000</td>
</tr>
<tr>
<td>KEARNS-SAYRE SYNDROME (KSS): OPHTHALMOPLEGIA, PIGMENTAL</td>
<td>545000</td>
</tr>
<tr>
<td>DEGENERATION OF THE RETINA, CARDIOMYOPATHY</td>
<td>551500</td>
</tr>
<tr>
<td>MERRF SYNDROME: MYOCLOCALIC EPILEPSEY AND RAGGED-RED FIBERS</td>
<td>258470</td>
</tr>
<tr>
<td>NEUROGENIC MUSCULAR WEAKNESS WITH ATAXIA AND RETINITIS</td>
<td>557000</td>
</tr>
<tr>
<td>PIGMENTOSA (NARP)</td>
<td>157640</td>
</tr>
<tr>
<td>PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA (CEOP)</td>
<td>516020</td>
</tr>
<tr>
<td>PEARSON SYNDROME (PEAR): BONE MARROW AND PANCREATIC</td>
<td>---------</td>
</tr>
<tr>
<td>FAILURE</td>
<td>---------</td>
</tr>
<tr>
<td>AUTOSOMAL DOMINANT INHERITED MITOCHONDRIAL MYOPATHY</td>
<td>---------</td>
</tr>
<tr>
<td>WITH MITOCHONDRIAL DELETION (ADMIMY)</td>
<td>---------</td>
</tr>
<tr>
<td>SOMATIC MUTATIONS IN CYTOCHROME B GENE: EXERCISE INTOLER-</td>
<td>---------</td>
</tr>
<tr>
<td>ANCE, LACTIC ACIDOSIS, COMPLEX III DEFICIENCY, MUSCLE</td>
<td>---------</td>
</tr>
<tr>
<td>PAIN, RAGGED-RED FIBERS</td>
<td>---------</td>
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</tbody>
</table>

FOR EXAMPLE, TREATMENT WITH THE ANTIRETROVIRAL COMPOUND AZIDOTHYMIDINE (AZT) CAUSES AN ACQUIRED MITOCHONDRIAL MYOPATHY THROUGH
DEPLETION OF MUSCULAR MTDNA.

MOSAICISM  MOSAICISM REFERS TO THE PRESENCE OF TWO OR MORE GENETICALLY DISTINCT CELL LINES IN THE TISSUES OF AN INDIVIDUAL. IT RESULTS FROM A MUTATION THAT OCCURS DURING EMBRYONIC, FETAL, OR EXTRAUTERINE DEVELOPMENT. THE DEVELOPMENTAL STAGE AT WHICH THE MUTATION ARISES WILL DETERMINE WHETHER GERM CELLS AND/OR SOMATIC CELLS ARE INVOLVED. CHROMOSOMAL MOSAICISM RESULTS FROM NON-DISJUNCTION AT AN EARLY EMBRYONIC MITOTIC DIVISION, LEADING TO THE PERSISTENCE OF MORE THAN ONE CELL LINE, AS EXEMPLIFIED BY SOME PATIENTS WITH TURNER SYNDROME (CHAP. 343). SOMATIC MOSAICISM IS CHARACTERIZED BY A PATCHY DISTRIBUTION OF GENETICALLY ALTERED SOMATIC CELLS. THE MCCUNE-ALBRIGHT SYNDROME, FOR EXAMPLE, IS CAUSED BY ACTIVATING MUTATIONS IN THE STIMULATORY G PROTEIN *(G###S*) THAT OCCUR EARLY IN DEVELOPMENT (CHAP. 347). THE CLINICAL PHENOTYPE VARIES DEPENDING ON THE TISSUE DISTRIBUTION OF THE MUTATION; MANIFESTATIONS INCLUDE OVARIAN CYSTS THAT SECRETE SEX STEROIDS AND CAUSE PRECOCIOUS PUBERTY, POLYOSTOTIC FIBROUS DYSPLASIA, CAFE-AU-LAIT SKIN PIGMENTATION, GROWTH HORMONE-SECRETING PITUITARY ADENOMAS, AND HYPERSECRETING AUTONOMOUS THYROID NODULES (CHAP. 341).

X-INACTIVATION, IMPRINTING, AND UNIPARENTAL DISOMY  ACCORDING TO TRADITIONAL MENDELIAN PRINCIPLES, THE PARENTAL ORIGIN OF A MUTANT GENE IS IRRELEVANT FOR THE EXPRESSION OF THE PHENOTYPE. THERE ARE, HOWEVER, IMPORTANT EXCEPTIONS TO THIS RULE. X-INACTIVATION PREVENTS THE EXPRESSION OF MOST GENES ON ONE OF THE TWO X-CHROMOSOMES IN EVERY CELL OF A FEMALE. GENE INACTIVATION ALSO OCCURS ON SELECTED CHROMOSOMAL REGIONS OF AUTOSOMES. THIS PHENOMENON, REFERRED TO AS GENOMIC IMPRINTING, LEADS TO INHERITABLE PREFERENTIAL EXPRESSION OF ONE OF THE PARENTAL ALLELES. IT IS OF PATHOPHYSIOLOGIC IMPORTANCE IN DISORDERS WHERE THE TRANSMISSION OF DISEASE IS DEPENDENT ON THE SEX OF THE TRANSMITTING PARENT AND, THUS, PLAYS AN IMPORTANT ROLE IN THE EXPRESSION OF CERTAIN GENETIC DISORDERS. TWO CLASSIC EXAMPLES ARE THE PRADER-WILLI SYNDROME AND ANGELMAN SYNDROME (CHAP. 63). PRADER-WILLI SYNDROME IS CHARACTERIZED BY DIMINISHED FETAL ACTIVITY, OBESITY, HYPOTONIA, MENTAL RETARDATION, SHORT STATURE, AND HYPOGONADOTROPIC HYPOGONADISM. DELETIONS OF THE PATERNAL COPY OF THE PRADER-WILLI LOCUS LOCATED ON THE SHORT ARM OF CHROMOSOME 15 RESULT IN A CONTIGUOUS GENE SYNDROME INVOLVING MISSING PATERNAL COPIES OF THE *NECDIN AND SNRPN* GENES, AMONG OTHERS. IN CONTRAST, PATIENTS WITH ANGELMAN SYNDROME, CHARACTERIZED BY MENTAL RETARDATION, SEIZURES, ATAXIA, AND HYPOTONIA, HAVE DELETIONS INVOLVING THE MATERNAL COPY OF THIS REGION ON CHROMOSOME 15. THESE TWO SYNDROMES MAY ALSO RESULT FROM UNIPARENTAL DISOMY. IN THIS CASE, THE SYNDROMES ARE NOT CAUSED BY DELETIONS ON CHROMOSOME 15 BUT BY THE INHERITANCE OF EITHER TWO MATERNAL CHROMOSOMES (PRADER-WILLI SYNDROME) OR TWO PA-
TERNAL CHROMOSOMES (ANGELMAN SYNDROME). IMPRINTING AND THE RELATED PHENOMENON OF ALLELIC EXCLUSION MAY BE MORE COMMON THAN CURRENTLY DOCUMENTED, AS IT IS DIFFICULT TO EXAMINE.

401 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS

TABLE 62-6 SELECTED TRINUCLEOTIDE REPEAT DISORDERS

DISEASE

X-CHROMOSOMAL SPINOBULBAR MUSCULAR ATROPHY (SBMA)
FRAGILE X-SYNDROME (FRAXA)
FRAGILE X-SYNDROME (FRAXE)
DYSTROPHIA MYOTONICA (DM)

HUNTINGTON DISEASE (HD)
SPINOCEREBELLAR ATAXIA TYPE 1 (SCA1)
SPINOCEREBELLAR ATAXIA TYPE 2 (SCA2)
SPINOCEREBELLAR ATAXIA TYPE 3 (SCA3); MACHADO JOSEPH DISEASE (MD)
SPINOCEREBELLAR ATAXIA TYPED (SCA6, CACNA1A)

SPINOCEREBELLAR ATAXIA TYPE 7 (SCA7)
SPINOCEREBELLAR ATAXIA TYPE 12 (SCA12)
DENTORUBRAL PALLIDOLUYSIANE ATROPHY (DRPLA)
FRIEDREICH ATAXIA (FRDA1)

LOCUS

XQ11-Q12

XQ27.3
XQ28
19Q 13.2-Q13.3

4P16.3
6P21.3-21.2
12Q24.1
14Q21

19P13.1-13.2
3P21.1-P12
5Q31
12P
9Q13-21

REPEAT

CAG
<table>
<thead>
<tr>
<th>CGG</th>
<th>GCC</th>
<th>CTG</th>
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<tr>
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<td>GAA</td>
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</table>

**TRIPLET LENGTH (NORMAL/DISEASE)**

<table>
<thead>
<tr>
<th>11-34/40-62</th>
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<tbody>
<tr>
<td>6-50/200-300</td>
</tr>
<tr>
<td>6-25/&gt;200</td>
</tr>
<tr>
<td>5-30/200-1000</td>
</tr>
<tr>
<td>6-34/37-180</td>
</tr>
<tr>
<td>6-39/40-88</td>
</tr>
<tr>
<td>15-31/34-400</td>
</tr>
<tr>
<td>13-36/55-86</td>
</tr>
<tr>
<td>4-16/20-33</td>
</tr>
<tr>
<td>4-19/37 TO &gt;300</td>
</tr>
<tr>
<td>6-26/66-78</td>
</tr>
<tr>
<td>7-23/49-75</td>
</tr>
<tr>
<td>7-22/200-900</td>
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**INHERITANCE**

<table>
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<tr>
<th>XR</th>
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<tr>
<td>XR</td>
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<tr>
<td>XR</td>
</tr>
<tr>
<td>AD, VARIABLE</td>
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<tr>
<td>PENET RANCE</td>
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<tr>
<td>AD</td>
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<tr>
<td>AD</td>
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<td>AD</td>
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</tbody>
</table>
GENE PRODUCT

ANDROGEN RECEPTOR

FMR-1 PROTEIN
FMR-2 PROTEIN
MYOTONIN PROTEIN KINASE

HUNTINGTIN
ATAXIN 1
ATAXIN 2
ATAXIN 3

ALPHA 1A VOLTAGE-DEPENDENT
L-TYPE CALCIUM CHANNEL
ATAXIN 7
PROTEIN PHOSPHATASE 2A
ATROPHIN 1
FRATAxin

NOTE: AD, AUTOSOMAL DOMINANT; AR, AUTOSOMAL RECESSIVE; XR, X-LINKED RECESSIVE.

LEVELS OF MRNA EXPRESSION FROM THE MATERNAL AND PATERNAL ALLELES IN SPECIFIC TISSUES OR IN INDIVIDUAL CELLS. GENOMIC IMPRINTING, OR UNIPARENTAL DISOMY, IS INVOLVED IN THE PATHOGENESIS OF SEVERAL OTHER DISORDERS AND MALIGNANCIES (CHAP. 63). FOR EXAMPLE, HYDATIDIFORM MOLES CONTAIN A NORMAL NUMBER OF DIPLOID CHROMOSOMES, BUT THEY ARE ALL OF PATERNAL ORIGIN. THE OPPOSITE SITUATION OCCURS IN OVARIAN TERATOMATA, WITH 46 CHROMOSOMES OF MATERNAL ORIGIN. EXPRESSION OF THE IMPRINTED GENE FOR INSULIN-LIKE GROWTH FACTOR II (IGF-II) IS INVOLVED IN THE PATHOGENESIS OF THE CANCER-PREDISPOSING BECKWITH-WIEDEMANN SYNDROME (BWS) (CHAP. 79). THESE CHILDREN SHOW SOMATIC OVERGROWTH WITH ORGANOMEgalIES AND HEMIHYPERTROPHY, AND THEY HAVE AN INCREASED RISK OF EMBRYONAL MALIGNANCIES SUCH AS WILM’S TUMOR. NORMALLY, ONLY THE PATERNALLY DERIVED COPY OF THE IGF-II GENE IS ACTIVE AND THE MATERNAL COPY IS INACTIVE. IMPRINTING OF THE IGF-II GENE IS REGULATED BY H19, WHICH ENCODES AN RNA TRANSCRIPT THAT IS NOT TRANSLATED INTO PROTEIN. DISRUPTION OR LACK OF H19 METHYLATION LEADS TO A RELAXATION OF IGF-II IMPRINTING AND EXPRESSION OF BOTH ALLELES.

MEIOTICALLY AND MITOTICALLY HERITABLE CHANGES IN GENE EXPRESSION NOT ASSOCIATED WITH DNA SEQUENCE ALTERATIONS ARE REFERRED TO AS EPIGENETIC EFFECTS. THESE CHANGES INVOLVE DNA METHYLATION, HISTONE MODIFICATIONS, AND RNA-MEDIATED SILENCING, RESULTING IN GENE REPRESSOR WITHOUT A CHANGE IN THE CODING SEQUENCE. EPIGENETIC ALTERATIONS ARE INCREASINGLY RECOGNIZED TO PLAY A ROLE IN HUMAN DISEASES SUCH AS CANCER, MENTAL RETARDATION, HEMATOLOGIC DISORDERS, AND POSSIBLY IN AGING. FOR EXAMPLE, DE NOVO METHYLATION OF CPG ISLANDS, REGIONS OF >500 BP IN SIZE WITH A GC CONTENT >55% IN PROMOTER REGIONS THAT ARE NORMALLY UNMETHYLATED, IS A HALLMARK OF HUMAN CANCERS. INHIBITORS OF ENZYMES CONTROLLING EPIGENETIC MODIFICATIONS SUCH AS HISTONE DEACETYLASES AND
DNA METHYLTRANSFERASES REVERSE GENE SILENCING AND REPRESENT A PROMISING NEW GROUP OF ANTINEOPLASTIC AGENTS.

**SOMATIC MUTATIONS** CANCER CAN BE DEFINED AS A GENETIC DISEASE AT THE CELLULAR LEVEL (CHAP. 79). CANCERS ARE MONOCLONAL IN ORIGIN, INDICATING THAT THEY HAVE ARisen FROM A SINGLE PRECURSOR CELL WITH ONE OR SEVERAL MUTATIONS IN GENES CONTROLLING GROWTH (PROLIFERATION OR APOPTOSIS) AND/OR DIFFERENTIATION. THESE ACQUIRED SOMATIC MUTATIONS ARE RESTRICTED TO THE TUMOR AND ITS METASTASES AND ARE NOT FOUND IN THE SURROUNDING NORMAL TISSUE. THE MOLECULAR ALTERATIONS INCLUDE DOMINANT GAIN-OF-FUNCTION MUTATIONS IN ONCogenes, RECESSIVE LOSS-OF-FUNCTION MUTATIONS IN TUMOR-SUPPRESSOR GENES AND DNA REPAIR GENES, GENE AMPLIFICATION, AND CHROMOSOME REARRANGEMENTS. RARELY, A SINGLE MUTATION IN CERTAIN GENES MAY BE SUFFICIENT TO TRANSFORM A NORMAL CELL INTO A MALIGNANT CELL. IN MOST CANCERS, HOWEVER, THE DEVELOPMENT OF A MALIGNANT PHENOTYPE REQUIRES SEVERAL GENETIC ALTERATIONS FOR THE GRADUAL PROGRESSION FROM A NORMAL CELL TO A CANCEROUS CELL, A PHENOMENON TERMED *MULTI-STEP CARCINOGENESIS* (CHAPS. 79, 80). MOST HUMAN TUMORS EXPRESS TELOMERASE, AN ENZYME FORMED OF A PROTEIN AND AN RNA COMPONENT, WHICH ADDS TELOMERE REPEATS AT THE ENDS OF CHROMOSOMES DURING REPLICATION. THIS MECHANISM IMPEDES SHORTENING OF THE TELOMERS, WHICH IS ASSOCIATED WITH SENESCENCE IN NORMAL CELLS, AND IS ASSOCIATED WITH ENHANCED REPLICATIVE CAPACITY IN CANCER CELLS. TELOMERASE INHIBITORS MAY PROVIDE A NOVEL STRATEGY FOR TREATING ADVANCED HUMAN CANCERS. IN MANY CANCER SYNDROMES, THERE IS AN INHERITED *PREDISPOSITION* TO TUMOR FORMATION. IN THESE INSTANCES, A GERMLINE MUTATION IS INHERITED IN AN AUTOSOMAL DOMINANT FASHION INACTIVATING ONE ALLELE OF AN AUTOSOMATIC TUMOR-SUPPRESSOR GENE. IF THE SECOND ALLELE IS INACTIVATED BY A SOMATIC MUTATION OR BY EPIGENETIC SILENCING IN A GIVEN CELL, THIS WILL LEAD TO NEOPLASTIC GROWTH (KNUDSON TWO-HIT MODEL). THUS, THE DEFECTIVE ALLELE IN THE GERMLINE IS TRANSMITTED IN A DOMINANT MODE, THOUGH TUMORIGENESIS RESULTS FROM A BIALLELIC LOSS OF THE TUMOR-SUPPRESSOR GENE IN AN AFFECTED TISSUE. THE CLASSIC EXAMPLE TO ILLUSTRATE THIS PHENOMENON IS RETINOBLASTOMA, WHICH CAN OCCUR AS A SPORADIC OR HEREDITARY TUMOR. IN SPORADIC RETINOBLASTOMA, BOTH COPIES OF THE RETINOBLASTOMA (*RB*) GENE ARE INACTIVATED THROUGH TWO SOMATIC EVENTS. IN HEREDITARY RETINOBLASTOMA, ONE MUTATED OR DELETED *RB* ALLELE IS INHERITED IN AN AUTOSOMAL DOMINANT MANNER AND THE SECOND ALLELE IS INACTIVATED BY A SUBSEQUENT SOMATIC MUTATION. THIS TWO-HIT MODEL APPLIES TO OTHER INHERITED CANCER SYNDROMES SUCH AS MEN-1 (CHAP. 345) AND NEUROFIBROMATOSIS TYPE 2 (CHAP. 374).

**NUCLEOTIDE REPEAT EXPANSION DISORDERS** SEVERAL DISEASES ARE ASSOCIATED WITH AN INCREASE IN THE NUMBER OF NUCLEOTIDE REPEATS ABOVE A CERTAIN THRESHOLD (TABLE 62-6). THE REPEATS ARE SOMETIMES LOCATED WITHIN THE CODING REGION OF THE GENES, AS IN HUNTINGTON DISEASE OR THE X-LINKED FORM OF SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA, KENNEDY SYNDROME). IN OTHER INSTANCES, THE REPEATS PROBABLY ALTER GENE
REGULATORY SEQUENCES. IF AN EXPANSION IS PRESENT, THE DNA FRAGMENT IS UNSTABLE AND TENDS TO EXPAND FURTHER DURING CELL DIVISION. THE LENGTH OF THE NUCLEOTIDE REPEAT OFTEN CORRELATES WITH THE SEVERITY OF THE DISEASE. WHEN REPEAT LENGTH INCREASES FROM ONE GENERATION TO THE NEXT, DISEASE MANIFESTATIONS MAY WORSEN OR BE OBSERVED AT AN EARLIER AGE; THIS PHENOMENON IS REFERRED TO AS ANTICIPATION. IN HUNTINGTON DISEASE, FOR EXAMPLE, THERE IS A CORRELATION BETWEEN AGE OF ONSET AND LENGTH OF THE TRIPLET CODON EXPANSION (CHAP. 360). ANTICIPATION HAS ALSO BEEN DOCUMENTED IN OTHER DISEASES CAUSED BY DYNAMIC MUTATIONS IN TRINUCLEOTIDE REPEATS (TABLE 62-6). THE REPEAT NUMBER MAY ALSO VARY IN A TISSUE-SPECIFIC MANNER. IN MYOTONIC DYSTROPHY, THE CTG REPEAT MAY BE TENFOLD GREATER IN MUSCLE TISSUE THAN IN LYMPHOCYTES (CHAP. 382).

**COMPLEX GENETIC DISORDERS** THE EXPRESSION OF MANY COMMON DISEASES SUCH AS CARDIOVASCULAR DISEASE, HYPERTENSION, DIABETES, ASTHMA, PSYCHIATRIC DISORDERS, AND CERTAIN CANCERS IS DETERMINED BY A COMBINATION OF GE-

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**402 PART 3: GENETICS AND DISEASE**

**TABLE 62-7 GENES AND LOCI INVOLVED IN MONO- AND POLYGENIC FORMS OF DIABETES**

**DISORDER**

**MONOGENIC FORMS OF DIABETES**
MODY 1
MODY 1
MODY 1
MODY 1
MODY 5 (RENA L CYSTS, DIABETES)
MODY 6
DIABETES MELLITUS TYPE 2; LOCI AND GENES LINKED AND/OR ASSOCIATED WITH SUSCEPTIBILITY FOR DIABETES MELLITUS TYPE 2

**GENES OR SUSCEPTIBILITY LOCUS**
HNF4* (HEPATOCYTE NUCLEAR FACTOR 4*)
GCK (GLUEOKINASE)
HNF1* (HEPATOCYTE NUCLEAR FACTOR 1*)
IPF1 (INSULIN RECEPTOR SUBSTRATE)
HNF1* (HEPATOCYTE NUCLEAR FACTOR 1*)
NEUROD1 (NEUROGENIC DIFFERENTIATION FACTOR 1)
GENES AND LOCI IDENTIFIED BY LINKAGE/ASSOCIATION STUDIES CPN10 (CALPAIN-10)
HNF4* (HEPATOCYTE NUCLEAR FACTOR 4*)
PTPN1 (PROTEIN-TYROSINE PHOSPHATASE)
PKLR (LIVER PYRUVATE KINASE)
CASQ1 (CALSEQUESTRIN 1)
APM1 (ADIPONECTIN)
TCF7L2 (TRANSCRIPTION FACTOR 7-LIKE2)
1Q21-23
2Q
3Q22-27
8P21-23
11Q
12Q24
15
18P11
20Q
20P
SELECTED CANDIDATE GENES WITH POSSIBLE CONTRIBUTION
PPAR* (PEROXISOME PROLIFERATOR RECEPTOR *)
KCNJ11 (ATP-SENSITIVE K CHANNEL KIR6.2)
ABCC8 (ATP-BINDING CASSETTE, SUBFAMILY C, MEMBER 8)
INSULIN VNTR
IRS-1 (INSULIN RECEPTOR SUBSTRATE)
PGC1 * (PPAR *Y COACTIVATORY *)
ENPP1 (ECTONUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE 1)

CHROMOSOMAL LOCATION

20Q 12-Q13.1
7P15-P13
12Q24.2
13Q 12.1
17CEN-Q21.3
2Q32
2Q3.73
20Q 12-Q 13.1
20Q13.1-Q13.2
1Q21
1Q21
3Q27
10Q25.3
1Q21-23
2Q
3Q22-27
8P21-23
11Q
12Q24
15
18P11
20Q
20P
3P25
11P15.1
OTHER FACTORS

AD INHERITANCE

DIET
ENERGY EXPENDITURE
OBESITY

NOTE: MODY, MATURITY ONSET DIABETES OF THE YOUNG; AD, AUTOSOMAL DOMINANT; VNTR, VARIABLE NUMBER OF TANDEM REPEATS.

NETIC BACKGROUND, ENVIRONMENTAL FACTORS, AND LIFESTYLE. A TRAIT IS CALLED POLYGENIC IF MULTIPLE GENES CONTRIBUTE TO THE PHENOTYPE OR MULTIFACTORIAL IF MULTIPLE GENES ARE ASSUMED TO INTERACT WITH ENVIRONMENTAL FACTORS. GENETIC MODELS FOR THESE COMPLEX TRAITS NEED TO ACCOUNT FOR GENETIC HETEROGENEITY AND INTERACTIONS WITH OTHER GENES AND THE ENVIRONMENT. COMPLEX GENETIC TRAITS MAY BE INFLUENCED BY MODIFYING GENES THAT ARE NOT LINKED TO THE MAIN GENE INVOLVED IN THE PATHOGENESIS OF THE TRAIT. THIS TYPE OF GENE-GENE INTERACTION, OR EPISTASIS, PLAYS AN IMPORTANT ROLE IN POLYGENIC TRAITS THAT REQUIRE THE SIMULTANEOUS PRESENCE OF VARIATIONS IN MULTIPLE GENES TO RESULT IN A PATHOLOGIC PHENOTYPE. TYPE 2 DIABETES MELLITUS PROVIDES A PARADIGM FOR CONSIDERING A MULTIFACTORIAL DISORDER, AS GENETIC, NUTRITIONAL, AND LIFESTYLE FACTORS ARE INTIMATELY INTERRELATED IN DISEASE PATHOGENESIS (TABLE 62-7) (CHAP. 338). THE IDENTIFICATION OF GENETIC VARIATIONS AND ENVIRONMENTAL FACTORS THAT EITHER PREDISPOSE TO OR PROTECT AGAINST DISEASE IS ESSENTIAL FOR PREDICTING DISEASE RISK, DESIGNING PREVENTIVE STRATEGIES, AND DEVELOPING NOVEL THERAPEUTIC APPROACHES. THE STUDY OF RARE MONOGENIC DISEASES MAY PROVIDE INSIGHT INTO SOME OF GENETIC AND MOLECULAR MECHANISMS IMPORTANT IN THE PATHOGENESIS OF COMPLEX DISEASES. FOR EXAMPLE, THE IDENTIFICATION OF THE HEPATOCYTE NUCLEAR FACTOR * (HNF*) IN MATURITY-ONSET OF DIABETES TYPE 4 DEFINED IT AS A CANDIDATE GENE IN THE PATHOGENESIS OF DIABETES MELLITUS TYPE 2 (TABLES 62-2 AND 62-8). GENOME SCANS HAVE IDENTIFIED VARIOUS LOCI THAT MAY BE ASSOCIATED WITH SUSCEPTIBILITY TO DEVELOPMENT OF DIABETES MELLITUS IN CERTAIN POPULATIONS. EFFORTS TO IDENTIFY SUSCEPTIBILITY GENES REQUIRE VERY LARGE SAMPLE SIZES, AND POSITIVE RESULTS MAY DEPEND ON ETHNICITY, ASCERTAINMENT CRITERIA, AND STATISTICAL ANALYSIS. ASSOCIATION STUDIES ANALYZING THE POTENTIAL INFLUENCE OF (BIOLOGICALLY
FUNCTIONAL SNPs and SNP haplotypes on a particular phenotype are a promising approach for the detection of involved genes.

**LINKAGE AND ASSOCIATION STUDIES** There are two primary strategies for mapping genes that cause or increase susceptibility to human disease: (1) classic linkage can be performed based on a known genetic model or, when the model is unknown, by studying pairs of affected relatives; or (2) disease genes can be mapped using allelic association studies (Table 62-8).

**GENETIC LINKAGE** Genetic linkage refers to the fact that genes are physically connected, or linked, to one another along the chromosomes. Two fundamental principles are essential for understanding the concept of linkage: (1) when two genes are close together on a chromosome, they are usually transmitted together, unless a recombination event separates them (Figs. 62-3, 62-8); and (2) the odds of a crossover, or recombination event, between two linked genes is proportional to the distance that separates them. Thus, genes that are further apart are more likely to undergo a recombination event than genes that are very close together. The detection of chromosomal loci that segregate with a disease by linkage can be used to identify the gene responsible for the disease (positional cloning) and to predict the odds of disease gene transmission in genetic counseling. Polymorphisms are essential for linkage studies because they provide a means to distinguish the maternal and paternal chromosomes in an individual. On average, 1 out of every 1000 bp varies from one person to the next. Although this degree of variation seems low (99.9% identical), it means that >3 million sequence differences exist between any two unrelated individuals and the probability that the sequence at such loci will differ on the two homologous chromosomes is high (often >70-90%). These sequence variations include VNTRs, short tandem repeats (STRs), and SNPs. Most STRs, also called polymorphic microsatellite markers, consist of di-, tri-, or tetranucleotide repeats that can be measured readily using PCR (Fig. 62-12). Characterization of SNPs, using DNA chips, provides an important new tool for comprehensive analyses of genetic variation, linkage, and association studies. Although these sequence variations usually have no apparent functional consequences, they provide much of the basis for variation in genetic traits.

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403 CHAPTER 62 PRINCIPLES OF HUMAN GENETICS

GENETIC APPROACHES FOR IDENTIFYING DISEASE GENES

METHOD

LINKAGE STUDIES
CLASSICAL LINKAGE ANALYSIS
(PARAMETRIC METHODS)

ALLELLE-SHARING METHODS
(NON PARAMETRIC METHODS)
AFFECTED SIB AND RELATIVE PAIR
ANALYSES SIB PAIR ANALYSIS

ASSOCIATION STUDIES

CASE-CONTROL STUDIES
LINKAGE DISEQUILIBRIUM
TRANSMISSION DISEQUILIBRIUM
TEST (TDT)
WHOLE-GENOME ASSOCIATION
STUDIES

INDICATIONS AND ADVANTAGES

ANALYSIS OF MONOGENIC TRAITS
SUITABLE FOR GENOME SCAN
CONTROL POPULATION NOT REQUIRED
USEFUL FOR MULTIFACTORIAL DISORDERS IN
ISOLATED POPULATIONS
SUITABLE FOR IDENTIFICATION OF SUSCEPTIBILITY GENES IN POLYGENIC AND
MULTIFACTORIAL DISORDERS
SUITABLE FOR GENOME SCAN
CONTROL POPULATION NOT REQUIRED IF
ALLELLE FREQUENCIES ARE KNOWN
STATISTICAL POWER CAN BE INCREASED BY
INCLUDING PARENTS AND RELATIVES

SUITABLE FOR IDENTIFICATION OF SUSCEPTIBILITY GENES IN POLYGENIC AND
MULTIFACTORIAL DISORDERS
SUITABLE FOR TESTING SPECIFIC ALLELIC
VARIANTS OF KNOWN CANDIDATE LOCI
FACILITATED BY HAPMAP DATA, MAKING
WHOLE-GENOME STUDIES MORE
FEASIBLE
DOES NOT NECESSARILY NEED RELATIVES

LIMITATIONS

DIFFICULT TO COLLECT LARGE INFORMATIVE
PEDIGREES
DIFFICULT TO OBTAIN SUFFICIENT STATISTICAL
POWER FOR COMPLEX TRAITS

DIFFICULT TO COLLECT SUFFICIENT NUMBER
OF SUBJECTS
DIFFICULT TO OBTAIN SUFFICIENT STATISTICAL
POWER FOR COMPLEX TRAITS
REDUCED POWER COMPARED TO CLASSICAL LINKAGE, BUT NOT SENSITIVE TO SPECIFICATION OF GENETIC MODE

REQUIRES LARGE SAMPLE SIZE AND MATCHED CONTROL POPULATION
FALSE-POSITIVE RESULTS IN THE ABSENCE OF SUITABLE CONTROL POPULATION
CANDIDATE GENE APPROACH DOES NOT PERMIT TO DETECT NOVEL GENES AND PATHWAYS
WHOLE-GENOME ASSOCIATION STUDIES VERY EXPENSIVE

IN ORDER TO IDENTIFY A CHROMOSOMAL LOCUS THAT SEGREGATES WITH A DISEASE, IT IS NECESSARY TO CHARACTERIZE POLYMORPHIC DNA MARKERS FROM AFFECTED AND UNAFFECTED INDIVIDUALS OF ONE OR SEVERAL PEDIGREES. ONE CAN THEN ASSESS WHETHER CERTAIN MARKER ALLELES COSEGREGATE WITH THE DISEASE. MARKERS THAT ARE CLOSEST TO THE DISEASE GENE ARE LESS LIKELY TO UNDERGO RECOMBINATION EVENTS AND THEREFORE RECEIVE A HIGHER LINKAGE SCORE. LINKAGE IS EXPRESSED AS A LOD (LOGARITHM OF ODDS) SCORE - THE RATIO OF THE PROBABILITY THAT THE DISEASE AND MARKER LOCI ARE LINKED RATHER THAN UNLINKED. LOD SCORES OF +3 (1000:1) ARE GENERALLY ACCEPTED AS SUPPORTING LINKAGE, WHEREAS A SCORE OF -2 IS CONSISTENT WITH THE ABSENCE OF LINKAGE. AN EXAMPLE OF THE USE OF LINKAGE ANALYSIS IS SHOWN IN FIG. 62-12. IN THIS CASE, THE GENE FOR THE Autosomal Dominant Disorder MEN-1 IS KNOWN TO BE LOCATED ON CHROMOSOME 11 Q 13. USING POSITIONAL CLONING, THE MEN1 GENE WAS IDENTIFIED AND SHOWN TO ENCODE MENIN, A TUMOR SUPPRESSOR. AFFECTED INDIVIDUALS INHERIT A MUTANT FORM OF THE MEN1 GENE, PREDISPOSING THEM TO CERTAIN TYPES OF TUMORS (PARATHYROID, PITUITARY, PANCREATIC ISLET) (CHAP. 345). IN THE TISSUES THAT DEVELOP A TUMOR, A “SECOND HIT” OCCURS IN THE NORMAL COPY OF THE MEN1 GENE. THIS SOMATIC MUTATION MAY BE A POINT MUTATION, A MICRODELETION, OR LOSS OF A CHROMOSOMAL FRAGMENT (DETECTED AS LOSS OF HETEROZYGOSITY, LOH). WITHIN A GIVEN FAMILY, LINKAGE TO THE MEN1 GENE LOCUS CAN BE ASSESSED WITHOUT NECESSARILY KNOWING

FIGURE 62-12 CAG REPEAT LENGTH AND LINKAGE ANALYSIS IN MULTIPLE ENDOCRINE NEOPLASIA
**UPPER PANEL.** DETECTION OF DIFFERENT ALLELES USING POLYMORPHIC MICROSATELLITE MARKERS. THE EXAMPLE DEPICTS A CAG TRINUCLEOTIDE REPEAT. PCR WITH PRIMERS FLANKING THE POLYMORPHIC REGION RESULTS IN PRODUCTS OF VARIABLE LENGTH, DEPENDING ON THE NUMBER OF CAG REPEATS. AFTER CHARACTERIZATION OF THE ALLELES IN THE PARENTS, TRANSMISSION OF THE PATERNAL AND MATERNAL ALLELES CAN BE DETERMINED.

**LOWER PANEL.** GENOTYPE ANALYSIS USING MICROSATELLITE MARKERS IN A FAMILY WITH MEN-1. TWO MICROSATELLITE MARKERS, A AND B, ARE LOCATED IN CLOSE PROXIMITY TO THE MEN1 GENE ON CHROMOSOME 11Q13. FOR EACH INDIVIDUAL, THE A AND B ALLELES HAVE BEEN DETERMINED. BASED ON THIS ANALYSIS, THE GENOTYPE A3, B4 IS LINKED TO THE DISEASE BECAUSE IT OCCURS IN THE TWO AFFECTED INDIVIDUALS I-1 AND II-1 BUT NOT IN UNAFFECTED SIBLINGS. BECAUSE THE DISEASE ALLELE IS LINKED TO A3,B4 WITHIN THE AFFECTED FAMILY, IT IS LIKELY THAT THE INDIVIDUAL III-1 IS A CARRIER OF THE MUTATED MEN1 GENE. ALTHOUGH III-5 ALSO HAS THE A3,B4 GENOTYPE, SHE HAS INHERITED THE ALLELE FROM HER UNAFFECTED FATHER (11-4), WHO IS NOT RELATED TO THE ORIGINAL FAMILY. THE A3, B4 GENOTYPE IS ONLY ASSOCIATED WITH MEN-1 IN THE ORIGINAL FAMILY, BUT NOT IN THE GENERAL POPULATION. THEREFORE, INDIVIDUAL III-5 IS NOT AT RISK FOR DEVELOPING THE DISEASE.

404 PART 3: GENETICS AND DISEASE

THE SPECIFIC MUTATION IN THE MEN1 GENE. USING POLYMORPHIC STRS THAT ARE CLOSE TO THE MEN1 GENE, ONE CAN ASSESS TRANSMISSION OF THE DIFFERENT MEN1 ALLELES AND COMPARE THIS PATTERN TO DEVELOPMENT OF THE DISORDER TO DETERMINE WHICH ALLELE IS ASSOCIATED WITH RISK OF MEN-1. IN THE PEDIGREE SHOWN, THE AFFECTED GRANDFATHER IN GENERATION I CARRIES ALLELES 3 AND 4 ON THE CHROMOSOME WITH THE MUTATED MEN1 GENE AND ALLELES 2 AND 2 ON HIS OTHER CHROMOSOME 11. CONSISTENT WITH LINKAGE OF THE 3/4 GENOTYPE TO THE MEN1 LOCUS, HIS SON IN GENERATION II IS AFFECTED, WHEREAS HIS DAUGHTER (WHO INHERITS THE 2/2 GENOTYPE FROM HER FATHER) IS UNAFFECTED. IN THE THIRD GENERATION, TRANSMISSION OF THE 3/4 GENOTYPE INDICATES RISK OF DEVELOPING MEN-1, ASSUMING THAT NO GENETIC RECOMBINATION BETWEEN THE 3/4 ALLELES AND THE MEN1 GENE HAS OCCURRED. AFTER A SPECIFIC MUTATION IN THE MEN1 GENE IS IDENTIFIED WITHIN A FAMILY, IT IS POSSIBLE TO TRACK TRANSMISSION OF THE MUTATION ITSELF, THEREBY ELIMINATING UNCERTAINTY CAUSED BY RECOMBINATION.

**ALLELIC ASSOCIATION, LINKAGE DISEQUILIBRIUM, AND HAPLOTYPES** ALLELIC ASSOCIATION REFERS TO A SITUATION IN WHICH THE FREQUENCY OF AN ALLELE IS SIG-
NIFICANTLY INCREASED OR DECREASED IN INDIVIDUALS AFFECTED BY A PARTICULAR DISEASE IN COMPARISON TO CONTROLS. LINKAGE AND ASSOCIATION DIFFER IN SEVERAL ASPECTS. GENETIC LINKAGE IS DEMONSTRABLE IN FAMILIES OR SIBSHIPS. ASSOCIATION STUDIES, ON THE OTHER HAND, COMPARE A POPULATION OF AFFECTED INDIVIDUALS WITH A CONTROL POPULATION. ASSOCIATION STUDIES CAN BE PERFORMED AS CASE-CONTROL STUDIES THAT INCLUDE UNRELATED AFFECTED INDIVIDUALS AND MATCHED CONTROLS, OR AS FAMILY-BASED STUDIES THAT COMPARE THE FREQUENCIES OF ALLELES TRANSMITTED OR NOT TRANSMITTED TO AFFECTED CHILDREN. ALLELIC ASSOCIATION STUDIES ARE PARTICULARLY USEFUL FOR IDENTIFYING SUSCEPTIBILITY GENES IN COMPLEX DISEASES. WHEN ALLELES AT TWO LOCI OCCUR MORE FREQUENTLY IN COMBINATION THAN WOULD BE PREDICTED (BASED ON KNOWN ALLELE FREQUENCIES AND RECOMBINATION FRACTIONS), THEY ARE SAID TO BE IN LINKAGE DISEQUILIBRIUM. IN FIG. 62-13, A MUTATION, Z, HAS OCCURRED AT A SUSCEPTIBILITY LOCUS WHERE THE NORMAL ALLELE IS Y. THE MUTATION IS IN CLOSE PROXIMITY TO A GENETIC POLYMORPHISM WITH ALLELE A OR B. WITH TIME, THE CHROMOSOMES CARRYING THE A AND Z ALLELES ACCUMULATE AND REPRESENT 10% OF THE CHROMOSOMES IN THE POPULATION. THE FACT THAT THE DISEASE SUSCEPTIBILITY GENE, Z, IS FOUND PREFERENTIALLY, OR EXCLUSIVELY, IN ASSOCIATION WITH THE A ALLELE ILLUSTRATES LINKAGE DISEQUILIBRIUM. THOUGH NOT ALL CHROMOSOMES CARRYING THE A ALLELE CARRY THE DISEASE GENE, THE A ALLELE IS ASSOCIATED WITH AN INCREASED RISK BECAUSE OF ITS POSSIBLE ASSOCIATION WITH THE Z ALLELE. THIS MODEL IMPLIES THAT IT MAY BE POSSIBLE IN THE FUTURE TO IDENTIFY Z DIRECTLY TO PROVIDE A MORE ACCURATE PREDICTION OF DISEASE SUSCEPTIBILITY. EVIDENCE FOR LINKAGE DISEQUILIBRIUM CAN BE HELPFUL IN MAPPING DISEASE GENES BECAUSE IT SUGGESTS THAT THE TWO LOCI, IN THIS CASE A AND Z, ARE TIGHTLY LINKED.

FIGURE 62-13 LINKAGE DISEQUILIBRIUM.

DETECTING THE GENETIC FACTORS CONTRIBUTING TO THE PATHOGENESIS OF COMMON COMPLEX DISORDERS REMAINS A GREAT CHALLENGE. IN MANY INSTANCES, THESE ARE LOW-PENETRANCE ALLELES, I.E., VARIATIONS THAT INDIVIDUALLY ONLY HAVE A SUBTLE EFFECT ON DISEASE DEVELOPMENT, AND THEY CAN ONLY BE IDENTIFIED BY UNBIASED GENOME-WIDE ASSOCIATION STUDIES. MOST VARIANTS ARE IN NONCODING OR REGULATORY SEQUENCES BUT DO NOT ALTER PROTEIN STRUCTURE. THE ANALYSIS OF COMPLEX DISORDERS IS FURTHER COMPLICATED BY ETHNIC DIFFERENCES IN DISEASE PREVALENCE, DIFFERENCES IN ALLELE FREQUENCIES IN KNOWN SUSCEPTIBILITY GENES AMONG DIFFERENT POPULATIONS, LOCUS AND ALLELIC HETEROGENEITY, GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS, AND THE POSSIBILITY OF PHENOCOPIES. THE HAPMAP PROJECT IS NOW MAKING GENOME-WIDE ASSOCIATION STUDIES FOR THE CHARACTERIZATION OF COMPLEX DISORDERS MORE REALISTIC. ADJACENT SNPS ARE INHERITED TOGETHER AS BLOCKS, AND THESE BLOCKS CAN BE IDENTIFIED BY
GENOTYPING SELECTED MARKER SNPs, SO-CALLED TAG SNPs, THEREBY REDUCING COST AND WORKLOAD (FIG. 62-8). THE AVAILABILITY OF THIS INFORMATION PERMITS THE CHARACTERIZATION OF A LIMITED NUMBER OF SNPs TO IDENTIFY THE SET OF HAPLOTYPES PRESENT IN AN INDIVIDUAL, E.G., IN CASES AND CONTROLS. THIS, IN TURN, PERMITS GENOME-WIDE ASSOCIATION STUDIES BY SEARCHING FOR ASSOCIATIONS OF CERTAIN HAPLOTYPES WITH A DISEASE PHENOTYPE OF INTEREST, AN ESSENTIAL STEP FOR UNRAVELING THE GENETIC FACTORS CONTRIBUTING TO COMPLEX DISORDERS.

**POPULATION GENETICS** IN POPULATION GENETICS, THE FOCUS CHANGES FROM ALTERATIONS IN AN INDIVIDUAL’S GENOME TO THE DISTRIBUTION PATTERN OF DIFFERENT GENOTYPES IN THE POPULATION. IN A CASE WHERE THERE ARE ONLY TWO ALLELES, A AND A, THE FREQUENCY OF THE GENOTYPES WILL BE $P^2 + 2PQ + Q^2 = 1$, WITH $P^2$ CORRESPONDING TO THE FREQUENCY OF AA, $2PQ$ TO THE FREQUENCY OF AA, AND $Q^2$ TO AA. WHEN THE FREQUENCY OF AN ALLELE IS KNOWN, THE FREQUENCY OF THE GENOTYPE CAN BE CALCULATED. ALTERNATIVELY, ONE CAN DETERMINE AN ALLELE FREQUENCY, IF THE GENOTYPE FREQUENCY HAS BEEN DETERMINED. ALLELE FREQUENCIES VARY AMONG ETHNIC GROUPS AND GEOGRAPHICAL REGIONS. FOR EXAMPLE, HETEROZYGOUS MUTATIONS IN THE CFTR GENE ARE RELATIVELY COMMON IN POPULATIONS OF EUROPEAN ORIGIN BUT ARE RARE IN THE AFRICAN POPULATION. ALLELE FREQUENCIES MAY VARY BECAUSE CERTAIN ALLELIC VARIANTS CONFER A SELECTIVE ADVANTAGE. FOR EXAMPLE, HETEROZYGOTES FOR THE SICKLE CELL MUTATION, WHICH IS PARTICULARLY COMMON IN WEST AFRICA, ARE MORE RESISTANT TO MALARIAL INFECTION BECAUSE THE ERYTHROCYTES OF HETEROZYGOTES PROVIDE A LESS FAVORABLE ENVIRONMENT FOR PLASMODIUM PARASITES. THOUGH HOMOZYGOSITY FOR THE SICKLE CELL GENE IS ASSOCIATED WITH SEVERE ANEMIA AND SICKLE CRISSES (CHAP. 99), HETEROZYGOTES HAVE A HIGHER PROBABILITY OF SURVIVAL BECAUSE OF THE REDUCED MORBIDITY AND MORTALITY FROM MALARIA; THIS PHENOMENON HAS LED TO AN INCREASED FREQUENCY OF THE MUTANT ALLELE. RECESSIVE CONDITIONS ARE MORE PREVALENT IN GEOGRAPHICALLY ISOLATED POPULATIONS BECAUSE OF THE MORE RESTRICTED GENE POOL.

**APPROACH TO THE PATIENT:**
**INHERITED DISORDERS**

FOR THE PRACTICING CLINICIAN, THE FAMILY HISTORY REMAINS AN ESSENTIAL STEP IN RECOGNIZING THE POSSIBILITY OF A HEREDITARY COMPONENT. WHEN TAKING THE HISTORY, IT IS USEFUL TO DRAW A DETAILED PEDIGREE OF THE FIRST-DEGREE RELATIVES (E.G., PARENTS, SIBLINGS, AND CHILDREN), SINCE THEY SHARE 50% OF GENES WITH THE PATIENT. STANDARD SYMBOLS FOR PEDIGREES ARE DEPICTED IN FIG. 62-9. THE FAMILY HISTORY SHOULD INCLUDE INFORMATION ABOUT ETHNIC BACKGROUND, AGE, HEALTH STATUS, AND (INFANT) DEATHS. NEXT, THE PHYSICIAN SHOULD EXPLORE WHETHER THERE IS A FAMILY HISTORY OF THE SAME OR RELATED ILLNESSES TO THE CURRENT PROB-
LEM. AN INQUIRY FOocused ON COMMONLY OCCURRING DISORDERS SUCH AS CANCERS, HEART DISEASE, AND DIABETES MELLITUS SHOULD FOLLOW. BECAUSE OF THE POSSIBILITY OF AGE-DEPENDENT EXPRESSIVITY AND PENETRANCE, THE FAMILY HISTORY WILL NEED INTERMITTENT UPDATING. IF THE FINDINGS SUGGEST A GENETIC DISORDER, THE CLINICIAN WILL HAVE TO ASSESS WHETHER SOME OF THE PATIENT’S RELATIVES MAY BE AT RISK OF CARRYING OR TRANSMITTING THE DISEASE. IN THIS CIRCUMSTANCE, IT IS USEFUL TO CONFIRM AND EXTEND THE PEDIGREE BASED ON INPUT FROM SEVERAL FAMILY MEMBERS. THIS INFORMATION MAY FORM THE BASIS FOR CARRIER DETECTION, GENETIC COUNSELING, EARLY INTERVENTION, AND PREVENTION OF A DISEASE IN RELATIVES OF THE INDEX PATIENT (CHAP. 64).


IDENTIFYING THE DISEASE-CAUSING GENE GENOMIC MEDICINE AIMS TO ENHANCE THE QUALITY OF MEDICAL CARE THROUGH THE USE OF GENOTYPIC ANALYSIS (DNA TESTING) TO IDENTIFY GENETIC PREDISPOSITION TO DISEASE, TO SELECT MORE SPECIFIC PHARMACOTHERAPY, AND TO DESIGN INDIVIDUALIZED MEDICAL CARE BASED ON GENOTYPE. GENOTYPE CAN BE DEDUCED BY ANALYSIS OF PROTEIN (E.G., HEMOGLOBIN, APOPROTEIN E), MRNA, OR DNA. HOWEVER, TECHNOLOGICAL ADVANCES HAVE MADE DNA ANALYSIS PARTICULARLY USEFUL BECAUSE IT CAN BE READILY APPLIED TO ALL BUT THE LARGEST GENES (FIG. 62-14).

DNA TESTING IS PERFORMED BY MUTATIONAL ANALYSIS OR LINKAGE STUDIES IN INDIVIDUALS AT RISK FOR A GENETIC DISORDER KNOWN TO BE PRESENT IN A FAMILY. MASS SCREENING PROGRAMS REQUIRE TESTS OF HIGH SENSITIVITY AND SPECIFICITY TO BE COST-EFFECTIVE. PREREQUISITES FOR THE SUCCESS OF GENETIC SCREENING PROGRAMS INCLUDE THE FOLLOWING: THAT THE DISORDER IS POTENTIALLY SERIOUS; THAT IT CAN BE INFLUENCED AT A PRE-SYMPTOMATIC STAGE BY CHANGES IN BEHAVIOR, DIET, AND/OR PHARMA-
CEUTICAL MANIPULATIONS; AND THAT THE SCREENING DOES NOT RESULT IN ANY HARM OR DISCRIMINATION. SCREENING IN JEWISH POPULATIONS FOR THE AUTOSOMAL RECESSIVE NEURODEGENERATIVE STORAGE DISEASE TAY-SACHS HAS REDUCED THE NUMBER OF AFFECTED INDIVIDUALS. IN CONTRAST, SCREENING FOR SICKLE CELL TRAIT/DISEASE IN AFRICAN AMERICANS HAS LED TO UNANTICIPATED PROBLEMS OF DISCRIMINATION BY HEALTH INSURERS AND EMPLOYERS. MASS SCREENING PROGRAMS HARBOR ADDITIONAL POTENTIAL PROBLEMS. FOR EXAMPLE, SCREENING FOR THE MOST COMMON GENETIC ALTERATION IN CYSTIC FIBROSIS, THE *F508 MUTATION WITH A FREQUENCY OF ~70% IN NORTHERN EUROPE, IS FEASIBLE AND SEEMS TO BE EFFECTIVE. ONE HAS TO KEEP IN MIND, HOWEVER, THAT THERE IS PRONOUNCED ALLELIC HETEROGENEITY AND THAT THE DISEASE CAN BE CAUSED BY >1400 OTHER MUTATIONS. THE SEARCH FOR THESE LESS COMMON MUTATIONS WOULD SUBSTANTIALLY INCREASE COSTS BUT NOT THE EFFECTIVENESS OF THE SCREENING PROGRAM AS A WHOLE. OCCUPATIONAL SCREENING PROGRAMS AIM TO DETECT INDIVIDUALS WITH INCREASED RISK FOR CERTAIN PROFESSIONAL ACTIVITIES (E.G., *###1 ANTITRYPSIN DEFICIENCY AND SMOKE OR DUST EXPOSURE).

MUTATIONAL ANALYSES DNA SEQUENCE ANALYSIS IS INCREASINGLY USED AS A DIAGNOSTIC TOOL AND HAS SIGNIFICANTLY ENHANCED DIAGNOSTIC ACCURACY. IT IS USED FOR DETERMINING CARRIER STATUS AND FOR PRENATAL TESTING IN MONOGENIC DISORDERS (CHAP. 64). NUMEROUS TECHNIQUES ARE AVAILABLE FOR THE DETECTION OF MUTATIONS (TABLE 62-9). IN A VERY BROAD SENSE, ONE CAN DISTINGUISH BETWEEN TECHNIQUES THAT ALLOW FOR SCREENING THE ABSENCE OR PRESENCE OF KNOWN MUTATIONS (SCREENING MODE) OR TECHNIQUES THAT DEFINITIVELY CHARACTERIZE MUTATIONS. ANALYSES OF LARGE ALTERATIONS IN THE GENOME ARE POSSIBLE USING CLASSIC METHODS SUCH AS CYTOGENETICS, FLUORESCENT IN SITU HYBRIDIZATION (FISH), AND SOUTHERN BLOTTING (CHAP. 63), AS WELL AS MORE SENSITIVE NOVEL TECHNIQUES THAT SEARCH FOR MULTIPLE SINGLE EXON DELETIONS OR DUPLICATIONS. MORE DISCRETE SEQUENCE ALTERATIONS RELY HEAVILY ON THE USE OF THE PCR, WHICH ALLOWS RAPID GENE AMPLIFICATION AND ANALYSIS. MOREOVER, PCR MAKES IT POSSIBLE TO PERFORM GENETIC TESTING AND MUTATIONAL ANALYSIS WITH SMALL AMOUNTS OF DNA EXTRACTED FROM LEUKOCYTES OR EVEN FROM SINGLE CELLS, BUCCELLS, OR HAIR ROOTS. SCREENING FOR POINT MUTATIONS CAN BE PERFORMED BY NUMEROUS METHODS (TABLE 62-9); MOST ARE BASED ON THE RECOGNITION OF MISMATCHES BETWEEN NUCLEIC ACID DUPLEXES, ELECTROPHORETIC SEPARATION OF SINGLE- OR DOUBLE-STRANDED DNA, OR SEQUENCING OF DNA FRAGMENTS AMPLIFIED BY PCR. DNA SEQUENCING CAN BE PERFORMED DIRECTLY ON PCR PRODUCTS OR ON FRAGMENTS CLONED INTO PLASMID VECTORS AMPLIFIED IN BACTERIAL HOST CELLS. RT-PCR MAY BE USEFUL TO DETECT ABSENT OR REDUCED LEVELS OF MRNA EXPRESSION DUE TO A MUTATED ALLELE. PROTEIN TRUNCATION TESTS (PTT) CAN BE USED TO DETECT THE BROAD ARRAY OF MUTATIONS THAT RESULT IN PREMATURE TERMINATION OF A POLYPEPTIDE DURING ITS SYNTHESIS. THE ISOLATED CDNA IS TRANSCRIBED AND TRANSLATED IN VITRO, AND THE PROTEINS ARE ANALYZED BY GEL ELECTROPHORESIS. COMPARISON OF ELECTROPHORETIC MOBILITY WITH THE WILD-TYPE PROTEIN ALLOWS DETECTION OF TRUNCATED MUTANTS. THE MAJORITY OF TRADITIONAL DIAGNOSTIC METHODS ARE GEL-BASED.
NOVEL TECHNOLOGIES FOR THE ANALYSIS OF MUTATIONS, GENOTYPING, LARGE-SCALE SEQUENCING, AND MRNA EXPRESSION PROFILES ARE IN RAPID DEVELOPMENT. DNA CHIP TECHNOLOGIES ALLOW HYBRIDIZATION OF DNA OR RNA TO HUNDREDS OF THOUSANDS OF PROBES SIMULTANEOUSLY. MICROARRAYS ARE BEING USED CLINICALLY FOR MUTATIONAL ANALYSIS OF SEVERAL HUMAN DISEASE GENES, AS WELL AS FOR THE IDENTIFICATION OF VIRAL SEQUENCE VARIATIONS. TOGETHER WITH THE KNOWLEDGE GAINED FROM THE HGP, THESE TECHNOLOGIES PROVIDE THE FOUNDATION TO EXPAND FROM A FOCUS ON SINGLE GENES TO ANALYSES AT THE SCALE OF THE GENOME. FASTER AND CHEAPER SEQUENCING TECHNOLOGIES ARE UNDER DEVELOPMENT, AND IT HAS BEEN ANTICIPATED THAT SEQUENCING THE WHOLE GENOME OF AN INDIVIDUAL FOR A COST OF <$1000 WILL BECOME A REALITY WITHIN THIS DECADE. THE AVAILABILITY OF COMPREHENSIVE INDIVIDUAL SEQUENCE INFORMATION IS EXPECTED TO HAVE A SIGNIFICANT IMPACT ON MEDICAL CARE AND PREVENTATIVE STRATEGIES, BUT IT ALSO RAISES ETHICAL AND LEGAL CONCERNS HOW SUCH INFORMATION MAY BE USED BY INSURERS AND EMPLOYERS.

A GENERAL ALGORITHM FOR THE APPROACH TO MUTATIONAL ANALYSIS IS OUTLINED IN FIG. 62-14. THE IMPORTANCE OF A DETAILED CLINICAL PHENOTYPE CANNOT BE OVEREMPHASIZED. THIS IS THE STEP WHERE ONE SHOULD ALSO CONSIDER THE POSSIBILITY OF GENETIC HETEROGENEITY AND PHENO-COPIES. IF OBVIOUS CANDIDATE GENES ARE SUGGESTED BY THE PHENOTYPE, THEY CAN BE ANALYZED DIRECTLY. AFTER IDENTIFICATION OF A MUTATION, IT IS ESSENTIAL TO DEMONSTRATE THAT IT SEGREGATES WITH THE PHENOTYPE.

THE FUNCTIONAL CHARACTERIZATION OF NOVEL MUTATIONS IS LABOR INTEN-

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406 PART 3: GENETICS AND DISEASE

TABLE 62-9 METHODS USED FOR THE DETECTION OF MUTATIONS

METHOD

COMMONLY USED TECHNIQUES

CYTOGENETIC ANALYSIS

FLUORESCENT IN SITU HYBRIDIZATION (FISH)
SOUTHERN BLOT

POLYMERASE CHAIN REACTION (PCR)

REVERSE TRANSCRIPTASE PCR (RT-PCR)

DNA SEQUENCING

RESTRICTION FRAGMENT POLYMORPHISM (RFLP)
OTHER TECHNIQUES

SINGLE-STRAND CONFORMATIONAL POLYMORPHISM (SSCP)
DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)
RNASE CLEAVAGE

OLIGONUCLEOTIDE SPECIFIC HYBRIDIZATION (OSH)
MICROARRAYS

PROTEIN TRUNCATION TEST (PTT)
PYRO SEQUENCING

MULTIPEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA)

PRINCIPLE

UNIQUE VISUAL APPEARANCE OF VARIOUS CHROMOSOMES
HYBRIDIZATION TO CHROMOSOMES WITH FLUORESCENTLY LABELED PROBES
HYBRIDIZATION WITH GENOMIC PROBE OR CDNA PROBE AFTER DIGESTION OF HIGH MOLECULAR DNA
AMPLIFICATION OF DNA SEGMENT

REVERSE TRANSCRIPTION, AMPLIFICATION OF DNA SEGMENT * ABSENCE OR REDUCTION OF MRNA TRANSCRIPTION
DIRECT SEQUENCING OF PCR PRODUCTS
SEQUENCING OF DNA SEGMENTS CLONED INTO PLASMID VECTORS
DETECTION OF ALTERED RESTRICTION PATTERN OF GENOMIC DNA (SOUTHERN BLOT) OR PCR PRODUCTS

PCR OF DNA SEGMENT: MUTATIONS RESULT IN CONFORMATIONAL CHANGE AND ALTERED MOBILITY
PCR OF DNA SEGMENT: MUTATIONS RESULT IN CONFORMATIONAL CHANGE AND ALTERED MOBILITY
CLEAVAGE OF MISMATCH BETWEEN MUTATED AND WILD-TYPE SEQUENCE
HYBRIDIZATION OF PCR PRODUCTS TO WILD-TYPE OR MUTATED OLIGONUCLEOTIDES IMMOBILIZED ON CHIPS OR SLIDES
HYBRIDIZATION OF PCR PRODUCTS TO WILD-TYPE OR MUTATED OLIGONUCLEOTIDES

TRANSCRIPTION/TRANSLATION OF CDNA ISOLATED FROM TISSUE SAMPLE
CLONAL AMPLIFICATION OF SINGLE DNA FRAGMENTS ON MICROPARTICLES FOLLOWED BY MASSIVE PARALLEL SEQUENCING
QUANTIFICATION OF PCR-GENERATED AMPLICONS REFLECTING THE NUMBER OF COPIES OF A SPECIFIC DNA SEQUENCE

TYPE OF MUTATION DETECTED

NUMERICAL OR STRUCTURAL ABNORMALITIES IN CHROMOSOMES
LARGE DELETION, INSERTION, REARRANGEMENT, EXPANSIONS OF TRIPLET REPEAT, AMPLIFICATION
EXPANSION OF TRIPLET REPEATS, VARIABLE NUMBER OF TANDEM REPEATS (VNTR), GENE REARRANGEMENTS, TRANSLOCATIONS,
PREPARE DNA FOR OTHER MUTATION METHODS
ANALYZE EXPRESSED MRNA (CDNA) SEQUENCE; DETECT LOSS OF EXPRESSION
POINT MUTATIONS, SMALL DELETIONS AND INSERTIONS

POINT MUTATIONS, SMALL DELETIONS AND INSERTIONS
POINT MUTATIONS, SMALL DELETIONS AND INSERTIONS
POINT MUTATIONS, SMALL DELETIONS AND INSERTIONS

POINT MUTATIONS, SMALL DELETIONS AND INSERTIONS
GENOTYPING OF SNPS
MUTATIONS LEADING TO PREMATURE TRUNCATIONS
SEQUENCING OF WHOLE GENOMES OF MICROORGANISMS, RESEQUENCING OF AMPLICONS
COPY NUMBER VARIATIONS
SIVE AND MAY REQUIRE ANALYSES IN VITRO OR IN TRANSGENIC MODELS IN ORDER TO DOCUMENT THE RELEVANCE OF THE GENETIC ALTERATION. **Prenatal Diagnosis** of numerous genetic diseases in instances with a high risk for certain disorders is now possible by direct DNA analysis. Amniocentesis involves the removal of a small amount of amniotic fluid, usually at 16 weeks of gestation. Cells can be collected and submitted for karyotype analyses, FISH, and mutational analysis of selected genes. The main indications for amniocentesis include advanced maternal age above age 35, abnormal serum triple marker test (*-fetoprotein, *-human chorionic gonadotropin, pregnancy-associated plasma protein A, or unconjugated estriol), a family history of chromosomal abnormalities, or a mendelian disorder amenable to genetic testing. Prenatal diagnosis can also be performed by chorionic villus sampling (CVS), in which a small amount of the chorion is removed by a transcervical or transabdominal biopsy. Chromosomes and DNA obtained from these cells can be submitted for cytogenetic and mutational analyses. CVS can be performed earlier in gestation (weeks 9-12) than amniocentesis, an aspect that may be of relevance when termination of pregnancy is a consideration. Later in pregnancy, beginning at about 18 weeks of gestation, percutaneous umbilical blood sampling (PUBS) permits collection of fetal blood for lymphocyte culture and analysis. In combination with in vitro fertilization (IVF) techniques, it is even possible to perform genetic diagnoses in a single cell removed from the four- to eight-cell embryo or to analyze the first polar body from an oocyte. Preconceptual diagnosis thereby avoids therapeutic abortions but is extremely costly and labor intensive. Lastly, it has to be emphasized that excluding a specific disorder by any of these approaches is never equivalent to the assurance of having a normal child. Mutations in certain cancer susceptibility genes, such as BRCA1 and BRCA2, may identify individuals with an increased risk for the development of malignancies and result in risk-reducing interventions. The detection of mutations is an important diagnostic and prognostic tool in leukemias and lymphomas. The demonstration of the presence or absence of mutations and polymorphisms is also relevant for the rapidly evolving field of pharmacogenomics, including the identification of differences in drug treatment response or metabolism as
A FUNCTION OF GENETIC BACKGROUND. FOR EXAMPLE, THE THIOPURINE DRUGS 6-
MERCAPTOPURINE AND AZATHIOPRINE ARE COMMONLY USED CYTOTOXIC AND IM-
MUNOSUPPRESSIVE AGENTS. THEY ARE METABOLIZED BY THIOPURINE METHYL-
TRANSFERASE (TPMT), AN ENZYME WITH VARIABLE ACTIVITY ASSOCIATED WITH GE-
NETIC POLYMORPHISMS IN 10% OF CAU-
CASIANS AND COMPLETE DEFICIENCY IN ABOUT 1/300 INDIVIDUALS. PATIENTS WITH INTERMEDIATE OR DEFICIENT TPMT AC-
TIVITY ARE AT RISK FOR EXCESSIVE TOXICITY, INCLUDING FATAL MYELOSUPPRESSION.
CHARACTERIZATION OF THESE POLYMOR-
PHISMS ALLOWS MERCAPTOPURINE DOSES TO BE MODIFIED BASED ON TPMT GENOTYPE. PHARMACOGENOMICS MAY INCREASINGLY PERMIT INDIVIDUALIZED DRUG THERAPY, IMPROVE DRUG EFFEC-
TIVENESS, REDUCE ADVERSE SIDE EFFECTS, AND PROVIDE COST-EFFECTIVE PHAR-
MACEUTICAL CARE.

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PAGE NO. 53

407 CHAPTER 63 CHROMOSOME DISORDERS

63 CHROMOSOME DISORDERS
TERRY HASSOLD, STUART SCHWARTZ

IN HUMANS, THE NORMAL DIPLOID NUMBER OF CHROMOSOMES IS 46, CON-
SISTING OF 22 PAIRS OF AUTOSOMAL CHROMOSOMES (NUMBERED 1-22 IN DE-
CREASING SIZE) AND ONE PAIR OF SEX CHROMOSOMES (XX IN FEMALES AND XY
IN MALES). THE GENOME IS ESTIMATED TO CONTAIN BETWEEN 30,000 AND
40,000 GENES. EVEN THE SMALLEST AUTOSOME CONTAINS BETWEEN 200 AND
300 GENES. NOT SURPRISINGLY, DUPLICATIONS OR DELETIONS OF CHROMO-
SOMES, OR EVEN SMALL CHROMOSOME SEGMENTS, HAVE PROFOUND CONSE-
SEQUENCES ON NORMAL GENE EXPRESSION, LEADING TO SEVERE DEVELOPMENTAL AND PHYSIOLOGIC ABNORMALITIES.

DEVIATIONS IN NUMBER OR STRUCTURE OF THE 46 HUMAN CHROMOSOMES ARE ASTONISHINGLY COMMON, DESPITE SEVERE DELETERIOUS CONSEQUENCES. CHROMOSOMAL DISORDERS OCCUR IN AN ESTIMATED 10-25% OF ALL PREGNANCIES. THEY ARE THE LEADING CAUSE OF FETAL LOSS AND, AMONG PREGNANCIES SURVIVING TO TERM, THE LEADING KNOWN CAUSE OF BIRTH DEFECTS AND MENTAL RETARDATION.

IN RECENT YEARS, THE PRACTICE OF CYTOGENETICS HAS SHIFTED FROM CONVENTIONAL CYTOGENETIC METHODOLOGY TO A UNION OF CYTOGENETIC AND MOLECULAR TECHNIQUES. FORMERLY THE PROVINCE OF RESEARCH LABORATORIES, FLUORESCENCE IN SITU HYBRIDIZATION (FISH) AND RELATED MOLECULAR CYTOGENETIC TECHNOLOGIES HAVE BEEN INCORPORATED INTO EVERYDAY PRACTICE IN CLINICAL LABORATORIES. AS A RESULT, THERE IS AN INCREASED APPRECIATION OF THE IMPORTANCE OF “SUBLTE” CONSTITUTIONAL CYTOGENETIC ABNORMALITIES, SUCH AS MICRODELETIONS AND IMPRINTING DISORDERS, AS WELL AS PREVIOUSLY RECOGNIZED TRANSLOCATIONS AND DISORDERS OF CHROMOSOME NUMBER.

VISUALIZING CHROMOSOMES
CONVENTIONAL CYTOGENETIC ANALYSIS

IN THEORY, CHROMOSOME PREPARATIONS CAN BE OBTAINED FROM ANY ACTIVELY DIVIDING TISSUE BY CAUSING THE CELLS TO ARREST IN METAPHASE, THE STAGE OF THE CELL CYCLE WHEN CHROMOSOMES ARE MAXIMALLY CONDENSED. IN PRACTICE, ONLY A SMALL NUMBER OF TISSUES ARE USED FOR ROUTINE CHROMOSOME ANALYSIS: AMNIOCYTES OR CHORIONIC VILLI FOR PRENATAL TESTING AND BLOOD, BONE MARROW, OR SKIN FIBROBLASTS FOR POSTNATAL STUDIES. SAMPLES OF BLOOD, BONE MARROW, AND CHORIONIC VILLI CAN BE PROCESSED USING SHORT-TERM CULTURE TECHNIQUES THAT YIELD RESULTS IN 1-3 DAYS. ANALYSIS OF OTHER TISSUE TYPES TYPICALLY INVOLVES LONG-TERM CELL CULTURE, REQUIRING 1-3 WEEKS OF PROCESSING BEFORE CYTOGENETIC ANALYSIS IS POSSIBLE. CELLS ARE ISOLATED AT METAPHASE OR PROMETAPHASE AND TREATED CHEMICALLY OR ENZYMATICALLY TO REVEAL CHROMOSOME “BANDS” (FIG. 63-1). ANALYSIS OF THE NUMBER OF CHROMOSOMES IN THE CELL AND THE DISTRIBUTION OF BANDS ON INDIVIDUAL CHROMOSOMES ALLOW THE IDENTIFICATION OF NUMERICAL OR STRUCTURAL ABNORMALITIES. THIS STRATEGY IS USEFUL FOR CHARACTERIZING THE NORMAL CHROMOSOME COMPLEMENT AND DETERMINING THE INCIDENCE AND TYPES OF MAJOR CHROMOSOME ABNORMALITIES.

EACH HUMAN CHROMOSOME CONTAINS TWO SPECIALIZED STRUCTURES: A CENTROMERE AND TWO TELOMERES. THE CENTROMERE, OR PRIMARY CONSTRUCTION, DIVIDES THE CHROMOSOME INTO SHORT (P) AND LONG (Q) ARMS AND IS RESPONSIBLE FOR THE SEGREGATION OF CHROMOSOMES DURING CELL DIVISION. THE TELOMERES, OR CHROMOSOME ENDS, “CAP” THE P AND Q ARMS AND ARE IMPORTANT FOR ALLOWING DNA REPLICATION AT THE ENDS OF THE CHROMOSOMES. PRIOR TO DNA REPLICATION, EACH CHROMOSOME CONSISTS OF A SINGLE CHROMATID COPY OF THE DNA DOUBLE HELIX. AFTER DNA REPLICATION AND CONTINUING UNTIL THE TIME OF CELL DIVISION (INCLUDING METAPHASE,
WHEN CHROMOSOMES ARE TYPICALLY VISUALIZED, EACH CHROMOSOME CONSISTS OF TWO IDENTICAL SISTER CHROMATIDS (FIG. 63-1).

MOLECULAR CYTOGENETICS


FIGURE 63-1 A. AN IDEALIZED HUMAN CHROMOSOME, SHOWING THE CENTROMERE (CEN), LONG (Q) AND SHORT (P) ARMS, AND TELOMERES (TEL). B. A G-BANDED HUMAN KARYOTYPE FROM A NORMAL (46, XX) FEMALE.

PAGE NO. 54

409 CHAPTER 63 CHROMOSOME DISORDERS

TERPHASE ANALYSIS INVOLVES THE APPLICATION OF FISH TO PARAFFIN-EMBEDDED SECTIONS, THEREBY PRESERVING THE ARCHITECTURE OF THE TISSUE. THE USE OF INTERPHASE FISH HAS INCREASED RECENTLY, ESPECIALLY FOR ANALYSES OF AMNIOCENTESIS SAMPLES. THESE STUDIES ARE PERFORMED ON UNCULTURED AMNIOTIC FLUID, TYPICALLY USING DNA PROBES SPECIFIC FOR THE CHROMOSOMES MOST COMMONLY IDENTIFIED IN TRISOMIES (CHROMOSOMES 13, 18, 21, AND THE X AND Y). THESE STUDIES CAN BE PERFORMED RAPIDLY (24-72 H) AND WILL ASCERTAIN ABOUT 60% OF THE ABNORMALITIES DETECTED PRENATALLY. ANOTHER AREA IN WHICH INTERPHASE ANALYSIS IS ROUTINELY UTILIZED IS CANCER CYTOGENETICS (CHAP. 79). MANY SITE-SPECIFIC TRANSLOCATIONS ARE ASSOCIATED WITH SPECIFIC TYPES OF MALIGNANCIES. FOR EXAMPLE, THERE ARE PROBES AVAILABLE FOR BOTH THE ABELS ON (ABL) ONCOGENE AND BREAKPOINT CLUSTER REGION (BCR) INVOLVED IN CHRONIC MYELOGENOUS LEUKEMIA (CML); THESE PROBES ARE LABELED IN RED AND GREEN, RESPECTIVELY, THE FUSION OF THESE GENES IN CML COMBINES THE FLUORESCENT COLORS AND APPEARS AS A YELLOW HYBRIDIZATION SIGNAL.

IN ADDITION TO STANDARD METAPHASE AND INTERPHASE FISH ANALYSES, A NUMBER OF ENHANCED TECHNIQUES HAVE BEEN DEVELOPED FOR SPECIFIC TYPES OF ANALYSIS, INCLUDING MULTICOLOR FISH TECHNIQUES, REVERSE PAINTING, COMPARATIVE GENOMIC HYBRIDIZATION, AND FIBER FISH. SPECTRAL KARYOTYPING (SKY) AND MULTICOLOR FISH (M-FISH) TECHNIQUES USE COMBINATORIALLY LABELED PROBES THAT CREATE A UNIQUE COLOR FOR INDIVIDUAL CHROMOSOMES. THIS TECHNOLOGY IS USEFUL IN THE IDENTIFICATION OF UNKNOWN CHROMOSOME MATERIAL (SUCH AS MARKERS OF DUPLICATIONS) BUT IS MOST COMMONLY USED WITH THE COMPLEX REARRANGEMENTS SEEN IN CANCER SPECIMENS.
**Fiber Fish** is a technique in which chromosomes are mechanically stretched, using a variety of different methods. It provides a higher resolution of analysis than conventional fish.

**Comparative Genomic Hybridization (CGH)** is a method that can be used only when DNA is available from a specimen of interest. The entire DNA specimen from the sample of interest is labeled in one color (e.g., green), and the normal control DNA specimen is indicated by another color (e.g., red). These are mixed in equal amounts and hybridized to normal metaphase chromosomes. The red-to-green ratio is analyzed by a computer program, which determines where the DNA of interest may have gains or losses of material. This technique is useful in the analysis of tumors, particularly in those cases where cytogenetic analysis is not possible.

An extension of CGH promises to yield another major advance for examining human chromosomes. Specifically, the development of CGH “arrays” uses protocols that are similar to standard CGH, except that test DNA is hybridized to DNAs that are spread on arrays, rather than hybridized to normal chromosomes. Depending on the type of array (most are constructed utilizing either BACS or oligonucleotides), the resolution can be up to 150 KB, far greater than for standard chromosome analysis. This technology has been used to study cryptic chromosomal imbalances in patients with mental retardation and multiple congenital anomalies, as well as in prenatal diagnosis. It has also been used to detect microdeletions and microduplications in cancer and in previously unidentified genomic disorders. Although this technology is still in development, its use is anticipated to increase in the near term.

**Indications for Cytogenetic Analysis**

Primary indications for karyotypic analysis vary according to the developmental stage/age of the conceptus/individual under investigation. One especially important application is in prenatal diagnosis (particularly for pregnancies involving older women), assaying for chromosomal abnormalities in either chorionic villi of first-trimester fetuses or amniotic fluid of second-trimester fetuses. Tissue specimens from spontaneously aborted fetuses or stillbirths can also be examined for chromosome abnormalities. Interphase cytogenetics (using FISH) is increasingly being used to study individual blastomeres of preimplantation embryos (with in vitro fertilization-derived pregnancies). This makes it possible to detect aneuploid or structurally unbalanced embryos or, in the case of sex-linked disorders, to identify male conceptuses; such embryos would not be used to initiate pregnancies.

Among infants and children, peripheral blood is examined, most often in individuals with specific phenotypic abnormalities. For example, karyotypic analysis can be used for the confirmation or exclusion of a specific chromosomal syndrome (e.g., trisomy 21); in patients with unexplained psychomotor retardation with or without dysmorphic features; in cases of monogenic disorders associated with mental...
RETARDATION AND/OR DYSMORPHIC FEATURES; AND WITH ABNORMALITIES OF SEXUAL DIFFERENTIATION AND DEVELOPMENT. IN ADULTS, PERIPHERAL BLOOD CAN BE EXAMINED IN PATIENTS WITH INFERTILITY OR RECURRENT MISCARRIAGES, SINCE CHROMOSOME ABNORMALITIES CAN LEAD TO MEIOtic ARREST OR TO GENETICALLY UNBALANCED GAMES. AN IMPORTANT BRANCH OF CYTOGENETICS IS CONCERNED WITH ANALYSES OF BONE MARROW, UNSTIMULATED PERIPHERAL BLOOD, AND LYMPH NODES OF TUMORS, AS CHROMOSOMAL ABNORMALITIES ARE A COMMON CORRELATE OF LEUKEMIA, LYMPHOMA, AND SOLID TUMORS (CHAP. 79).

CYTOGENETIC TESTING IN PRENATAL DIAGNOSIS

THE VAST MAJORITY OF PRENATAL DIAGNOSTIC STUDIES ARE PERFORMED TO RULE OUT A CHROMOSOMAL ABNORMALITY, BUT CELLS MAY ALSO BE PROPAGATED FOR BIOCHEMICAL STUDIES OR MOLECULAR ANALYSES OF DNA. THREE PROCEDURES ARE USED TO OBTAIN SAMPLES FOR PRENATAL DIAGNOSIS: AMNIOCENTESIS, CHORIONIC VILLUS SAMPLING (CVS), AND FETAL BLOOD SAMPLING. AMNIOCENTESIS IS THE MOST COMMON PROCEDURE AND IS ROUTINELY PERFORMED AT 15-17 WEEKS OF GESTATION. ON SOME OCCASIONS, EARLY AMNIOCENTESIS AT 12-14 WEEKS IS PERFORMED TO EXPEDITE RESULTS, ALTHOUGH LESS FLUID IS OBTAINED AT THIS TIME. EARLY AMNIOCENTESIS CARRIES A GREATER RISK OF SPONTANEOUS ABORTION OR FETAL INJURY BUT PROVIDES RESULTS AT AN EARLIER STAGE OF PREGNANCY. THE VAST MAJORITY OF AMNIOCENTESIS ARE PERFORMED IN THE CONTEXT OF ADVANCED MATERNAL AGE, THE BEST-KNOWN CORRELATE OF TRISOMY (SEE BELOW). ADDITIONAL REASONS FOR AMNIOCENTESIS REFERRAL INCLUDE AN ABNORMAL “TRIPLE- OR QUAD-MARKER ASSAY” AND/OR DETECTION OF ULTRASOUND ABNORMALITIES. IN THIS ASSAY, LEVELS OF HUMAN CHORIONIC GONADOTROPIN, *-FETOPROTEIN, AND UNCONJUGATED ESTRIOL (AND, IN THE QUAD ASSAY, INHIBIN) IN THE MATERNAL SERUM ARE QUANTIFIED AND USED TO ADJUST THE MATERNAL AGE-PREDICTED RISK OF A TRISOMY 21 OR TRISOMY 18 FETUS. SPECIFIC ULTRASOUND ABNORMALITIES, WHEN DETECTED AT MIDTRIMESTER, CAN ALSO BE ASSOCIATED WITH CHROMOSOMAL DEFECTS. WHEN A NONSPECIFIC ULTRASOUND ABNORMALITY IS PRESENT, THE ESTIMATED RISK OF A CHROMOSOMAL DEFECT IS ~16%. ASSOCIATIONS OF CHROMOSOMAL ABNORMALITIES AND SPECIFIC TYPES OF ABNORMAL ULTRASOUND FINDINGS ARE LISTED IN TABLE 63-1. CVS IS THE SECOND MOST COMMON PROCEDURE FOR GENETIC PRENATAL DIAGNOSIS. BECAUSE THIS PROCEDURE IS ROUTINELY PERFORMED AT ABOUT 10-12 WEEKS OF GESTATION, IT ALLOWS FOR AN EARLIER DETECTION OF ABNORMALITIES AND A SAFER PREGNANCY TERMINATION, IF DESIRED. CVS IS A RELATIVELY SAFE PROCEDURE (SPONTANEOUS; ABORTIONS, <0.5-1%). BECAUSE THERE IS AN INCREASED ASSOCIATION OF LIMB DEFECTS WHEN THE PROCEDURE IS PERFORMED EARLIER (<10 WEEKS OF GESTATION), CVS IS APPLICABLE DURING A NARROW TIME FRAME OF GESTATION. CVS INVOLVES THE USE OF A CATHETER INSERTED TRANSVAGINALLY; ~25 MG OF VILLI ARE ASPIRATED FROM THE CHORION FRONDosUM (THE FETAL PORTION OF THE PLACENTA). BY ADDING COLCHICINE DIRECTLY TO THE RAPIDLY DIVIDING CYTOTROPHOBLASTS, RESULTS CAN BE OBTAINED WITHIN 24-48 H. FINDINGS FROM THESE PROCEDURES SHOULD BE CONFIRMED
### TABLE 63-1 FREQUENCY OF CHROMOSOME ABNORMALITIES, IDENTIFIED ON THE BASIS OF ABNORMAL ULTRASOUND FINDINGS

<table>
<thead>
<tr>
<th>ULTRASOUND FINDING</th>
<th>CHROMOSOMAL ABNORMALITIES (FREQUENCY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABNORMAL ULTRASOUND (NONSPECIFIC)</td>
<td>AVERAGE, %</td>
</tr>
<tr>
<td>OMPHALOCELE</td>
<td>16</td>
</tr>
<tr>
<td>CYSTIC HYGROMA</td>
<td>39</td>
</tr>
<tr>
<td>CONGENITAL HEART DISEASE</td>
<td>68</td>
</tr>
<tr>
<td>CHOROID PLEXUS CYST</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

**RANGE IN DIFFERENT STUDIES, %**

| 13-35 |
| 26-54 |
| 46-78 |
| 8-40 |
| 4-10 |

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**410 PART 3: GENETICS AND DISEASE**

By analyses of cultured mesenchymal cells, as they are more reliably derived from the fetus.

Percutaneous umbilical blood sampling (PUBS) is a method for obtaining fetal blood during the second and third trimesters of pregnancy. PUBS is usually performed when ultrasound abnormalities are detected late in the second trimester. PUBS is also used when cytogenetic results from amniocentesis need clarification, such as in the detection of mosaicism.
CHROMOSOME ABNORMALITIES

CHROMOSOMES IN CELL DIVISION


INCIDENCE AND TYPES OF CHROMOSOME ABNORMALITIES

ERRORS IN MEIOSIS, OR IN EARLY CLEAVAGE DIVISIONS, OCCUR WITH EXTRAORDINARY FREQUENCY. AT LEAST 10-25% OF ALL PREGNANCIES, FOR EXAMPLE, INVOLVE CHROMOSOMALLY ABNORMAL CONCEPTIONS. A LARGE PROPORTION OF THESE TERMINATE IN THE EARLIEST STAGES OF PREGNANCY, MANY OF WHICH GO UNRECOGNIZED. NEVERTHELESS, EVEN AMONG CLINICALLY RECOGNIZED PREGNANCIES, NEARLY 10% OF FETUSES ARE CHROMOSOMALLY UNBALANCED. FOR THE THREE TYPES OF CLINICALLY RECOGNIZED PREGNANCIES-SPONTANEOUS ABORTIONS, STILLBIRTHS, AND LIVEBIRTHS-THE FREQUENCIES OF DIFFERENT CHROMOSOMAL ABNORMALITIES ARE SUM-
The most common abnormalities are numerical, involving fetuses with additional (trisomy) or missing (monosomy) chromosomes, or those with one (triploidy) or two (tetraploidy) additional sets of chromosomes. Structural chromosome abnormalities are much less common, although several of the most important clinical chromosomal disorders involve structural rearrangements (see below). By far the most common abnormality is trisomy, which is identified in ~25% of spontaneous abortions and 0.3% of newborns. Trisomies for all chromosomes have now been identified in embryos or fetuses, but there is considerable variation in frequency for various chromosomes. For example, trisomy 16 is extraordinarily common, accounting for about one-third of all trisomies in spontaneous abortions, whereas trisomies 1, 5, 11, and 19 have been identified less often. Available evidence suggests two reasons for this variation: (1) some chromosomes (e.g., chromosome 16) are more likely to segregate abnormally or undergo nondisjunction during meiosis than are others; and (2) the potential for development varies widely among different trisomic conditions, with some being eliminated very early in gestation, others surviving to the time of clinical pregnancy recognition, and some (e.g., trisomies 13, 18, and 21 and sex chromosome trisomies) being compatible with survival to term.

Table 63-2 Frequency and distribution of chromosomal abnormalities in different types of clinically recognizable pregnancies

<table>
<thead>
<tr>
<th>Chromosome Abnormality</th>
<th>Frequency</th>
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</tbody>
</table>

Figure 63-3 Chromosome segregation in meiosis. A. In meiosis I, each of the 23 pairs of chromosomes finds its "partner," or homologue, and exchanges genetic material (recombines) with it. At metaphase, each homologous pair aligns on the equatorial plate, at anaphase, each member of the homologous pair segregates from its partner thus, at the end of meiosis I, each daughter cell contains 23 chromosomes, with each chromosome consisting of two sister chromatids. B. In meiosis II, each chromosome aligns on the metaphase plate, and at anaphase, each of the two sister chromatids divides from the other. Thus, at the end of meiosis II, each daughter cell (e.g., the oocyte or spermatoocyte) contains 23 chromosomes, with each chromosome consisting of one sister chromatid. In mitosis, the chromosomes behave exactly as they do in meiosis II, except that somatically dividing cells contain 46 chromosomes, not the 23 that are present in the meiosis II cell.
TRISOMY, ALL
+ 13, 18, 21
+ 16
SEX CHROMOSOME MONOSOMY (45, X) TRIPLOIDY TETRAPLOIDY STRUCTURAL ABNORMALITY TOTAL ABNORMALITIES

FREQUENCY OF ABNORMALITY,

SPONTANEOUS ABORTION

<table>
<thead>
<tr>
<th></th>
<th>25.1</th>
<th>45</th>
<th>75</th>
<th>8.7</th>
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<tr>
<td>6.4</td>
<td>2.4</td>
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STILLBIRTH

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<tr>
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<td>-</td>
<td>0.8</td>
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LIVEBIRTH

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<tr>
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<th>0.3</th>
<th>0.14</th>
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<tr>
<td>-</td>
<td>0.3</td>
<td>0.6</td>
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PROBABILITY OF SURVIVING TO TERM, %

<table>
<thead>
<tr>
<th></th>
<th>5</th>
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<tbody>
<tr>
<td>15</td>
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CHAPTER 63 CHROMOSOME DISORDERS

CHROMOSOMAL SYNDROMES

While most chromosomally abnormal conceptions perish in utero, several conditions are compatible with survival to term. The best-characterized of these are numerical abnormalities, involving loss or gain of individual chromosomes, and abnormalities resulting from unbalanced translocations. Fish and other molecular studies have led to the identification of two “new” types of chromosome abnormalities, commonly referred to as microdeletion syndromes and imprinting syndromes.

NUMERICAL ABNORMALITIES

Virtually all types of numerical abnormalities are eliminated prenatally, so that only those involving small, gene-poor autosomes or the sex chromosomes are identified with any frequency among live-borns. Clinically, the most important of these is trisomy 21, the most frequent cause of Down syndrome. Depending on the maternal age structure of the population and the utilization of prenatal testing, the incidence of trisomy 21 ranges from 1/600 to 1/1000 live births, making it the most common chromosome abnormality in live-born individuals. Like most trisomies, the incidence of trisomy 21 is highly correlated with maternal age, increasing from about 1/1500 live births for women 20 years of age to 1/30 for women *45 years.

In addition to trisomy 21, only two other autosomal trisomies, 13 and 18, occur with any frequency in livebirths. Incidence rates for trisomies 13 and 18 in livebirths are 1/20,000 and 1/10,000, respectively. Unlike trisomy 21, which is associated with near-normal life expectancy, both trisomies 13 and 18 are associated with death in infancy, typically occurring during the first year of life.

Three sex chromosome trisomies—the 47, XXX, 47, XXY (Klinefelter syndrome), and 47, XYY conditions—are quite common, with each occurring in about 1/2000 newborns. Of all the trisomic conditions, these three have the fewest phenotypic complications. In fact, with the excep-
TION OF INFERTILITY IN KLINEFELTER SYNDROME (CHAP. 343), IT IS LIKELY THAT MOST INDIVIDUALS WITH SUCH TRISOMIC CONDITIONS WOULD GO UNDETECTED. THE ADDITIONAL Y CHROMOSOME IN THE 47, XXY CONDITION IS SMALL AND CONTAINS ONLY A FEW GENES. MOST Y-LINKED GENES ARE INVOLVED IN TESTICULAR DEVELOPMENT OR SPERMATOGENESIS. THUS, DOSAGE IMBALANCE OF Y-LINKED GENES HAS RELATIVELY LITTLE EFFECT ON OTHER DEVELOPMENTAL PROCESSES. THE 47, XYY GENOTYPE IS ASSOCIATED WITH INCREASED HEIGHT. ITS ROLE IN ANTISOCIAL BEHAVIOR, POSTULATED INITIALLY BECAUSE OF AN INCREASED PREVALENCE AMONG SOME PENALIZED POPULATIONS, IS UNCLEAR.

FOR THE 47, XXX AND 47, XXY CONDITIONS, THE SITUATION IS DIFFERENT - THE X CHROMOSOME CONTAINS >1000 GENES, MANY OF THEM ESSENTIAL FOR NORMAL DEVELOPMENT. HOW, THEN, ARE 47, XXX AND 47, XXY INDIVIDUALS SPARED FROM THE CATASTROPHIC CONSEQUENCES OF DOSAGE IMBALANCE? THE ANSWER LIES IN THE BIOLOGY OF X CHROMOSOME GENE EXPRESSION. IN NORMAL FEMALES, ONE OF THE CHROMOSOMES UNDERGOES X INACTIVATION IN SOMATIC CELLS. THE INACTIVATION OF THE PATERNAL OR MATERNAL X CHROMOSOME OCCURS RANDOMLY IN EACH SOMATIC CELL AND THEREBY SERVES AS A MECHANISM OF DOSAGE COMPENSATION, ENSURING THAT MALES AND FEMALES HAVE EQUAL EXPRESSION OF MOST X-LINKED GENES. THE INACTIVATION PROCESS OCCURS AT THE BLASTOCYST STAGE OF DEVELOPMENT; PRIOR TO THIS, BOTH X CHROMOSOMES ARE ACTIVE. IN ADDITION, NOT ALL X-LINKED GENES ARE INACTIVATED. SOME GENES ON THE X CHROMOSOME “ESCAPE” THE INACTIVATING MECHANISM AND ARE EXPRESSED FROM BOTH X CHROMOSOMES. IN DISORDERS SUCH AS KLINEFELTER SYNDROME, SOME GENES MAY BE EXPRESSED FROM BOTH X CHROMOSOMES, RESULTING IN ITS PHENOTYPIC FEATURES.

AS A RULE, MONOSOMIC CONDITIONS ARE INCOMPATIBLE WITH FETAL DEVELOPMENT AND, CONSEQUENTLY, AUTOSOMAL MONOSOMIES ARE ONLY RARELY IDENTIFIED IN SPONTANEOUS ABORTIONS AND ARE NOT FOUND AMONG LIVE-BORN INDIVIDUALS. IN FACT, THE ONLY MONOSOMY COMPATIBLE WITH LIVE BIRTH IS THE 45, X CONDITION, WHICH CAUSES TURNER SYNDROME. THE 45, X CHROMOSOME CONSTITUTION OCCURS WITH SURPRISINGLY HIGH FREQUENCY, PRESENT IN AT LEAST 1-2% OF ALL PREGNANCIES. MORE THAN 99% OF ALL 45, X CONCEPTIONS ARE SPONTANEOUSLY ABORTED. THUS, LIVE-BORN INDIVIDUALS WITH A 45, X CHROMOSOME CONSTITUTION REPRESENT A RARE GROUP OF SURVIVORS. THE 45, X PHENTYPE IS MILD, PRESUMABLY BECAUSE THE SECOND COPY OF MANY X CHROMOSOMAL GENES IS NORMALLY INACTIVATED. NONETHELESS, TURNER SYNDROME CAUSES GONADAL DYSGENESIS, RESULTING IN INFERTILITY AND FAILURE TO

### TABLE 63-3 STUDIES OF THE PARENT AND MEIOTIC/MITOTIC STAGE OF ORIGIN OF HUMAN TRISOMIES AND SEX CHROMOSOME MONOSOMY
TRISOMY

2
7
15
16
18
21
22
XXY
XXX

MONOSOMY

X###A

ORIGIN, %

PATERNAL

I

28
-
-
-
-
3
3
3
46
-

II

-
-
-
15
1
-
5
-
-
6

* 80

MATERNAL

I

54
17
RESULTS PERTAIN TO NON MOSAIC 45, X INDIVIDUALS.

UNDERGO SECONDARY SEXUAL DEVELOPMENT, ALONG WITH A NUMBER OF OTHER PHENOTYPIC FEATURES (CHAP. 343). SEVERAL OTHER STRUCTURAL ABNORMALITIES OF THE X CHROMOSOME SUCH AS DELETIONS, ISOCHROMOSOME X, OR RING CHROMOSOMES CAN CAUSE TURNER SYNDROME. MOSAICISM, INCLUDING 45,X/45,XX, 45,X/45,XXX, 45,X/45,XY, AND OTHERS, ALSO OCCURS (SEE BELOW) AND CONTRIBUTES TO THE PHENOTYPIC SPECTRUM IN TURNER SYNDROME.

BECAUSE NUMERICAL ABNORMALITIES ORIGINATE IN MEIOSIS (TABLE 63-3), AFFECTED INDIVIDUALS HAVE MISSING OR EXTRA CHROMOSOMES IN ALL CELLS. IN A SMALL PROPORTION OF CASES, A MITOTIC NONDISJUNCTIONAL EVENT OCCURS AT AN EARLY STAGE IN AN INDIVIDUAL WITH AN INITIALLY NORMAL CHROMOSOME CONSTITUTION. ALTERNATIVELY, A “NORMALIZING” MITOTIC NONDISJUNCTIONAL EVENT MAY RESULT IN A NORMAL CHROMOSOME COMPLEMENT IN SOME CELLS.
OF AN EMBRYO. IN EITHER CASE, THE EMBRYO IS A MOSAIC, WITH SOME CELLS BEARING A NORMAL CHROMOSOME CONSTITUTION AND OTHERS AN ANEUPLOID NUMBER OF CHROMOSOMES. THE PHENOTYPIC CONSEQUENCES ARE DIFFICULT TO PREDICT BECAUSE THEY DEPEND ON THE TIMING OF NONDISJUNCTION AND THE DISTRIBUTION OF NORMAL AND ABNORMAL CELLS IN DIFFERENT TISSUES. NEVERTHELESS, MOSAICISM MAY LEAD TO CLINICAL ABNORMALITIES INDISTINGUISHABLE FROM THOSE OF NONMOSAIC INDIVIDUALS; FOR EXAMPLE, NEARLY 5% OF ALL CASES OF DOWN SYNDROME INVOLVE INDIVIDUALS WITH MOSAIC TRISOMY 21, AND ABOUT 15% OF INDIVIDUALS WITH TURNER SYNDROME ARE MOSAIC FOR VARIOUS SEX CHROMOSOMAL CONSTITUTIONS AS DESCRIBED ABOVE.

THE ORIGIN ABD ETIOLOGY OF NUMERICAL ABNORMALITIES OVER THE PAST DECADE, A NUMBER OF STUDIES HAVE USED DNA POLYMORPHISMS TO INVESTIGATE THE ORIGIN OF DIFFERENT TYPES OF CHROMOSOME ABNORMALITIES (FIG. 63-4). THE MOST THOROUGHLY INVESTIGATED TYPES HAVE BEEN NUMERICAL ABNORMALITIES (TABLE 63-3). SEX CHROMOSOME MONOSOMY USUALLY RESULTS FROM LOSS OF THE PATERNAL SEX CHROMOSOME, REGARDLESS OF WHETHER THE CONCEPTION IS LIVE-BORN OR SPONTANEOUSLY ABORTED. TRISOMIES SHOW REMARKABLE VARIATION IN PARENTAL ORIGIN. FOR EXAMPLE, PATERNAL NONDISJUNCTION IS RESPONSIBLE FOR NEARLY 50% OF 47,XXY BUT ONLY 5-10% OF CASES OF TRISOMIES 13, 14, 15,21, AND 22; IT IS RARELY, IF EVER, THE SOURCE OF THE ADDITIONAL CHROMOSOME IN TRISOMY 16. SIMILARLY, THERE IS CONSIDERABLE VARIABILITY IN THE MEIOTIC STAGE OF ORIGIN. FOR EXAMPLE, ALL CASES OF TRISOMY 16 MAY BE DUE TO MEIOSIS I ERRORS, WHEREAS FOR TRISOMY 21, ONE-THIRD OF CASES ARE ASSOCIATED WITH MEIOSIS II ERRORS, AND FOR TRISOMY 18, THE MAJORITY OF CASES ARE APPARENTLY DUE TO MEIOSIS II NONDISJUNCTION. IN SPITE OF THIS VARIATION IN PARENTAL AND MEIOTIC ORIGIN, NONDISJUNCTION AT MATERNAL MEIOSIS I APPEARS TO BE THE MOST COMMON SOURCE OF TRISOMY.

MATERNAL AGE AND TRISOMY THE ASSOCIATION BETWEEN INCREASING MATERNAL AGE AND TRISOMY IS THE MOST IMPORTANT ETIOLOGIC FACTOR IN CON-
GENITAL CHROMOSOMAL DISORDERS. AMONG WOMEN UNDER THE AGE OF 25, ~2% OF ALL CLINICALLY RECOGNIZED PREGNANCIES ARE TRISOMIC; BY THE AGE OF 36, HOWEVER, THIS FIGURE INCREASES TO 10% AND BY THE AGE OF 42, TO >33% (FIG. 63-5). THIS ASSOCIATION BETWEEN MATERNAL AGE AND TRISOMY IS EXERTED WITHOUT RESPECT TO RACE, GEOGRAPHY, OR SOCIOECONOMIC FACTORS AND LIKELY AFFECTS SEGREGATION OF ALL CHROMOSOMES. DESPITE THE IMPORTANCE OF INCREASING AGE, LITTLE IS KNOWN ABOUT THE MECHANISM BY WHICH AGING LEADS TO ABNORMAL CHROMOSOMAL SEGREGATION.

FIGURE 63-5 ESTIMATED MATERNAL AGE-ADJUSTED RATES OF TRISOMY AMONG ALL CLINICALLY RECOGNIZED PREGNANCIES (E.G., SPONTANEOUS ABORTIONS, STILLBIRTHS, AND LIVEBIRTHS). AMONG WOMEN IN THEIR FORTIES, OVER 25% OF ALL PREGNANCIES ARE ESTIMATED TO INVOLVE A TRISOMIC CONCEPTION, THE VAST MAJORITY OF THESE SPONTANEOUSLY ABORT, WITH ONLY TRISOMIES 13, 18, AND 21 AND SEX CHROMOSOME TRISOMIES SURVIVING TO TERM WITH ANY APPRECIABLE FREQUENCY.

AS NOTED ABOVE, IT IS THOUGHT TO ORIGINATE IN MATERNAL MEIOSIS I OWING TO THE PROTRACTED TIME TO COMPLETION (OFTEN >40 YEARS) IN FEMALES, AND RECENT STUDIES SUGGEST THAT IT MAY BE ASSOCIATED WITH ALTERATIONS IN MEIOTIC CROSSING-OVER. IN TRISOMY 21, FOR EXAMPLE, CROSSOVER PATTERNS APEAR TO BE SIMILARLY ABNORMAL IN YOUNGER AND OLDER MOTHERS OF TRISOMIC CONCEPTIONS. THUS, IT HAS BEEN SUGGESTED THAT TWO DISTINCT STEPS, OR “HITS,” MAY BE INVOLVED IN MATERNAL AGE-RELATED NONDISJUNCTION. THE FIRST HIT, WHICH IS AGE INDEPENDENT, INVOLVES THE ESTABLISHMENT OF A “VULNERABLE” CROSSOVER CONFIGURATION IN THE FETAL OOCYTE; THE SECOND HIT, WHICH IS AGE DEPENDENT, INVOLVES ABNORMAL PROCESSING OF THE VULNERABLE BIVALENT STRUCTURE AT METAPHASE I. IF THIS MODEL IS CORRECT, IT SUGGESTS THAT THE NONDISJUNCTIONAL PROCESS IS THE SAME IN YOUNGER AND OLDER WOMEN, BUT IT OCCURS MORE FREQUENTLY WITH AGING, POSSIBLY BECAUSE OF AGE-DEPENDENT DEGRADATION OF MEIOTIC PROTEINS.

STRUCTURAL CHROMOSOME ABNORMALITIES

STRUCTURAL REARRANGEMENTS INVOLVE BREAKAGE AND REUNION OF CHROMOSOMES. ALTHOUGH LESS COMMON THAN NUMERICAL ABNORMALITIES, THEY PRESENT ADDITIONAL CHALLENGES FROM A GENETIC COUNSELING STANDPOINT. THIS IS BECAUSE STRUCTURAL ABNORMALITIES, UNLIKE NUMERICAL ABNORMALITIES, CAN BE PRESENT IN “BALANCED” FORM IN CLINICALLY NORMAL INDIVIDUALS BUT TRANSMITTED IN “UNBALANCED” FORM TO PROGENY, THEREBY RESULTING IN A HEREDITARY FORM OF CHROMOSOME ABNORMALITY. REARRANGEMENTS MAY INVOLVE EXCHANGES OF MATERIAL BETWEEN DIFFERENT CHROMOSOMES (TRANSLOCATIONS) OR LOSS, GAIN, OR REARRANGEMENTS OF INDIVIDUAL CHROMOSOMES (E.G., DELETIONS, DUPLICATIONS, INVERSIONS,
OF PARTICULAR CLINICAL IMPORTANCE ARE TRANSLOCATIONS, WHICH INVOLVE TWO BASIC TYPES: ROBERTSONIAN AND RECIPROCAL. ROBERTSONIAN REARRANGEMENTS ARE A SPECIAL CLASS OF TRANSLOCATION, IN WHICH THE LONG ARMS OF TWO ACROCENTRIC CHROMOSOMES (CHROMOSOMES 13, 14, 15, 21, AND 22) JOIN TOGETHER, GENERATING A FUSION CHROMOSOME THAT CONTAINS VIRTUALLY ALL OF THE GENETIC MATERIAL OF THE ORIGINAL TWO CHROMOSOMES. IF THE ROBERTSONIAN TRANSLOCATION IS PRESENT IN UNBALANCED FORM, A MONOSOMIC OR TRISOMIC CONCEPTION EN-SUES. FOR EXAMPLE, ~3% OF DOWN SYNDROME CASES ARE ATTRIBUTABLE TO UNBALANCED ROBERTSONIAN TRANSLOCATIONS, MOST OFTEN INVOLVING CHROMOSOMES 14 AND 21. IN THIS INSTANCE, THE AFFECTED INDIVIDUAL HAS 46 CHROMOSOMES, INCLUDING ONE STRUCTURALLY NORMAL CHROMOSOME 14, TWO STRUCTURALLY NORMAL CHROMOSOMES 21, AND ONE FUSION 14/21 CHROMOSOME. THIS EFFECT LEADS TO A NORMAL DIPLOID DOSAGE FOR CHROMOSOME 14 AND TO A TRIPLICATION OF CHROMOSOME 21, THUS RESULTING IN DOWN SYNDROME. SIMILARLY, A SMALL PROPORTION OF INDIVIDUALS WITH TRISOMY 13 SYNDROME ARE CLINICALLY AFFECTED BECAUSE OF AN UNBALANCED ROBERTSONIAN TRANSLOCATION.

RECIPROCAL TRANSLOCATIONS INVOLVE MUTUAL EXCHANGES BETWEEN ANY TWO CHROMOSOMES. IN THIS CIRCUMSTANCE, THE PHENOTYPIC CONSEQUENCES ASSOCIATED WITH UNBALANCED TRANSLOCATIONS DEPEND ON THE LOCATION OF THE BREAKPOINTS, WHICH DICTATE THE AMOUNT OF MATERIAL THAT HAS BEEN “EXCHANGED” BETWEEN THE TWO CHROMOSOMES. BECAUSE MOST RECIPROCAL TRANSLOCATIONS INVOLVE UNIQUE SETS OF BREAKPOINTS, IT IS DIFFICULT TO PRE-DICT THE PHENOTYPIC CONSEQUENCES IN ANY ONE SITUATION. IN GENERAL, SEVERITY IS DETERMINED BY THE AMOUNT OF EXCESS OR MISSING CHROMOSOME MATERIAL IN INDIVIDUALS WITH UNBALANCED TRANSLOCATIONS.

IN ADDITION TO REARRANGEMENTS BETWEEN CHROMOSOMES, THERE ARE SEVERAL EXAMPLES OF INTRACHROMOSOME STRUCTURAL ABNORMALITIES. THE MOST COMMON AND DELETERIOUS OF THESE INVOLVE LOSS OF CHROMOSOME MATERIAL DUE TO DELETIONS. THE TWO BEST-CHARACTERIZED DELETION SYNDROMES, WOLF-HIRSCHHORN SYNDROME AND CRI-DU-CHAT SYNDROME, RESULT FROM LOSS OF RELATIVELY SMALL CHROMOSOMAL SEGMENTS ON CHROMOSOMES 4P AND 5P, RESPECTIVELY. NONETHELESS, EACH IS ASSOCIATED WITH MULTIPLE CONGENITAL ANOMALIES, DEVELOPMENTAL DELAYS, PROFOUND RETARDATION, AND REDUCED LIFESPAN.

MICRODELETION SYNDROMES THE TERM CONTIGUOUS GENE SYNDROME REFERS TO GENETIC DISORDERS THAT MIMIC A COMBINATION OF SINGLE-GENE DISORDERS. THEY RESULT FROM THE DELETION OF A SMALL NUMBER OF TIGHTLY CLUSTERED GENES. BECAUSE SOME ARE TOO SMALL TO BE DETECTED CYTOGENETICALLY, THEY ARE TERMED MICRODELETIONS. THE APPLICATION OF MOLECULAR TECH-
### TABLE 63-4 SOME COMMONLY IDENTIFIED MICRODELETION AND MICRODUPlication SYndromes

#### SYndrome

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<tr>
<th>Syndrome</th>
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<tr>
<td>Langer-Giedion Syndrome</td>
<td>8q24.1 (DEL)</td>
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<tr>
<td>WAGR Complex</td>
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<tr>
<td>Beckwith-Wiedemann Syndrome</td>
<td>11p13 (DEL)</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>11p15 (DUP)</td>
</tr>
<tr>
<td>Prader-Willi Syndrome</td>
<td>13q14.11 (DEL)</td>
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<tr>
<td>Angelman Syndrome</td>
<td>15q11-13 (DEL)</td>
</tr>
<tr>
<td>Thalassemia and Mental Retardation</td>
<td>15q11-13 (DEL)</td>
</tr>
<tr>
<td>Smith-Magenis Syndrome</td>
<td>16p13.3 (DEL)</td>
</tr>
<tr>
<td>Miller-Dieker Syndrome</td>
<td>17p11.2 (DEL)</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth Syndrome Type 1A</td>
<td>17p13 (DEL)</td>
</tr>
<tr>
<td>DiGeorge Syndrome/Velocardiofacial Syndrome</td>
<td>22q11 (DEL)</td>
</tr>
</tbody>
</table>
PRINCIPAL FEATURES

SPARSE HAIR, BULBOUS NOSE, VARIABLE MENTAL RETARDATION
WILMS' TUMOR, ANIRIDIA, GENITO-URINARY DISORDERS, MENTAL RETARDATION
MACROSOMIA, MACROGLOSSIA, OMPHALOCOELE

RETINOBlastOMA DUE TO HOMOZYGOUS LOSS OF FUNCTIONAL RB ALLELE
OBESITY, HYPOGONADISM, MENTAL RETARDATION
ATAXIC GAIT

*-THALASSEMIA AND MENTAL RETARDATION DUE TO DELETION OF DISTAL 16P, INCLUDING
*-GLOBIN LOCUS
BRACHYCEPHALY, MIDFACE HYPOPLASIA, MENTAL RETARDATION
DYSMORPHIC FACES, LISSENCEPHALY
PROGRESSIVE NEUROPATHY DUE TO MICRDUPICATION
ABNORMALITIES OF THIRD AND FOURTH BRANCHIAL ARCHES

IMPRINTING EFFECTS

NO

NO

YES, OCCASIONALLY ASSOCIATED WITH “PARENTAL UNIPARENTAL DISOMY” (SEE TEXT)
NO OBVIOUS EFFECT, ALTHOUGH ABNORMAL RB ALLELE MORE LIKELY TO BE PATERNAL
YES, PROTOTYPE IMPRINTING DISORDER (SEE TEXT)
WITH PRADER-WILLI SYNDROME, PROTOTYPIC IMPRINTING DISORDER (SEE TEXT)

NO

NO

NO
NIQUES HAS LED TO THE IDENTIFICATION OF AT LEAST 18 OF THESE MICRODELETION SYNDROMES (TABLE 63-4). SOME OF THE MORE COMMON ONES INCLUDE THE WILMS’ TUMOR-ANIRIDIA COMPLEX (WAGR), MILLER DIEKER SYNDROME (MDS), AND VELOCARDIOFACIAL (VCF) SYNDROME. WAGR IS CHARACTERIZED BY MENTAL RETARDATION AND INVOLVEMENT OF MULTIPLE ORGANS, INCLUDING KIDNEY (WILMS’ TUMOR), EYE (ANIRIDIA), AND THE GENITOURINARY SYSTEM. THE CYTOGENETIC ABNORMALITY INVOLVES A DELETION OF A PART OF THE SHORT ARM OF CHROMOSOME 11 (11 P13), WHICH TYPICALLY IS DETECTABLE ON WELL-BANDED CHROMOSOME PREPARATIONS. IN MDS, A DISORDER CHARACTERIZED BY MENTAL RETARDATION, DYSMORPHIC FACES, AND LISSENCEPHALY, THE DELETION INVOLVES CHROMOSOME 17 (17P13). USING FISH, 17P DELETIONS HAVE BEEN DETECTED IN >90% OF PATIENTS WITH MDS AS WELL AS IN 20% OF CASES OF ISOLATED LISSENCEPHALY.

DELETIONS INVOLVING THE LONG ARM OF CHROMOSOME 22 (22Q11) ARE THE MOST COMMON MICRODELETIONS IDENTIFIED TO DATE, PRESENT IN ~1/3000 NEWBORNS. VCF SYNDROME, THE MOST COMMONLY ASSOCIATED SYNDROME, CONSISTS OF LEARNING DISABILITIES OR MILD MENTAL RETARDATION, PALATAL DEFECTS, A HYPOPLASTIC ALOE NASI AND LONG NOSE, AND CONGENITAL HEART DEFECTS (CONOTRUNCAL DEFECT). SOME INDIVIDUALS WITH 22Q11 DELETION ARE MORE SEVERELY AFFECTED AND PRESENT WITH DIGEORGE SYNDROME, WHICH INVOLVES ABNORMALITIES IN THE DEVELOPMENT OF THE THIRD AND FOURTH BRANCHIAL ARCHES LEADING TO THYMIC HYPOPLASIA, PARATHYROID HYPOPLASIA, AND CONOTRUNCAL HEART DEFECTS. IN ~30% OF THESE CASES, A DELETION AT 22Q11 CAN BE DETECTED WITH HIGH-RESOLUTION BANDING, BY COMBING CONVENTIONAL CYTOGENETICS, FISH, AND MOLECULAR DETECTION TECHNIQUES (I.E., SOUTHERN BLOTTING OR POLYMERASE CHAIN REACTION ANALYSES), THESE RATES IMPROVE TO >90%. ADDITIONAL STUDIES HAVE DEMONSTRATED A SURPRISINGLY HIGH FREQUENCY OF 22Q11 DELETIONS IN INDIVIDUALS WITH NONSYNDROMIC CONOTRUNCAL DEFECTS. APPROXIMATELY 10% OF INDIVIDUALS WITH A 22Q11 DELETION INHERITED IT FROM A PARENT WITH A SIMILAR DELETION. SMITH-MAGENIS SYNDROME INVOLVES A MICRO DELETION LOCALIZED TO THE PROXIMAL REGION OF THE SHORT ARM OF CHROMOSOME 17 (17P11.2). AFFECTED INDIVIDUALS HAVE MENTAL RETARDATION, DYSMORPHIC FACIAL FEATURES, DELAYED SPEECH, PERIPHERAL NEUROPATHY, AND BEHAVIOR ABNORMALITIES. MOST OF THESE DELETIONS CAN BE DETECTED WITH CYTOGENETIC ANALYSIS, ALTHOUGH FISH IS AVAILABLE TO CONFIRM THESE FINDINGS. IN CONTRAST, WILLIAM SYNDROME, A CHROMOSOME 7 (7Q11.23) MICRODELETION, CANNOT BE DIAGNOSED WITH STAN-
IN ADDITION TO MICRODELETION SYNDROMES, THERE IS NOW AT LEAST ONE WELL-DESCRIBED MICRODUPlication SYNDROME, CHARCOT-MARIE-TOOTH TYPE 1A (CMT1A). THIS IS A NERVE CONDUCTION DISEASE PREVIOUSLY THOUGHT TO BE TRANSMITTED AS A SIMPLE AUTOSOMAL DOMINANT DISORDER. RECENT MOLECULAR STUDIES HAVE DEMONSTRATED THAT AFFECTED INDIVIDUALS ARE HETEROZYGOUS FOR DUPlication OF A SMALL REGION OF CHROMOSOME 17 (17P 11.2-12). ALTHOUGH IT IS NOT YET CLEAR WHY INCREASED GENE DOSAGE WOULD RESULT IN CMT1A, THE INHERITANCE PATTERN IS EXPLAINED BY THE FACT THAT ONE-HALF OF THE OFFSPRING OF AFFECTED INDIVIDUALS INHERIT THE DUPlication-CARRYING CHROMOSOME.

IMPRINTING DISORDERS

TWO OTHER MICRODELETION SYNDROMES, PRADER-WILLI SYNDROME (PWS) AND ANGELMAN SYNDROME (AS), EXHIBIT PARENT-OF-ORIGIN, OR “IMPRINTING,” EFFECTS. FOR MANY YEARS, IT HAS BEEN KNOWN THAT CYTOGENETICALLY DETECTABLE DELETIONS OF CHROMOSOME 15 OCCUR IN A PROPORTION OF PATIENTS WITH PWS, AS WELL AS IN THOSE WITH AS. THIS SEEMED CURIOUS, AS THE CLINICAL MANIFESTATIONS OF THE TWO SYNDROMES ARE VERY DISSIMILAR. PWS IS CHARACTERIZED BY OBESITY, HYPOGONADISM, AND MILD TO MODERATE MENTAL RETARDATION, WHEREAS AS IS ASSOCIATED WITH MICROCEPHALY, ATAXIC GAIT, SEIZURES, INAPPROPRIATE LAUGHTER, AND SEVERE MENTAL RETARDATION. NEW INSIGHT INTO THE PATHOGENESIS OF THESE DISORDERS HAS BEEN PROVIDED BY THE RECOGNITION THAT PARENTAL ORIGIN OF THE DELETION DETERMINES WHICH PHENOTYPE ENSUES: IF THE DELETION IS PATERNAL, THE RESULT IS PWS, WHEREAS IF THE DELETION IS MATERNAL, THE RESULT IS AS (FIG. 63-2). THIS SCENARIO IS COMPLICATED FURTHER BY THE RECOGNITION THAT NOT ALL INDIVIDUALS WITH PWS OR AS CARRY THE CHROMOSOME 15 DELETION. FOR SUCH INDIVIDUALS, THE PARENTAL ORIGIN OF THE CHROMOSOME 15 REGION IS AGAIN THE IMPORTANT DETERMINANT. IN PWS, FOR EXAMPLE, NONDELETION PATIENTS INVARIABLY HAVE TWO MATERNAL AND NO PATERNAL CHROMOSOMES [MATERNAL UNIPARENTAL DISOMY (UPD)], WHEREAS FOR SOME NONDELETION AS PATIENTS THE REVERSE IS TRUE (PATERNAL UPD). THIS INDICATES THAT AT LEAST SOME GENES ON CHROMOSOME 15 ARE DIFFERENTLY EXPRESSED, DEPENDING ON WHICH PARENT CONTRIBUTED THE CHROMOSOME. ADDITIONALLY, THIS MEANS THAT NORMAL FETAL DEVELOPMENT REQUIRES THE PRESENCE OF ONE MATERNAL AND ONE PATERNAL COPY OF CHROMOSOME 15. APPROXIMATELY 70% OF PWS CASES ARE DUE TO PATERNAL DELETIONS OF
15Q11-Q13, WHEREAS 25% ARE DUE TO MatERNal UPD, AND ABOUT 5% ARE CAUSED BY MUTATIONS IN A CHROMOSOME 15 IMPRINTING CENTER. IN AS, 75% OF CASES ARE DUE TO MATERNAL DELETIONS, AND ONLY 2% ARE DUE TO PA-TERNAL UPD. THE REMAINING CASES ARE PRESUMABLY CAUSED BY IMPRINTING MUTATIONS (5%), OR MUTATIONS IN THE UBE3A GENE, WHICH IS ASSOCIATED WITH AS. THE UPD CASES ARE MOSTLY CAUSED BY MEIOTIC NONDISJUNCTION RESULTING IN TRISOMY 15, SUBSEQUENTLY FOLLOWED BY A NORMALIZING MITOTIC NONDISJUNCTION EVENT (“TRISOMY RESCUE”) RESULTING IN TWO NORMAL CHROMOSOMES 15, BOTH FROM THE SAME PARENT. UBE3A IS THE ONLY MA-TERNALLY IMPRINTED GENE KNOWN IN THE CRITICAL REGION OF CHROMOSOME 15. HOWEVER, SEVERAL PATERNALLY IMPRINTED GENES, OR EXPRESSED-SEQUENCE TAGS (ESTS), HAVE BEEN IDENTIFIED, INCLUDING ZNF127, IPW, SNRPN, SNURF, PARI, AND PAR5.

414 PART 3: GENETICS AND DISEASE

CHROMOSOMAL REGIONS THAT BEHAVE IN THE MANNER OBSERVED IN PWS AND AS ARE SAID TO BE IMPRINTED. THIS PHENOMENON IS INVOLVED IN DIFFERENTIAL EXPRESSION OF CERTAIN GENES ON DIFFERENT CHROMOSOMES. CHROMOSOME 11 IS ONE OF THESE WITH AN IMPRINTED REGION, SINCE IT IS KNOWN THAT A SMALL PROPORTION OF INDIVIDUALS WITH THE BECKWITH-WIEDEMANN OVERGROWTH SYNDROME HAVE TWO PATERNAL BUT NO MATERNAL COPIES OF THIS CHROMOSOME.

ACQUIRED CHROMOSOME ABNORMALITIES IN CANCER

IN ADDITION TO THE CONSTITUTIONAL CYTOGENETIC CHROMOSOMAL ABNORMALITIES THAT ARE PRESENT AT BIRTH, SOMATIC CHROMOSOMAL CHANGES CAN BE ACQUIRED LATER IN LIFE AND ARE OFTEN ASSOCIATED WITH MALIGNANT CONDITIONS. AS WITH CONSTITUTIONAL ABNORMALITIES, SOMATIC CHANGES CAN INCLUDE THE NET LOSS OF CHROMOSOMAL MATERIAL (DUE TO A DELETION OR LOSS OF A CHROMOSOME), NET GAIN OF MATERIAL (DUPLICATION OR GAIN OF A CHROMOSOME), AND RELOCATION OF DNA SEQUENCES (TRANSLOCATION). CYTOGENETIC CHANGES HAVE BEEN PARTICULARLY WELL STUDIED IN (1) LEUKEMIAS, E.G., PHILADELPHIA CHROMOSOME TRANSLOCATION IN CML [T (9; 22) (Q34.1; Q11.2)]; AND (2) LYMPHOMAS, E.G., TRANSLOCATIONS OF MYC IN BURKITT’S [T (8; 14) (Q24; Q32)]. THESE AND OTHER TRANSLOCATIONS ARE USEFUL FOR DIAGNOSIS, CLASSIFI-CATION, AND PROGNOSIS. ANALYSES OF CYTOGENETIC CHANGES ARE ALSO USEFUL IN CERTAIN SOLID TUMORS. FOR EXAMPLE, A COMPLEX KARYOTYPE WITH WILMS’ TUMOR, DIPLOIDY IN MEDULLOBLASTOMA, AND HER-2/NEU AMPLIFI-
IMPLICATIONS OF MOLECULAR GENETICS FOR INTERNAL MEDICINE

THE FIELD OF MEDICAL GENETICS HAS TRADITIONALLY FOCUSED ON CHROMOSOMAL ABNORMALITIES (CHAP. 63) AND MENDELIAN DISORDERS (CHAP. 62). HOWEVER, THERE IS GENETIC SUSCEPTIBILITY TO MANY COMMON ADULT-ONSET DISEASES, INCLUDING ATHEROSCLEROSIS, CARDIAC DISORDERS, ASTHMA, HYPERTENSION, AUTOIMMUNE DISEASES, DIABETES MELLITUS, MACULAR DEGENERATION, ALZHEIMER’S DISEASE, PSYCHIATRIC DISORDERS, AND MANY FORMS OF CANCER. GENETIC CONTRIBUTIONS TO THESE COMMON DISORDERS INVOLVE MORE THAN THE ULTIMATE EXPRESSION OF AN ILLNESS; THESE GENES CAN ALSO INFLUENCE THE SEVERITY OF INFIRMITY, EFFECT OF TREATMENT, AND PROGRESSION OF DISEASE.

THE PRIMARY CARE CLINICIAN IS NOW FACED WITH THE ROLE OF RECOGNIZING AND COUNSELING PATIENTS AT RISK FOR A NUMBER OF GENETICALLY INFLUENCED ILLNESSES. AMONG THE GREATER THAN 20,000 GENES IN THE HUMAN GENOME, IT IS ESTIMATED THAT EACH OF US HARBORS SEVERAL POTENTIALLY DELETERIOUS MUTATIONS. FORTUNATELY, MANY OF THESE ALTERATIONS ARE RECESSIVE AND CLINICALLY SILENT. AN EVEN GREATER NUMBER, HOWEVER, REPRESENT GENETIC VARIANTS THAT ALTER DISEASE SUSCEPTIBILITY, SEVERITY, OR RESPONSE TO THERAPY.

GENETIC MEDICINE IS CHANGING THE WAY DISEASES ARE CLASSIFIED, ENHANCING OUR UNDERSTANDING OF PATHOPHYSIOLOGY, PROVIDING PRACTICAL INFORMATION CONCERNING DRUG METABOLISM AND THERAPEUTIC RESPONSES, AND ALLOWING FOR INDIVIDUALIZED SCREENING AND HEALTH CARE MANAGEMENT PROGRAMS. IN VIEW OF THESE CHANGES, THE PHYSICIAN MUST INTEGRATE PERSONAL MEDICAL HISTORY, FAMILY HISTORY, AND DIAGNOSTIC MOLECULAR TESTING INTO THE OVERALL CARE OF INDIVIDUAL PATIENTS AND THEIR FAMILIES. SURVEYS INDICATE THAT PATIENTS STILL TURN TO THEIR PRIMARY CARE INTERNIST FOR GUIDANCE ABOUT GENETIC DISORDERS, EVEN THOUGH THEY MAY BE SEEING OTHER SPECIALISTS. THE INTERNIST HAS AN IMPORTANT ROLE IN EDUCATING PATIENTS ABOUT THE INDICATIONS, BENEFITS, RISKS, AND LIMITATIONS OF GENETIC TESTING IN THE MANAGEMENT OF A NUMBER OF DIVERSE DISEASES. THIS IS A DIFFICULT TASK, AS SCIENTIFIC ADVANCES IN GENETIC MEDICINE HAVE OUTPACED THE TRANSLATION OF THESE DISCOVERIES INTO STANDARDS OF CLINICAL CARE.

RATION IN BREAST CANCER ARE POOR PROGNOSTIC SIGNS. FOR DETAILED DISCUSSION OF CANCER GENETICS, SEE CHAP. 79.

FURTHER READINGS


COMMON ADULT-ONSET GENETIC DISORDERS
MULTIFACTORIAL INHERITANCE

The risk for many adult-onset disorders reflects the combined effects of genetic factors at multiple loci that may function independently or in combination with other genes or environmental factors. Our understanding of the genetic basis of these disorders is incomplete, despite the clear recognition of genetic susceptibility. In type 2 diabetes mellitus, for example, the concordance rate in monozygotic twins ranges between 50 and 90%. Diabetes or impaired glucose tolerance occurs in 40% of siblings and in 30% of the offspring of an affected individual. Despite the fact that diabetes affects 5% of the population and exhibits a high degree of heritability, only a few genetic mutations (most of which are rare) that might account for the familial nature of the disease have been identified. They include certain mitochondrial DNA disorders (Chap. 62), mutations in a cascade of genes that control pancreatic islet cell development and function (HNF4α, HNF1α, IPF1, TCF7L2, glucokinase), insulin receptor mutations, and others (Chap. 338). Superimposed on this genetic background are environmental influences such as diet, exercise, pregnancy, and medications. Identifying susceptibility genes associated with multifactorial adult-onset disorders is a formidable task. Nonetheless, a reasonable goal for these types of diseases is to identify genes that increase (or decrease) disease risk by a factor of two or more. For common diseases such as diabetes or heart disease, this level of risk has important implications for health. In much the same way that cholesterol is currently used as a biochemical marker of cardiovascular risk, we can anticipate the development of genetic panels with similar predictive power. The advent of DNA-sequencing chips represents an important technical advance that promises to make large-scale testing more feasible (Chap. 62). Whether to perform a genetic test for a particular inherited adult-onset disorder, such as hemochromatosis, multiple endocrine neoplasia (MEN) type 1, prolonged QT syndrome, or Huntington disease, is a complex decision; it depends on the clinical features of the disorder, the desires of the patient and family, and whether the results of
GENETIC TESTING WILL ALTER MEDICAL DECISION-MAKING OR TREATMENT (SEE BELOW).

POPULATION SCREENING  MASS GENETIC SCREENING PROGRAMS REQUIRE TESTS OF HIGH ENOUGH SENSITIVITY AND SPECIFICITY TO BE COST-EFFECTIVE. AN EFFECTIVE SCREENING PROGRAM SHOULD FULFILL THE FOLLOWING CRITERIA: THAT THE TESTED DISORDER IS PREVALENT AND SERIOUS; THAT IT CAN BE INFLUENCED PRE-SYMPPTOMATICALLY THROUGH LIFESTYLE CHANGES, SCREENING, OR MEDICATIONS;

PAGE NO. 60

415 CHAPTER 64 THE PRACTICE OF GENETICS IN CLINICAL MEDICINE

AND THAT IDENTIFICATION OF RISK DOES NOT RESULT IN UNDUE DISCRIMINATION OR HARM. SCREENING INDIVIDUALS OF JEWISH DESCENT FOR THE AUTOSOMAL RECESSIVE NEURODEGENERATIVE DISORDER TAY-SACHS DISEASE HAS RESULTED IN A DRAMATIC DECLINE IN THE INCIDENCE OF THIS SYNDROME IN THE UNITED STATES. ON THE OTHER HAND, SCREENING FOR SICKLE CELL DISEASE OR TRAIT IN THE AFRICAN-AMERICAN POPULATION HAS SOMETIMES RESULTED IN INSURANCE AND EMPLOYMENT DISCRIMINATION. MASS SCREENING FOR COMPLEX GENETIC DISORDERS CAN RESULT IN POTENTIAL PROBLEMS. FOR EXAMPLE, CYSTIC FIBROSIS IS MOST COMMONLY ASSOCIATED WITH ALTERATIONS IN *F508. THIS VARIANT ACCOUNTS FOR 30-80% OF MUTANT ALLELES DEPENDING ON THE ETHNIC GROUP. NEVERTHELESS, CYSTIC FIBROSIS IS ASSOCIATED WITH PRONOUNCED GENETIC HETEROGENEITY WITH MORE THAN 1000 DISEASE-RELATED MUTATIONS. THE AMERICAN COLLEGE OF MEDICAL GENETICS RECOMMENDS A PANEL OF 23 ALLELES, INCLUDING THE *F508 ALLELE, FOR ROUTINE DIAGNOSTIC AND CARRIER TESTING. ANALYSIS FOR THE LESS COMMON CYSTIC FIBROSIS-ASSOCIATED MUTATIONS WOULD GREATLY IMPACT THE COST OF TESTING WITHOUT SIGNIFICANTLY INFLUENCING THE EFFECTIVENESS OF MASS SCREENING. NEVERTHELESS, THE INDIVIDUAL WHO CARRIES ONE OF THE LESS COMMON CYSTIC FIBROSIS-ASSOCIATED ALTERATIONS WILL NOT BENEFIT IF TESTING IS LIMITED TO A ROUTINE PANEL. OCCUPATIONAL HEALTH SCREENING PROGRAMS HOLD PROMISE BUT ALSO RAISE CONCERNS ABOUT EMPLOYMENT DISCRIMINATION. THESE CONCERNS WERE BROUGHT TO LIGHT IN 2001 WHEN IT WAS DISCOVERED THAT A RAILROAD COMPANY WAS TESTING ITS EMPLOYEES, WITHOUT CONSENT, FOR A RARE GENETIC CONDITION THAT RESULTS IN SUSCEPTIBILITY TO CARPAL TUNNEL SYNDROME. THE EQUAL EMPLOYMENT OPPORTUNITY COMMISSION ARGUED THAT THE TESTS WERE UNLAWFUL UNDER THE AMERICANS WITH DISABILITIES ACT.

THE FAMILY HISTORY

WHEN TWO OR MORE FIRST-DEGREE RELATIVES ARE AFFECTED WITH ASTHMA, CAR-
DIOVASCULAR DISEASE, TYPE 2 DIABETES, BREAST CANCER, COLON CANCER, OR MELANOMA, THE RELATIVE RISK RANGES FROM TWO- TO FIVEFOLD, UNDERSCORING THE IMPORTANCE OF FAMILY HISTORY FOR THESE PREVALENT DISORDERS. PENDING FURTHER ADVANCES IN GENETIC TESTING, THE KEY TO ASSESSING THE INHERITED RISK FOR COMMON ADULT-ONSET DISEASES RESTS IN THE COLLECTION AND INTERPRETATION OF A DETAILED PERSONAL AND FAMILY MEDICAL HISTORY IN CONJUNCTION WITH A DIRECTED PHYSICAL EXAMINATION. FOR EXAMPLE, A HISTORY OF MULTIPLE FAMILY MEMBERS WITH EARLY-ONSET CORONARY ARTERY DISEASE, GLUCOSE INTOLERANCE, AND HYPERTENSION SHOULD SUGGEST INCREASED RISK FOR GENETIC, AND PERHAPS ENVIRONMENTAL, PREDISPOSITION TO METABOLIC SYNDROME (CHAP. 236). INDIVIDUAL PATIENTS WITH THIS FAMILY HISTORY SHOULD BE MONITORED FOR THE POSSIBLE DEVELOPMENT OF HYPERTENSION, DIABETES, AND HYPERLIPIDEMIA. THEY SHOULD BE COUNSELED ABOUT THE IMPORTANCE OF AVOIDING ADDITIONAL RISK FACTORS SUCH AS OBESITY AND CIGARETTE SMOKING.

Presenting with xanthomas at a young age should prompt consideration of familial hypercholesterolemia. Some adult-onset disease-causing mutations are more prevalent in certain ethnic groups. For instance, >2% of the Ashkenazi population carry one of three specific mutations in the BRCA1 or BRCA2 genes. The prevalence of the Factor V Leiden allele ranges from 3 to 7% in Caucasians but is much lower in Africans or Asians.

Recall of family history is often inaccurate. This is especially so when the history is remote and families become more dispersed geographically. It can be helpful to ask patients to fill out family history forms before or after their visits, as this provides them with an opportunity to contact relatives. Attempts should be made to confirm the illnesses reported in the family history before making important and, in certain circumstances, irreversible management decisions. This process is often labor intensive and ideally involves interviews of additional family members or reviewing medical records, autopsy reports, and death certificates.

Although many inherited disorders will be suggested by the clustering of relatives with the same or related conditions, it is important to note that disease penetrance is incomplete for most multifactorial genetic disorders. As a result, the pedigree obtained in such families may not exhibit a clear mendelian inheritance pattern, as not all family members carrying the disease-associated alleles will manifest a clinical disorder. Furthermore, genes associated with some of these disorders often exhibit variable expression of disease. For example, the breast cancer-associated gene BRCA1 can predispose to several different malignancies in the same family, including cancers of the breast, ovary, and prostate (Chap. 79). For common diseases such as breast cancer, some family members without the disease-causing mutation may also develop breast cancer, representing another confounding variable in the pedigree analysis.

Some of the aforementioned features of the family history are illustrated in Fig. 64.1. In this example, the proband, a 36-year-old woman (IV-L), has a strong history of breast and ovarian cancer on the paternal side of her family. The early age of onset, as well as the co-occurrence of breast and ovarian cancer in this family, suggests the

**Figure 64.1** A 36-year-old woman (arrow) seeks consultation because of her family history of cancer. The patient expresses concern that the multiple cancers in her relatives imply an inherited predisposition to develop cancer. The family history is recorded and records of the patient's relatives confirm the reported diagnoses.
POSSIBILITY OF AN INHERITED MUTATION IN *BRCA1* OR *BRCA2*. IT IS UNCLEAR
THOUGH-WITHOUT GENETIC TESTING-WHETHER HER FATHER INHERITED SUCH A
MUTATION AND TRANSMITTED IT TO HER. AFTER APPROPRIATE GENETIC
COUNSELING OF THE PROBAND AND HER FAMILY, ONE APPROACH TO DNA ANALYSIS IN
THIS FAMILY IS TO TEST THE CANCER-AFFECTED 42-YEAR-OLD LIVING COUSIN FOR
THE PRESENCE OF A *BRCA1* OR *BRCA2* MUTATION. IF A MUTATION IS FOUND,
THEN IT IS POSSIBLE TO TEST FOR THIS PARTICULAR ALTERATION IN THE PROBAND
AND OTHER FAMILY MEMBERS, IF THEY SO DESIRE. IN THE EXAMPLE SHOWN, IF
THE PROBAND’S FATHER HAS THE *BRCA1* MUTATION, THERE IS A 50:50 PROBABILITY
THAT THE MUTATION WAS TRANSMITTED TO HER, AND GENETIC TESTING CAN
BE USED TO ESTABLISH THE ABSENCE OR PRESENCE OF THIS ALTERATION.

**GENETIC TESTING FOR ADULT-ONSET DISORDERS**

A CRITICAL FIRST STEP BEFORE INITIATING GENETIC TESTING IS TO ENSURE THAT THE
CORRECT CLINICAL DIAGNOSIS HAS BEEN MADE, WHETHER BASED ON FAMILY HISTORY, CHARACTERISTIC PHYSICAL FINDINGS, OR BIOCHEMICAL TESTING.
CAREFUL CLINICAL ASSESSMENT CAN DEFINE THE PHENOTYPE, THEREBY PREVENTING UNNECESSARY TESTING AND DIRECTING TESTING TOWARD THE MOST PROBABLE CANIDATE GENES (FIG. 64-2). FOR PATIENTS IDENTIFIED BY POPULATION-BASED SCREENING (E.G., DIABETES, HYPERCHOLESTEROLEMIA), TESTING MIGHT INVOLVE KNOWN CANDIDATE GENES, OR GENOME-WIDE LINKAGE STUDIES (HAPMAP) OF THE POPULATION COULD BE USED AS PART OF A RESEARCH STUDY TO IDENTIFY SUSCEPTIBILITY ALLELES. FOR PATIENTS WITH A STRONG FAMILY HISTORY (E.G., BREAST CANCER, HEMOCHROMATOSIS), TESTING OFTEN INCLUDES KNOWN CANDIDATE GENES, OR TRADITIONAL LINKAGE ANALYSES WITHIN PEDIGREES CAN IDENTIFY DISEASE-CAUSING GENES. ONCE CANDIDATE GENES ARE KNOWN, MUTATIONAL ANALYSES CAN BE PERFORMED AFTER PRETEST GENETIC COUNSELING (SEE BELOW).

MANY DISORDERS EXHIBIT THE FEATURE OF LOCUS HETEROGENEITY, WHICH REFERS TO THE FACT THAT MUTATIONS IN DIFFERENT GENES CAN CAUSE PHENotypically SIMILAR DISORDERS. FOR EXAMPLE, OSTEOGENESIS IMPERFECTA (CHAP. 357), LONG QT SYNDROME (CHAP. 226), MUSCULAR DYSTROPHY (CHAP. 382), HOMOCYSTINURIA (CHAP. 358), RETINITIS PIGMENTOSA (CHAP. 29), AND HEREDITARY PREDISPOSITION TO COLON CANCER (CHAP. 87) OR BREAST CANCER (CHAP. 86) CAN EACH BE CAUSED BY MUTATIONS IN DISTINCT GENES. THE PATTERN OF DISEASE TRANSMISSION, CLINICAL COURSE, AND TREATMENT MAY DIFFER SIGNIFICANTLY, DEPENDING ON THE SPECIFIC GENE AFFECTED. IN THESE CASES, THE CHOICE OF WHICH GENES TO TEST IS OFTEN DETERMINED BY UNIQUE CLINICAL AND FAMILY HISTORY FEATURES, THE RELATIVE PREVALENCE OF MUTATIONS IN VARIOUS GENES, OR TEST AVAILABILITY.

**METHODOLOGIC APPROACHES TO GENETIC TESTING**

GENETIC TESTING IS PERFORMED IN MUCH THE SAME WAY AS OTHER SPECIALIZED LABORATORY TESTS. IN THE UNITED STATES, GENETIC TESTING LABORATORIES
FIGURE 64-2 APPROACH TO IDENTIFYING A DISEASE-CAUSING GENE.

ARE CLIA (CLINICAL LABORATORY IMPROVEMENT ACT) APPROVED TO ENSURE THAT THEY MEET QUALITY AND PROFICIENCY STANDARDS. A USEFUL SOURCE FOR VARIOUS GENETIC TESTS IS WWW.GENETESTS.ORG.

DNA TESTING IS MOST COMMONLY PERFORMED BY DNA SEQUENCE ANALYSIS FOR MUTATIONS, ALTHOUGH GENOTYPE CAN ALSO BE DEDUCED THROUGH THE STUDY OF RNA OR PROTEIN (E.G., APOPROTEIN E, HEMOGLOBIN, IMMUNOHISTOCHEMISTRY). THE DETERMINATION OF DNA SEQUENCE ALTERATIONS RELIES HEAVILY ON THE USE OF POLYMERASE CHAIN REACTION (PCR), WHICH ALLOWS RAPID AMPLIFICATION AND ANALYSIS OF THE GENE OF INTEREST. IN ADDITION, PCR ENABLES GENETIC TESTING ON MINIMAL AMOUNTS OF DNA EXTRACTED FROM A WIDE RANGE OF TISSUE SOURCES INCLUDING LEUKOCYTES, FIBROBLASTS, EPITHELIAL CELLS IN SALIVA OR HAIR, AND ARCHIVAL TISSUES. AMPLIFIED DNA CAN BE ANALYZED DIRECTLY BY DNA SEQUENCING OR IT CAN BE HYBRIDIZED TO DNA CHIPS OR BLOTS TO DETECT THE PRESENCE OF NORMAL AND MUTANT DNA SEQUENCES. DIRECT DNA SEQUENCING IS INCREASINGLY USED FOR PRENATAL DIAGNOSIS AS WELL AS FOR DETERMINATION OF HEREDITARY DISEASE SUSCEPTIBILITY.

ANALYSES OF LARGE ALTERATIONS IN THE GENOME ARE POSSIBLE USING CYTOGENETICS, FLOUORESCENT IN SITU HYBRIDIZATION (FISH), OR SOUTHERN BLOTTING (CHAP. 63). PROTEIN TRUNCATION TESTS (PTTS) ARE USED TO DETECT MUTATIONS THAT RESULT IN THE PREMATURE TERMINATION OF A POLYPEPTIDE OCCURRING DURING PROTEIN SYNTHESIS. IN THIS ASSAY, THE ISOLATED COMPLEMENTARY DNA (CDNA) IS TRANSCRIBED AND TRANSLATED IN VITRO, AND THE PROTEIN IS ANALYZED BY GEL ELECTROPHORESIS. THE TRUNCATED (MUTANT) GENE PRODUCT IS READILY IDENTIFIED AS ITS ELECTROPHORETIC MOBILITY DIFFERS FROM THAT OF THE NORMAL PROTEIN. THIS TEST IS USED MOST COMMONLY FOR ANALYSES OF LARGE GENES WITH SIGNIFICANT GENETIC HETEROGENEITY SUCH AS DMD, APC, AND THE BRCA GENES. LIKE ALL LABORATORY TESTS, THERE ARE LIMITATIONS TO THE ACCURACY AND INTERPRETATION OF GENETIC TESTS. IN ADDITION TO TECHNICAL ERRORS, GENETIC TESTS ARE SOMETIMES DESIGNED TO DETECT ONLY THE MOST COMMON MUTATIONS. IN THIS CASE, A NEGATIVE RESULT MUST BE QUALIFIED BY THE POSSIBILITY THAT THE INDIVIDUAL MAY HAVE A MUTATION THAT IS NOT INCLUDED IN THE TEST. IN ADDITION, A NEGATIVE RESULT DOES NOT MEAN THAT THERE IS NOT A MUTATION IN SOME OTHER GENE THAT CAUSES A SIMILAR INHERITED DISORDER. IN ADDITION TO MOLECULAR TESTING FOR ESTABLISHED DISEASE, GENETIC TESTING FOR SUSCEPTIBILITY TO CHRONIC DISEASE IS BEING INCREASINGLY INTEGRATED INTO THE PRACTICE OF MEDICINE. IN MOST CASES, HOWEVER, THE DISCOVERY OF DISEASE-ASSOCIATED GENES HAS GREATLY OUTPACED STUDIES THAT ASSESS CLINICAL
OUTCOMES AND THE IMPACT OF INTERVENTIONS. UNTIL SUCH EVIDENCE-BASED STUDIES ARE AVAILABLE, PREDICTIVE MOLECULAR TESTING MUST BE APPROACHED WITH CAUTION AND SHOULD BE OFFERED ONLY TO PATIENTS WHO HAVE BEEN ADEQUATELY COUNSELED AND HAVE PROVIDED INFORMED CONSENT. IN THE MAJORITY OF CASES, GENETIC TESTING SHOULD BE OFFERED ONLY TO INDIVIDUALS WITH A SUGGESTIVE PERSONAL OR FAMILY MEDICAL HISTORY OR IN THE CONTEXT OF A CLINICAL TRIAL. PREDICTIVE GENETIC TESTING FALLS INTO TWO DISTINCT CATEGORIES. PRESYMPTOMATIC TESTING APPLIES TO DISEASES WHERE A SPECIFIC GENETIC ALTERATION IS ASSOCIATED WITH A NEAR 100% LIKELIHOOD OF DEVELOPING DISEASE. IN CONTRAST, PREDISPOSITION TESTING PREDICTS A RISK FOR DISEASE THAT IS LESS THAN 100%. FOR EXAMPLE, PRESYMPTOMATIC TESTING IS AVAILABLE FOR THOSE AT RISK FOR HUNTINGTON’S DISEASE, WHEREAS PREDISPOSITION TESTING IS CONSIDERED FOR THOSE AT RISK FOR HEREDITARY BREAST CANCER. IT IS IMPORTANT TO NOTE THAT, FOR THE MAJORITY OF ADULT-ONSET, MULTIFACTORIAL GENETIC DISORDERS, TESTING IS PURELY PREDICTIVE. TEST RESULTS CANNOT REVEAL WITH CONFIDENCE WHETHER, WHEN, OR HOW THE DISEASE WILL MANIFEST ITSELF. FOR EXAMPLE, NOT EVERYONE WITH THE APOLIPOPROTEIN E ALLELE (*4) WILL DEVELOP ALZHEIMER’S DISEASE, AND INDIVIDUALS WITHOUT THIS GENETIC MARKER CAN STILL DEVELOP THE DISORDER (CHAP. 365). MOLECULAR ANALYSIS IS GENERALLY MORE INFORMATIVE IF TESTING IS INITIATED IN A SYMPTOMATIC FAMILY MEMBER, SINCE THE IDENTIFICATION OF A MUTATION CAN DIRECT THE TESTING OF OTHER AT-RISK FAMILY MEMBERS (WHETHER THEY ARE SYMPTOMATIC OR NOT). IN THE ABSENCE OF ADDITIONAL FAMILIAL OR ENVIRONMENTAL RISK FACTORS, INDIVIDUALS WHO TEST NEGATIVE FOR THE MUTATION FOUND IN THE AFFECTED FAMILY MEMBER CAN BE INFORMED THAT THEY ARE AT GENERAL POPULATION RISK FOR THAT PARTICULAR DISEASE. FURTHERMORE, THEY CAN BE REASSURED THAT THEY ARE NOT AT RISK FOR PASSING ON THE MUTATION TO THEIR CHILDREN. ON THE OTHER HAND, ASYMPTOMATIC FAMILY MEMBERS WHO TEST POSITIVE FOR THE KNOWN MUTATION MUST BE INFORMED THAT THEY ARE AT INCREASED RISK FOR DISEASE DEVELOPMENT AND FOR TRANSMITTING THE ALTERATION TO THEIR CHILDREN.

417 CHAPTER 64 THE PRACTICE OF GENETICS IN CLINICAL MEDICINE

CLINICIANS PROVIDING PRETEST COUNSELING AND EDUCATION SHOULD ASSESS THE PATIENT’S ABILITY TO COPE WITH TEST RESULTS. INDIVIDUALS WHO DEMONSTRATE SIGNS AND SYMPTOMS OF EMOTIONAL DISTRESS SHOULD HAVE THEIR PSYCHOSOCIAL NEEDS ADDRESSED BEFORE PROCEEDING WITH MOLECULAR TESTING. GENERALLY, GENETIC TESTING SHOULD NOT BE OFFERED AT A TIME OF PERSONAL CRISIS OR ACUTE ILLNESS WITHIN THE FAMILY. PATIENTS WILL DERIVE MORE BENE-
FIT FROM TEST RESULTS IF THEY ARE EMOTIONALLY ABLE TO COMPREHEND AND AB-SORB THE INFORMATION. IT IS IMPORTANT TO ASSESS PATIENTS’ PRECONCEIVED NOTIONS OF THEIR PERSONAL LIKELIHOOD OF DISEASE IN PREPARING PRETEST EDUCATIONAL STRATEGIES. OFTEN, PATIENTS HARBOR UNWARRANTED FEAR OR DENIAL OF THEIR LIKELIHOOD OF GENETIC RISK.

GENETIC TESTING HAS THE POTENTIAL OF AFFECTING THE WAY INDIVIDUAL FAMILY MEMBERS RELATE TO ONE ANOTHER, BOTH NEGATIVELY AND POSITIVELY. AS A RESULT, PATIENTS ADDRESSING THE OPTION OF MOLECULAR TESTING MUST CONSIDER HOW TEST RESULTS MIGHT IMPACT THEIR RELATIONSHIPS WITH RELATIVES, PARTNERS, SPOUSES, AND FRIENDS. IN FAMILIES WITH A KNOWN GENETIC MUTATION, THOSE WHO TEST POSITIVE MUST CONSIDER THE IMPACT OF THEIR CARRIER STATUS ON THEIR PRESENT AND FUTURE LIFESTYLES; THOSE WHO TEST NEGATIVE MAY MANIFEST SURVIVOR GUILT. FAMILY MEMBERS ARE LIKELY TO DIFFER IN THEIR EMOTIONAL AND SOCIAL RESPONSES TO THE SAME INFORMATION. COUNSELING SHOULD ALSO ADDRESS THE POTENTIAL CONSEQUENCES OF TEST RESULTS ON RELATIONSHIPS WITH A SPOUSE OR CHILD. PARENTS WHO ARE FOUND TO HAVE A DISEASE-ASSOCIATED MUTATION OFTEN EXPRESS CONSIDERABLE ANXIETY AND DESPAIR AS THEY ADDRESS THE ISSUE OF RISK TO THEIR CHILDREN. WHEN A CONDITION DOES NOT MANIFEST UNTIL ADULTHOOD, CLINICIANS WILL BE FACED WITH THE QUESTION OF WHETHER AT-RISK CHILDREN SHOULD BE OFFERED MOLECULAR TESTING AND, IF SO, AT WHAT AGE. ALTHOUGH THE MATTER IS DEBATED, SEVERAL PROFESSIONAL ORGANIZATIONS HAVE CAUTIONED THAT GENETIC TESTING FOR ADULT-ONSET DISORDERS SHOULD NOT BE OFFERED TO CHILDREN. MANY OF THESE CONDITIONS ARE NOT PREVENTABLE; CONSEQUENTLY, SUCH INFORMATION CAN POSE SIGNIFICANT PSYCHOSOCIAL RISK TO THE CHILD. IN ADDITION, THERE IS CONCERN THAT TESTING DURING CHILDHOOD VIOLATES A CHILD’S RIGHT TO MAKE AN INFORMED DECISION REGARDING TESTING UPON REACHING ADULTHOOD. ON THE OTHER HAND, TESTING SHOULD BE OFFERED IN CHILDHOOD FOR DISORDERS THAT MAY MANIFEST EARLY IN LIFE, ESPECIALLY WHEN MANAGEMENT OPTIONS ARE AVAILABLE. FOR EXAMPLE, CHILDREN AT RISK FOR FAMILIAL ADENOMATOUS POLYPOSIS (FAP), ASSOCIATED WITH ALTERATIONS IN THE APC GENE, MAY DEVELOP POLYPS AS EARLY AS THEIR TEENS, AND PROGRESSION TO AN INVASIVE CANCER CAN OCCUR BY THEIR TWENTIES. LIKewise, children at risk for men type 2, which is caused by mutations in the RET proto-onco-gene, may develop medullary thyroid cancer early in childhood, and the issue of prophylactic thyroidectomy should be addressed with the parents of children with documented mutations (chap. 345).

**INFORMED CONSENT**

WHEN THE ISSUE OF TESTING IS ADDRESSED, PATIENTS SHOULD BE STRONGLY ENCOURAGED TO INVOLVE OTHER RELATIVES IN THE DECISION-MAKING PROCESS, AS MOLECULAR DIAGNOSTICS WILL LIKELY HAVE AN IMPACT ON THE ENTIRE FAMILY. INFORMED CONSENT FOR MOLECULAR TESTING BEGINS WITH DETAILED EDUCATION AND COUNSELING (FIG. 64-3). THE PATIENT MUST FULLY UNDERSTAND THE RISKS, BENEFITS, AND LIMITATIONS OF UNDERGOING THE ANALYSIS. INFORMED CONSENT SHOULD INCLUDE A WRITTEN DOCUMENT, DRAFTED CLEARLY AND CON-
CISELY IN A LANGUAGE AND FORMAT THAT IS COMPREHENSIBLE TO THE PATIENT, WHO SHOULD BE MADE AWARE OF THE DISPOSITION OF TEST RESULTS. INFORMED CONSENT SHOULD ALSO INCLUDE A DISCUSSION OF THE MECHANICS OF TESTING. MOST MOLECULAR TESTING FOR HEREDITARY DISEASE INVOLVES DNA-BASED ANALYSIS OF PERIPHERAL BLOOD. IN THE MAJORITY OF CIRCUMSTANCES, TEST RESULTS SHOULD BE GIVEN ONLY TO THE INDIVIDUAL, IN PERSON, AND WITH A SUPPORT PERSON IN THE ROOM. BECAUSE MOLECULAR TESTING OF AN ASYMPTOMATIC INDIVIDUAL OFTEN ALLOWS PREDICTION OF FUTURE RISK, THE PATIENT SHOULD UNDERSTAND ANY POTENTIAL LONG-TERM MEDICAL, PSYCHOLOGICAL, AND SOCIAL IMPLICATIONS OF THIS DECISION. IN THE UNITED STATES, LEGISLATION AFFECTING THIS AREA IS STILL EVOLVING, AND IT IS IMPORTANT TO EXPLORE WITH THE PATIENT THE POTENTIAL IMPACT THAT TEST RESULTS MAY HAVE ON EMPLOYMENT AND FUTURE HEALTH, AS WELL AS DISABILITY AND LIFE INSURANCE COVERAGE. PATIENTS SHOULD UNDERSTAND THAT ALTERNATIVES TO MOLECULAR ANALYSIS REMAIN AVAILABLE IF THEY DECIDE NOT TO PROCEED WITH THIS OPTION. THEY SHOULD ALSO BE NOTIFIED THAT TESTING IS AVAILABLE IN THE FUTURE IF THEY ARE NOT CURRENTLY PREPARED TO UNDERGO ANALYSIS. THE OPTION OF DNA BANKING SHOULD BE PRESENTED SO THAT SAMPLES ARE READILY AVAILABLE FOR FUTURE USE BY FAMILY MEMBERS, IF NEEDED.

**FIGURE 64-3 ALGORITHM FOR GENETIC COUNSELING** IN ASSOCIATION WITH GENETIC TESTING.

DEPENDING ON THE NATURE OF THE GENETIC DISORDER, POSTTEST INTERVENTIONS MAY INCLUDE (1) CAUTIOUS SURVEILLANCE AND APPROPRIATE HEALTH CARE SCREENING, (2) SPECIFIC MEDICAL INTERVENTIONS, (3) CHEMOPREVENTION, (4) RISK AVOIDANCE, AND (5) REFERRAL TO SUPPORT SERVICES. FOR EXAMPLE, PATIENTS WITH KNOWN PATHOLOGIC MUTATIONS IN BRCA1 OR BRCA2 ARE OFFERED INTENSIVE SCREENING AS WELL AS THE OPTION OF PROPHYLACTIC MASTECTOMY AND OOPHORECTOMY. IN ADDITION, SUCH WOMEN MAY BE ELIGIBLE FOR PREVENTIVE TREATMENT WITH TAMOXIFEN, OR ENROLLMENT IN A CHEMOPREVENTION CLINICAL TRIAL. IN CONTRAST, THOSE AT KNOWN RISK FOR HUNTINGTON’S DISEASE ARE OFFERED CONTINUED FOLLOW-UP AND SUPPORTIVE SERVICES, INCLUDING PHYSICAL AND OCCUPATIONAL THERAPY, AND SOCIAL SERVICES OR SUPPORT GROUPS, AS INDICATED. SPECIFIC INTERVENTIONS WILL CHANGE AS TRANSLATIONAL RESEARCH CONTINUES TO ENHANCE OUR UNDERSTANDING OF THESE GENETIC DISEASES AND AS MORE IS LEARNED ABOUT THE FUNCTIONS OF THE GENE PRODUCTS INVOLVED. INDIVIDUALS WHO TEST NEGATIVE FOR A MUTATION IN A DISEASE-ASSOCIATED GENE IDENTIFIED IN AN AffECTED FAMILY MEMBER MUST BE REMINDED THAT
THEY MAY STILL BE AT RISK FOR THE DISEASE. THIS IS OF PARTICULAR IMPORTANCE FOR COMMON DISEASES SUCH AS DIABETES MELLITUS, CANCER, AND CORONARY ARTERY DISEASE. FOR EXAMPLE, A WOMAN WHO FINDS THAT SHE DOES NOT CARRY THE DISEASE-ASSOCIATED MUTATION IN \textit{BRCA2} PREVIOUSLY DISCOVERED IN HER FAMILY MUST BE REMINDED THAT SHE STILL REQUIRES THE SAME BREAST CANCER SCREENING RECOMMENDED FOR THE GENERAL POPULATION.

PAGE NO. 63

418 PART 3: GENETICS AND DISEASE

TABLE 64-1 INDICATIONS FOR GENETIC COUNSELING

ADVANCED MATERNAL (>35) OR PATERNAL (>50) AGE
CONSANGUINITY
PREVIOUS HISTORY OF A CHILD WITH BIRTH DEFECTS OR A GENETIC DISORDER
PERSONAL OR FAMILY HISTORY SUGGESTIVE OF A GENETIC DISORDER
HIGH-RISK ETHNIC GROUPS; KNOWN CARRIERS OF GENETIC ALTERATIONS
DOCUMENTED GENETIC ALTERATION IN A FAMILY MEMBER
ULTRASOUND OR PRENATAL TESTING SUGGESTING A GENETIC DISORDER

GENETIC COUNSELING AND EDUCATION

GENETIC COUNSELING SHOULD BE DISTINGUISHED FROM GENETIC TESTING AND SCREENING, EVEN THOUGH GENETIC COUNSELORS ARE OFTEN INVOLVED IN ISSUES RELATED TO TESTING. GENETIC COUNSELING REFERS TO A \textit{COMMUNICATION PROCESS THAT DEALS WITH HUMAN PROBLEMS ASSOCIATED WITH THE OCCURRENCE OR RISK OF A GENETIC DISORDER IN A FAMILY}. GENETIC RISK ASSESSMENT IS COMPLEX AND OFTEN INVOLVES ELEMENTS OF UNCERTAINTY. COUNSELING THEREFORE INCLUDES GENETIC EDUCATION AS WELL AS PSYCHOSOCIAL COUNSELING. GENETIC COUNSELORS MAY BE CALLED UPON BY OTHER HEALTH CARE PROFESSIONALS (OR BY INDIVIDUAL PATIENTS AND FAMILIES) TO ADDRESS A BROAD RANGE OF ISSUES DIRECTLY AND INDIRECTLY RELATED TO GENETIC DISEASE (\textbf{TABLE 64-1}). THE ROLE OF THE GENETIC COUNSELOR INCLUDES THE FOLLOWING:

* GATHER AND DOCUMENT A DETAILED FAMILY HISTORY;
* EDUCATE PATIENTS ABOUT GENERAL GENETIC PRINCIPLES RELATED TO DISEASE RISK, BOTH FOR THEMSELVES AND FOR OTHERS IN THEIR FAMILY;
* ASSESS AND ENHANCE THE PATIENT’S ABILITY TO COPE WITH THE GENETIC INFORMATION OFFERED;
* DISCUSS HOW NONGENETIC FACTORS MAY RELATE TO THE ULTIMATE EXPRESSION OF DISEASE;
* ADDRESS MEDICAL MANAGEMENT ISSUES;
* ASSIST IN DETERMINING THE ROLE OF GENETIC TESTING FOR THE INDIVIDUAL AND FAMILY;
* ENSURE THAT THE PATIENT IS AWARE OF THE INDICATIONS, PROCESS, RISKS,
BENEFITS, AND LIMITATIONS OF THE VARIOUS GENETIC TESTING OPTIONS; 
*ASSIST THE PATIENT, FAMILY, AND REFERRING PHYSICIAN IN THE INTERPRETA-
TION OF THE TEST RESULTS; AND 
*REFER THE PATIENT AND OTHER AT-RISK FAMILY MEMBERS FOR ADDITIONAL 
MEDICAL AND SUPPORT SERVICES, IF NECESSARY.

THE COMPLEXITY OF GENETIC COUNSELING AND THE BROAD SCOPE OF GENETIC 
DISEASES HAVE LED TO THE DEVELOPMENT OF SPECIALIZED, MULTIDISCIPLINARY 
CLINICS DESIGNED TO PROVIDE BROAD-BASED SUPPORT AND MEDICAL CARE FOR 
THOSE AT RISK AND THEIR FAMILY MEMBERS. SUCH SPECIALTY CLINICS ARE 
WELL ESTABLISHED IN THE AREAS OF CANCER AND NEURODEGENERATIVE DISORDERS. 
THE MULTIDISCIPLINARY TEAMS ARE OFTEN COMPOSED OF MEDICAL GENETICISTS, SPEC-
IALIST PHYSICIANS, GENETIC COUNSELORS, NURSES, PSYCHOLOGISTS, SOCIAL 
WORKERS, AND BIOMEDICAL ETHICISTS WHO WORK TOGETHER TO CONSIDER DIFFICULT 
DIAGNOSTIC, TREATMENT, AND TESTING DECISIONS. SUCH A FORMAT ALSO 
PROVIDES PRIMARY CARE PHYSICIANS WITH INVALUABLE SUPPORT AND ASSISTANCE AS 
THEY FOLLOW AND TREAT AT-RISK PATIENTS.

THE APPROACH TO GENETIC COUNSELING HAS IMPORTANT ETHICAL, SOCIAL, AND 
FINANCIAL IMPLICATIONS. PHILOSOPHIES RELATED TO GENETIC COUNSELING 
VARY WIDELY BY COUNTRY AND CENTER. IN NORTH AMERICAN CENTERS, FOR 
EXAMPLE, COUNSELING IS GENERALLY OFFERED IN A NONDIRECTIVE MANNER, WHEREIN 
PATIENTS LEARN TO UNDERSTAND HOW THEIR VALUES FACTOR INTO A PARTICULAR 
MEDICAL DECISION. NONDIRECTIVE COUNSELING IS PARTICULARLY APPROPRIATE WHEN THERE 
ARE NO DATA DEMONSTRATING A CLEAR BENEFIT ASSOCIATED WITH A PARTICULAR 
INTERVENTION OR WHEN AN INTERVENTION IS CONSIDERED EXPERIMENTAL. FOR 
EXAMPLE, NONDIRECTIVE GENETIC COUNSELING IS EMPLOYED WHEN A PERSON IS DECIDING 
WHETHER TO UNDERGO GENETIC TESTING FOR HUNTINGTON’S DISEASE (CHAP. 
365). AT THIS TIME, THERE IS NO CLEAR BENEFIT (IN TERMS OF MEDICAL OUTCOME) TO 
AN AT-RISK INDIVIDUAL UNDERGOING GENETIC TESTING FOR THIS DISEASE, AS ITS 
COURSE CANNOT BE ALTERED BY THERAPEUTIC INTERVENTIONS. HOWEVER, TESTING CAN 
HAVE AN IMPORTANT IMPACT ON THIS INDIVIDUAL’S PERCEPTION OF THE FUTURE 
AND HIS OR HER INTERPERSONAL RELATIONSHIPS AND PLANS FOR REPRODUCTION. 
THEREFORE, THE DECISION TO PURSUE TESTING RESTS ON THE INDIVIDUAL’S 
BELIEF SYSTEM AND VALUES. ON THE OTHER HAND, A MORE DIRECTIVE APPROACH IS 
APPROPRIATE WHEN A CONDITION CAN BE TREATED. IN A FAMILY WITH FAP, COLON 
CANCER SCREENING AND PROPHYLAXIS COLEDOMY SHOULD BE RECOMMENDED FOR
KNOWN APC MUTATION CARRIERS. THE COUNSELOR AND CLINICIAN FOLLOWING THIS FAMILY MUST ENSURE THAT THE AT-RISK FAMILY MEMBERS HAVE ACCESS TO THE RE-SOURCES NECESSARY TO ADHERE TO THESE RECOMMENDATIONS. GENETIC EDUCATION IS CENTRAL TO AN INDIVIDUAL’S ABILITY TO MAKE AN INFORMED DECISION REGARDING TESTING OPTIONS AND TREATMENT. ALTHOUGH GENETIC COUNSELORS REPRESENT ONE SOURCE OF GENETIC EDUCATION, OTHER HEALTH CARE PROVIDERS ALSO NEED TO CONTRIBUTE TO PATIENT EDUCATION. PATIENTS AT RISK FOR GENETIC DISEASE SHOULD UNDERSTAND FUNDAMENTAL MEDICAL GENETIC PRINCIPLES AND TERMINOLOGY RELEVANT TO THEIR SITUATION. THIS INCLUDES THE CONCEPT OF GENES, HOW THEY ARE TRANSMITTED, AND HOW THEY CONFER HEREDITARY DISEASE RISK. AN ADEQUATE KNOWLEDGE OF PATTERNS OF INHERITANCE WILL ALLOW PATIENTS TO UNDERSTAND THE PROBABILITY OF DISEASE RISK FOR THEMSELVES AND OTHER FAMILY MEMBERS. IT IS ALSO IMPORTANT TO IMPART THE CONCEPTS OF DISEASE PENETRANCE AND EXPRESSION. FOR MOST COMPLEX ADULT-ONSET GENETIC DISORDERS, ASYMPTOMATIC PATIENTS SHOULD BE ADVISED THAT A POSITIVE TEST RESULT DOES NOT ALWAYS TRANSLATE INTO FUTURE DISEASE DEVELOPMENT. IN ADDITION, THE ROLE OF NONGENETIC FACTORS, SUCH AS ENVIRONMENTAL EXPOSURES, MUST BE DISCUSSED IN THE CONTEXT OF MULTIFACTORIAL DISEASE RISK AND DISEASE PREVENTION. FINALLY, PATIENTS SHOULD UNDERSTAND THE NATURAL HISTORY OF THE DISEASE AS WELL AS THE POTENTIAL OPTIONS FOR INTERVENTION, INCLUDING SCREENING, PREVENTION, AND-IN CERTAIN CIRCUMSTANCES-PHARMACOLOGIC TREATMENT OR PROPHYLACTIC SURGERY.

THERAPEUTIC INTERVENTIONS BASED ON GENETIC RISK FOR DISEASE

SPECIFIC TREATMENTS ARE NOW AVAILABLE FOR AN INCREASING NUMBER OF GENETIC DISORDERS, WHETHER IDENTIFIED THROUGH POPULATION-BASED SCREENING OR DIRECTED TESTING (TABLE 64-2). ALTHOUGH THE STRATEGIES FOR THERAPEUTIC INTERVENTIONS ARE BEST DEVELOPED FOR CHILDHOOD HEREDITARY METABOLIC DISEASES, THESE PRINCIPLES ARE MAKING THEIR WAY INTO THE DIAGNOSIS AND

TABLE 64-2 EXAMPLES OF GENETIC TESTING AND POSSIBLE INTERVENTIONS GENETIC DISORDER
ONCOLOGIC

HEREDITARY NONPOLYPOSIS COLON CANCER

FAMILIAL ADENOMATOUS POLYPsis

FAMILIAL BREAST AND OVARIAN CANCER

FAMILIAL MELANOMA

BASAL CELL NEVUS SYNDROME

INHERITANCE

AD

AD

AD

AD

GENES

\textit{MSH2, MLH1, MSH6, PMS1, PMS2, TGFBRI2, APC, BRCA1, BRCA2, CDKN2A, PTCH}

INTERVENTIONS

EARLY ENDOSCOPIC SCREENING

EARLY ENDOSCOPIC SCREENING

NONSTERoidal ANTI-INFLAMMATORY DRUGS

COLECTOMY

ESTROGEN RECEPTOR ANTAGONISTS

EARLY SCREENING BY EXAMS AND MAMMOGRAPHY

CONSIDERATION OF PROPHELYACTIC SURGERY

AVOIDANCE OF UV LIGHT

SCREENING AND BIOPSIES

AVOIDANCE OF UV LIGHT

SCREENING AND BIOPSIES

(\textit{CONTINUED})
### Chapter 64: The Practice of Genetics in Clinical Medicine

#### Table 64-2: Examples of Genetic Testing and Possible Interventions (Continued)

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NEUROLOGIC

MALIGNANT HYPERThERMIA
HYPERkALEMIC PERIODIC PARALYSIS
ADRENOLEUKODYSTROPHY

DUCHENNE AND BECKER MUSCULAR DYSTROPHY

FAMILIAL PARKINSON DISEASE

WILSON DISEASE

INHERITANCE

AD

XL

XL

XL

AD

AD

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AD

XL, AR

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AD
AR
GENES
F5
F8C
F9
G6PD
MYH7, MYBPC3, TMSA, TNNT2, TPM1
KCNQ1, SCN5A, HERG, MIRP1, KCNE1, KCNE2
FBN1
MEFV
HFE
P1
BMPR2
PKHD1, PKHD2
AVPR2, AQP2
AVP
MULTIPLE GENES
CASR
KAL
RET
CYP21
Ryr1
SCN4A
ABCD1
DMD
SNCA, PARK2
ATP78

INTERVENTIONS
AVOIDANCE OF THROMBOGENIC RISK FACTORS AND ORAL CONTRACEPTIVES
FACTOR VIII REPLACEMENT
FACTOR IX REPLACEMENT POSSIBLE GENE THERAPY
AVOIDANCE OF OXIDANT DRUGS
ECHOCARDIOGRAPHIC SCREENING
EARLY PHARMACOLOGIC INTERVENTION
MYOMECTOMY
ELECTROCARDIOGRAPHIC SCREENING
EARLY PHARMACOLOGIC INTERVENTION
IMPLANTABLE CARDIOVERTER DEFIBRILLATOR DEVICES
ECHOCARDIOGRAPHIC SCREENING
PROPHYLACTIC BETA BLOCKERS

COLCHICINE TREATMENT
PHLEBOTOMY

AVOIDANCE OF SMOKING
AVOIDANCE OF OCCUPATIONAL AND ENVIRONMENTAL TOXINS
TREATMENT WITH PULMONARY VASODILATORS

PREVENTION OF HYPERTENSION
PREVENTION OF URINARY TRACT INFECTIONS
KIDNEY TRANSPLANTATION
FLUID REPLACEMENT
THIAZIDES, AMILORIDE

REPLACE VASOPRESSIN
SCREEN AND TREAT FOR DIABETES
AVOIDANCE OF PARATHYROIDECTOMY
INDUCTION OF PUBERTY WITH HORMONE REPLACEMENT
PROPHYLACTIC THYROIDECTOMY
SCREENING FOR PHEOCHROMOCYTOMA AND
HYPERPARATHYROIDISM
GLUCOCORTICOID AND MINERALOCORTICOID TREATMENT

AVOIDANCE OF PRECIPITATING ANESTHETICS
ACETAZOLAMIDE
POSSIBLE BONE MARROW TRANSPLANT FOR SEVERE CHILDHOOD
CNS FORM
CORTICOSTEROIDS
POSSIBLE FUTURE MYOBLAST TRANSFER
AMANTADINE, ANTICHOLINERGICS, LEVODOPA, MONOAMINE
OXIDASE B INHIBITORS
ZINC, TRIENTENE

ABBREVIATIONS: AD, AUTOSOMAL DOMINANT; AR, AUTOSOMAL RECESSIVE; CNS, CENTRAL NERVOUS SYSTEM; XL, X-LINKED.

MANAGEMENT OF ADULT-ONSET DISORDERS. HEREDITARY HEMOCHROMATOSIS ILLUSTRATES MANY OF THE ISSUES RAISED BY THE AVAILABILITY OF GENETIC SCREENING IN THE ADULT POPULATION. FOR INSTANCE, IT IS RELATIVELY COMMON (APPROXIMATELY 1 IN 200 INDIVIDUALS OF NORTHERN EUROPEAN DESCENT ARE HOMOZYGOUS), AND ITS COMPLICATIONS ARE POTENTIALLY PREVENTABLE THROUGH PHLEBOTOMY (CHAP. 351). THE IDENTIFICATION OF THE HFE GENE, MUTATIONS
OF WHICH ARE ASSOCIATED WITH THIS SYNDROME, HAS SPARKED INTEREST IN THE USE OF DNA-BASED TESTING FOR PRESYMPTOMATIC DIAGNOSIS OF THE DISORDER. HOWEVER, UP TO ONE-THIRD OF INDIVIDUALS WHO ARE HOMOZYGOUS FOR THE HFE MUTATION DO NOT HAVE EVIDENCE OF IRON OVERLOAD. CONSEQUENTLY, IN THE ABSENCE OF A POSITIVE FAMILY HISTORY, CURRENT RECOMMENDATIONS INCLUDE PHENOTYPIC SCREENING FOR EVIDENCE OF IRON OVERLOAD FOLLOWED BY GENETIC TESTING. WHETHER GENETIC SCREENING FOR HEMOCHROMATOSIS WILL SOMEDAY BE COUPLED TO ASSESSMENT OF PHENOTYPIC EXPRESSION AWAIT FURTHER STUDIES. IN CONTRAST TO THE ISSUE OF POPULATION SCREENING, IT IS IMPORTANT TO TEST AND COUNSEL OTHER FAMILY MEMBERS WHEN THE DIAGNOSIS OF HEMOCHROMATOSIS HAS BEEN MADE IN A PROBAND. TESTING ALLOWS THE PHYSICIAN TO EXCLUDE FAMILY MEMBERS WHO ARE NOT AT RISK. IT ALSO PERMITS PRE-SYMPTOMATIC DETECTION OF IRON OVERLOAD AND THE INSTITUTION OF TREATMENT (PHLEBOTOMY) BEFORE THE DEVELOPMENT OF ORGAN DAMAGE. PREVENTIVE MEASURES AND THERAPEUTIC INTERVENTIONS ARE NOT RESTRICTED TO METABOLIC DISORDERS. IDENTIFICATION OF FAMILIAL FORMS OF LONG QT SYNDROME, ASSOCIATED WITH VENTRICULAR ARRHYTHMIAS, ALLOWS EARLY ELECTROCARDIOGRAPHIC TESTING AND THE USE OF PROPHYLACTIC ANTIARRHYTHMIC THERAPY, OVERDRIVE PACEMAKERS, OR DEFIBRILLATORS (CHAP. 226). INDIVIDUALS WITH FAMILIAL HYPERTROPHIC CARDIOMYOPATHY CAN BE SCREENED BY ULTRASOUND, TREATED WITH BETA BLOCKERS OR OTHER DRUGS, AND COUNSELED ABOUT THE IMPORTANCE OF AVOIDING STRENuous EXERCISE AND DEHYDRATION (CHAP. 231). LIKEWISE, INDIVIDUALS WITH MARFAN SYNDROME CAN BE TREATED WITH BETA BLOCKERS AND MONITORED FOR THE DEVELOPMENT OF AORTIC ANEURYSMS (CHAP. 242). INDIVIDUALS WITH *###1 ANTITRYPSIN DEFICIENCY CAN BE STRONGLY COUNSELED TO AVOID CIGARETTE SMOKING AND EXPOSURE TO ENVIRONMENTAL PULMONARY AND HEPATOTOXINS. VARIOUS HOST GENES INFLUENCE THE PATHOGENESIS OF CERTAIN INFECTIOUS DISEASES IN HUMANS, INCLUDING HIV (CHAP. 182). THE FACTOR V LEIDEN ALLELE INCREASES RISK OF THROMBOSIS (CHAP. 59). APPROXIMATELY 3% OF THE WORLDWIDE POPULATION IS HETEROZYGOUS FOR THIS MUTATION. MOREOVER, IT IS FOUND IN UP TO 25% OF PATIENTS WITH RECURRENT DEEP-VEIN THROMBOSIS OR PULMONARY EMBOLISM. WOMEN WHO ARE HETEROZYGOUS OR HOMOZYGOUS FOR THIS ALLELE SHOULD THEREFORE AVOID THE USE OF ORAL CONTRA-
CEPTIVES AND RECEIVE HEPARIN PROPHYLAXIS AFTER SURGERY OR TRAUMA. THE FIELD OF PHARMACOGENOMICS SEEKS TO IDENTIFY GENES THAT ALTER DRUG METABOLISM OR CONFER SUSCEPTIBILITY TO TOXIC DRUG REACTIONS. PHARMACOGENOMICS PERMITS INDIVIDUALIZED DRUG THERAPY, RESULTING IN IMPROVED TREATMENT OUTCOMES, REDUCED TOXICITIES, AND MORE COST-EFFECTIVE PHARMACEUTICAL CARE. EXAMPLES INCLUDE SUCCINYLCHOLINE SENSITIVITY, THIOPURINE METHYLTRANSFERASE (TPMT) DEFICIENCY, MALIGNANT HYPERThERMIA, DIHYDROPYRIMIDINE DEHYDROGENASE DEFICIENCY, THE PORPHYRIAS, AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY.

AS NOTED ABOVE, THE IDENTIFICATION OF GENES THAT INCREASE THE RISK OF SPECIFIC TYPES OF NEOPLASIA IS RAPIDLY CHANGING THE MANAGEMENT OF MANY CANCERS. IDENTIFYING FAMILY MEMBERS WITH MUTATIONS THAT PREDISPOSE TO FAP OR HEREDITARY NONPOLYPOSIS COLON CANCER (HNPPC) CAN LEAD TO RECOMMENDATIONS OF EARLY CANCER SCREENING OR PROPHYLACTIC SURGERY (CHAP. 87). SIMILAR PRINCIPLES APPLY TO FAMILIAL FORMS OF MELANOMA, BASAL CELL CARCINOMA, AND CANCERS OF THE BREAST, OVARY, AND THYROID GLAND. IT SHOULD BE RECOGNIZED, HOWEVER, THAT MOST CANCERS HARBOR SEVERAL DISTINCT GENETIC ABNORMALITIES BY THE TIME THEY ACQUIRE INVASIVE OR METASTATIC POTENTIAL (CHAPS. 79 AND 80). CONSEQUENTLY, THE MAJOR IMPACT OF GENETIC TESTING IN THESE CASES IS TO ALLOW MORE INTENSIVE CLINICAL SCREENING, AS IT REMAINS VERY CHALLENGING TO PREDICT DISEASE PENETRANCE, EXPRESSION, OR CLINICAL COURSE.

ALTHOUGH GENETIC DIAGNOSIS OF THESE AND OTHER DISORDERS IS ONLY BEGINNING TO BE USED IN THE CLINICAL SETTING, PREDICTIVE TESTING HOLDS THE PROMISE OF ALLOWING EARLIER AND MORE TARGETED INTERVENTIONS THAT CAN REDUCE MORBIDITY AND MORTALITY. WE CAN EXPECT THE AVAILABILITY OF GENETIC TESTS TO EXPAND. A CRITICAL CHALLENGE FOR PHYSICIANS AND OTHER HEALTH CARE PROVIDERS IS TO KEEP PACE WITH THESE ADVANCES IN GENETIC MEDICINE AND TO IMPLEMENT TESTING JUDICIOUSLY.

FURTHER READINGS

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ENSENAUER RE: GENETIC TESTING: PRACTICAL, ETHICAL, AND COUNSELING CONSIDERATIONS. MAYO CLIN PROC 80:63, 2005
HARPER PS: PRACTICAL GENETIC COUNSELLING, 5TH ED. OXFORD, BUTTERWORTH-HEINMANN, 1998
MCCANDLESS SE ET AL: THE BURDEN OF GENETIC DISEASE ON INPATIENT CARE IN A CHILDREN’S HOSPITAL. AM J HUM GENET 74:121, 2004
GENE TRANSFER IS A NOVEL AREA OF THERAPEUTICS IN WHICH THE ACTIVE AGENT IS A NUCLEIC ACID SEQUENCE RATHER THAN A PROTEIN OR SMALL MOLECULE. BECAUSE DELIVERY OF NAKED DNA OR RNA TO A CELL IS AN INEFFECTIVE PROCESS, MOST GENE TRANSFER IS CARRIED OUT USING A VECTOR, OR GENE DELIVERY VEHICLE. THESE VEHICLES HAVE GENERALLY BEEN ENGINEERED FROM VIRUSES BY DELETING SOME OR ALL OF THE VIRAL GENOME AND REPLACING IT WITH THE THERAPEUTIC GENE OF INTEREST UNDER THE CONTROL OF A SUITABLE PROMOTER (TABLE 65-1). GENE TRANSFER STRATEGIES CAN BE DESCRIBED IN TERMS OF THREE ESSENTIAL ELEMENTS: (1) A VECTOR, (2) A GENE TO BE DELIVERED, AND

TABLE 65-1 CHARACTERISTICS OF GENE DELIVERY VEHICLES

FEATURES

VIRAL GENOME
CELL DIVISION
REQUIREMENT
PACKAGING
LIMITATION
IMMUNE RESPONSES TO VECTOR
GENOME INTEGRATION
LONG-TERM EXPRESSION
MAIN ADVANTAGES
MAIN DISADVANTAGES

VIRAL VECTORS

RETROVIRAL

RNA
YES

8 KB
FEW
YES
YES

PERSISTENT GENE TRANSFER IN
DIVIDING CELLS

THEORETICAL RISK OF INSERTIONAL MUTAGENESIS (OCCURRED IN 3 CASES)

LENTIVIRAL

RNA
G1 PHASE
8 KB
FEW
YES
YES

PERSISTENT GENE TRANSFER IN TRANS-DUCED TISSUES MIGHT INDUCE ONCOGENESIS IN SOME CASES

ADENOVIRAL

DNA
NO
8-30 KB
EXTENSIVE
POOR
NO

HIGHLY EFFECTIVE IN TRANSDUCING VARIOUS TISSUES VIRAL CAPSID ELICITS STRONG IMMUNE RESPONSES
AAV
DNA
NO
5 KB
FEW
POOR
YES

ELICITS FEW INFLAMMATORY RESPONSES, NONPATHOGENIC LIMITED PACKAGING CAPACITY

HUMAN FOAMY VIRUS
RNA
NO
85 KB
FEW
YES

YES

PERSISTENT GENE EXPRESSION IN BOTH DIVIDING AND NONDIVIDING CELLS IN NEED OF A STABLE PACKAGING SYSTEM

HSV-1
DNA
NO
40-150 KB
FEW IN RECOMBINANT VIRUS
NO
LARGE PACKAGING CAPACITY
WITH PERSISTENT GENE TRANSFER
RESIDUAL CYTOTOXICITY WITH
NEURON SPECIFICITY

SV-40

DNA
NO

5 KB
FEW
POOR
NO

WIDE HOST CELL RANGE;
LACK OF IMMUNOGENICITY
LIMITED PACKAGING
CAPACITY

ALPHA-VIRUSES

RNA
NO

5 KB
FEW
NO

NO

LIMITED IMMUNE RESPONSES AGAINST THE VECTOR TRANSFERED
GENE EXPRESSION IS TRANSIENT

NOTE: AAV, ADENO-ASSOCIATED VIRUS; HSV, HERPES SIMPLEX VIRUS; SV, SARCOMA VIRUS.

PAGE NO. 66

421 CHAPTER 65 GENE THERAPY IN CLINICAL MEDICINE

(3) A RELEVANT TARGET CELL TO WHICH THE DNA OR RNA IS DELIVERED. THE SERIES OF STEPS IN WHICH THE DONATED DNA ENTERS THE TARGET CELL AND BEGINS EXPRESSION IS REFERRED TO AS TRANSDUCTION. GENE DELIVERY CAN TAKE PLACE IN VIVO, IN WHICH THE VECTOR IS DIRECTLY INJECTED INTO THE PATIENT OR, IN THE CASE OF HEMATOPOIETIC AND SOME OTHER TARGET CELLS, EX VIVO, WITH REMOVAL OF THE TARGET CELLS FROM THE PATIENT, FOLLOWED BY RETURN OF THE MODIFIED AUTOLOGOUS CELLS AFTER GENE TRANSFER IN THE LABORATORY. THE LATER APPROACH OFFERS OPPORTUNITIES TO INTEGRATE GENE TRANSFER TECHNIQUES WITH CELLULAR THERAPIES (CHAP. 67).

GENE TRANSFER TECHNOLOGY IS STILL UNDER DEVELOPMENT AND PROTOCOLS ARE EXPERIMENTAL. GENE THERAPY IS ONE OF THE MOST COMPLEX THERAPEUTIC MODALITIES YET ATTEMPTED, AND EACH NEW DISEASE REresents A THERAPEUTIC PROBLEM FOR WHICH DOSING, SAFETY, AND EFFICACY MUST BE DEFINED. NONE-THELESS, GENE TRANSFER REMAINS ONE OF THE MOST POWERFUL CONCEPTS IN MODERN MOLECULAR MEDICINE AND HAS THE POTENTIAL TO ADDRESS A HOST OF DISEASES FOR WHICH THERE ARE CURRENTLY NO CURES OR, IN SOME CASES, NO AVAILABLE TREATMENT. OVER 5000 SUBJECTS HAVE BEEN ENROLLED IN GENE TRANSFER STUDIES, AND SERIOUS ADVERSE EVENTS HAVE BEEN RARE. GENE THERAPIES ARE BEING DEVELOPED FOR A WIDE VARIETY OF DISEASE ENTITIES (FIG. 65-1).

GENE TRANSFER FOR GENETIC DISEASE

GENE TRANSFER STRATEGIES FOR GENETIC DISEASE GENERALLY INVOLVE GENE ADDITION THERAPY. THIS APPROACH MOST COMMONLY INVOLVES TRANSFER OF THE MISSING GENE TO A PHYSIOLOGICALLY RELEVANT TARGET CELL. HOWEVER, OTHER STRATEGIES ARE POSSIBLE, INCLUDING SUPPLYING A GENE THAT ACHIEVES A SIMILAR BIOLOGIC EFFECT THROUGH AN ALTERNATIVE PATHWAY (E.G., FACTOR VIIA FOR HEMOPHILIA A); SUPPLYING AN ANTISENSE OLIGONUCLEOTIDE TO SPLICE OUT A MUTANT EXON IF THE SEQUENCE IS NOT CRITICAL TO THE FUNCTION OF THE PROTEIN (AS HAS BEEN DONE WITH THE DYSTROPHIN GENE IN DUCHENNE MUSCULAR DYSTROPHY); OR DOWNREGULATING A HARMFUL RESPONSE THROUGH AN
SIRNA. TWO DISTINCT STRATEGIES ARE USED TO ACHIEVE LONG-TERM GENE EXPRESSION: ONE IS TO TRANSDUCE STEM CELLS WITH AN INTEGRATING VECTOR, SO THAT ALL PROGENY CELLS WILL CARRY THE DONATED GENE; THE OTHER IS TO TRANSDUCE LONG-LIVED CELLS, SUCH AS SKELETAL MUSCLE OR NEURAL CELLS. IN THE CASE OF LONG-LIVED CELLS, INTEGRATION INTO THE TARGET CELL GENOME IS UNNECESSARY, PROVIDED THE DONATED DNA CAN BE STABILIZED IN AN EPISOMAL FORM.

IMMUNODEFICIENCY DISORDERS: PROOF OF PRINCIPLE EARLY ATTEMPTS TO PROVIDE GENE REPLACEMENT INTO HEMATOPOIETIC STEM CELLS (HSCS) WERE STYMIED BY THE RELATIVELY LOW TRANSDUCTION EFFICIENCY OF RETROVIRAL VECTORS.

FEATURES

VIRAL GENOME
CELL DIVISION
REQUIREMENT
PACKAGING
LIMITATION
IMMUNE
RESPONSES
TO VECTOR
GENOME
INTEGRATION
LONG-TERM
EXPRESSION
MAIN ADVANTAGES

MAIN DISADVANTAGES

NON-VIRAL VECTORS

TRANSPOSON/
TRANSPOSASE SYSTEM

N/A
NO

UNDETERMINED,

PROBABLY LARGE
NO

YES

YES

TRANSFECTS MANY CELL T
TYPES WITH LONG-TERM GENE EXPRESSION

EARLY STAGE IN DEVELOPMENT

LIPOSOMES

N/A
NO

UNDETERMINED, PROBABLY LARGE
NO

NO

TRANSFECTS MANY CELL TYPES. LARGE HOLDING CAPACITY TO ENABLE A HIGH NUMBER OF BASE PAIRS EXPENSIVE TO PRODUCE

NAKED DNA

N/A
NO

UNDETERMINED, PROBABLY LARGE
NO

NO

EFFICIENT IN GENE TRANSFER; LIMITED IMMUNOGENICITY

TRANSIENT AND LOW-LEVEL EXPRESSION

SITE-SPECIFIC INTEGRASE

N/A
NO
INDICATIONS IN GENE THERAPY CLINICAL TRIALS. The chart divides clinical gene transfer studies by disease classification. A majority of trials have addressed cancer, with monogenic disorders and cardiovascular diseases the next largest categories. (Reproduced with permission from J Gene Med. New Jersey, Wiley, 2006.)

TORS, which require dividing target cells for integration. Because HSCs are normally quiescent, they are a formidable transduction target. However, identification of cytokines that induced cell division without promoting differentiation of stem cells, along with technical improvements in the isolation and transduction of HSCs, led to modest but real gains in transduction efficiency.

The first convincing therapeutic effect from gene transfer occurred with X-linked severe combined immunodeficiency disease (SCID), which results from mutations in the gene (IL2RG) encoding the \( \alpha \) subunit of a cytokine receptor required for normal development of T and NK cells (Chap. 310). Affected infants present in the first few months of life with overwhelming infections and/or failure to thrive. In this disorder, it was recognized that the transduced cells, even if few in number, would have a proliferative advantage compared to the non-transduced cells, which lack receptors for the cytokines required for lymphocyte development and maturation. Complete reconstitution of the immune system, including documented responses to standard childhood vaccinations, clearing of infections, and remarkable gains in growth occurred in most of the treated children. However, two developed a syndrome similar to T cell acute lymphocytic leukemia, with splenomegaly, rising white counts, and the emergence of a single clone of T cells. In these children, the retroviral vector had integrated within a gene, LMO-2 (LIM only-2), which encodes a component of a transcription factor complex involved in hematopoietic development. Insertion of the retroviral long terminal repeat is thought to increase the
EXPRESSION OF *LMO-2.*

The *X*-linked SCID studies were a watershed event in the evolution of gene therapy. They demonstrated conclusively that gene therapy could cure disease; of the 16 infants eventually treated in these trials, 15 achieved correction of the immunodeficiency disorder. Unfortunately, 3 later developed a leukemia-like disorder, but 12 are alive and free of complications at time periods ranging up to 7 years after initial treatment. These studies also demonstrated that insertional mutagenesis leading to cancer was more than a hypothetical possibility. As a result of the experience in these trials, all protocols using integrating vectors in hematopoietic cells must include a plan for monitoring sites of insertion and clonal proliferation. Strategies to overcome this complication have included employing a “suicide” gene cassette in the vector, so that errant clones can be quickly ablated; or using “insulator” elements in the cassette, which can limit the activation of genes surrounding the insertion site.

More clear-cut success has been achieved in a gene therapy trial for another form of SCID, adenosine deaminase (ADA) deficiency (Chap. 310). ADA-SCID is clinically similar to *X*-linked SCID, although it can be treated by enzyme replacement therapy with a pegylated form of the enzyme (PEG-ADA), which leads to immune reconstitution but not always to normal T cell counts. Enzyme replacement therapy is expensive (annual costs: $200,000-$300,000 in U.S. dollars). Gene therapy protocols have evolved to include the use of HSCs rather than T cells as the target for transduction; discontinuation of PEG-ADA at the time of vector infusion, so that the transduced cells have a proliferative advantage over the non-transduced; and the use of a mild conditioning regimen to facilitate engraftment of the transduced cells. There have been no complications in the six children treated on this protocol, with a median follow-up of >4 years. Based on current data, the efficacy of gene transfer for ADA-SCID is convincing, but longer term follow-up will be required to determine whether this approach is sufficiently safe to be routinely recommended as an alternative to PEG-ADA.

Other diseases likely to be amenable to transduction of HSCs in-
CLIDE WISKOTT-ALDRICH SYNDROME (TRIALS UNDERWAY), CHRONIC GRANULOMATOUS DISEASE, SICKLE CELL DISEASE, AND THALASSEMA.

LONG-TERM EXPRESSION IN GENETIC DISEASE: IN VIVO GENE TRANSFER WITH RECOMBINANT ADENO-ASSOCIATED VIRAL (AAV) VECTORS RECOMBINANT AAV VECTORS HAVE EMERGED AS ATTRACTIVE GENE DELIVERY VEHICLES FOR GENETIC DISEASE. ENGINEERED FROM A SMALL REPLICATION-DEFECTIVE DNA VIRUS, THEY ARE DEVOID OF VIRAL CODING SEQUENCES AND TRIGGER VERY LITTLE IMMUNE RESPONSE IN EXPERIMENTAL ANIMALS. THEY ARE CAPABLE OF TRANSDUCING NON-DIVIDING TARGET CELLS, AND THE DONATED DNA IS STABILIZED PRIMARILY IN AN EPISOMAL FORM, THUS MINIMIZING RISKS ASSOCIATED WITH INSERTIONAL MUTAGENESIS. BECAUSE THE VECTOR HAS A TROPISM FOR CERTAIN LONG-LIVED CELL TYPES, SUCH AS SKELETAL MUSCLE, THE CENTRAL NERVOUS SYSTEM (CNS), AND HEPATOCYTES, LONG-TERM EXPRESSION CAN BE ACHIEVED EVEN IN THE ABSENCE OF INTEGRATION.

CLINICAL TRIALS USING RECOMBINANT AAV VECTORS ARE NOW ONGOING FOR MUSCULAR DYSTROPHIES, *###1-ANTITRYPSIN DEFICIENCY, LIPOPROTEIN LIPASE DEFICIENCY, HEMOPHILIA B, AND A FORM OF CONGENITAL BLINDNESS CALLED LEBER’S CONGENITAL AMAUROSIS. HEMOPHILIA IS OFTEN CONSIDERED A PROMISING DISEASE MODEL FOR GENE TRANSFER, AS THE GENE PRODUCT DOES NOT REQUIRE PRECISE REGULATION OF EXPRESSION AND BIOLOGICALLY ACTIVE CLOTTING FACTORS CAN BE SYNTHESIZED IN A VARIETY OF TISSUE TYPES, PERMITTING LATITUDE IN CHOICE OF TARGET TISSUE. MOREOVER, RAISING CIRCULATING FACTOR LEVELS FROM <1% (LEVELS SEEN IN THOSE SEVERELY AFFECTED) INTO THE RANGE OF 5% GREATLY IMPROVES THE PHENOTYPE OF THE DISEASE. PRECLINICAL STUDIES WITH RECOMBINANT AAV VECTORS INFUSED INTO SKELETAL MUSCLE OR LIVER HAVE RESULTED IN LONG-TERM (>5 YEARS) EXPRESSION OF FACTOR VIII OR FACTOR IX IN THE HEMOPHILIC DOG MODEL. ADMINISTRATION TO SKELETAL MUSCLE OF AN AAV VECTOR EXPRESSING FACTOR IX IN PATIENTS WITH HEMOPHILIA WAS SAFE AND RESULTED IN LONG-TERM EXPRESSION AS MEASURED BY MUSCLE BIOPSY, BUT CIRCULATING LEVELS NEVER ROSE >1% FOR SUSTAINED PERIODS, AND A LARGE NUMBER OF IM INJECTIONS (>80-100) WAS REQUIRED TO ACCESS A LARGE MUSCLE MASS. INTRAVASCULAR VECTOR DELIVERY HAS BEEN EMPLOYED TO ACCESS LARGE AREAS OF SKELETAL MUSCLE IN ANIMAL MODELS OF HEMOPHILIA AND WILL LIKELY BE TESTED IN UPCOMING TRIALS. ADMINISTRATION OF AN AAV VECTOR EXPRESSING FACTOR IX TO THE LIVER IN HUMANS WITH HEMOPHILIA RESULTED IN THERAPEUTIC CIRCULATING LEVELS AT THE HIGHEST DOSE TESTED, BUT EXPRESSION AT THESE LEVELS (>5%) LASTED FOR ONLY 6-10 WEEKS BEFORE DECLINING TO BASELINE (<1%). A MEMORY T CELL RESPONSE TO VIRAL CAPSID, PRESENT IN HUMANS BUT NOT IN OTHER ANIMAL SPECIES (WHICH ARE NOT NATURAL HOSTS FOR THE VIRUS), MAY BE A CONTRIBUTING FACTOR IN THE LOSS OF EXPRESSION. FORTUNATELY, TRIGGERING OF THE MEMORY T CELL RESPONSE APPEARS TISSUE-SPECIFIC, AND IT IS POSSIBLE THAT INTRODUCTION OF THE VECTOR INTO IMMUNOPRIVILEGED SITES, SUCH AS THE CNS (E.G., FOR PARKINSON’S DISEASE) OR THE RETINA, WILL AVOID THIS COMPLICATION.

LEBER’S CONGENITAL AMAUROSIS (LCA) IS A FORM OF RETINAL DEGENERATION...
TIVE DISEASE, CHARACTERIZED BY SEVERE EARLY-ON SET BLINDNESS. THIS DIS-
EASE, NOT CURRENTLY TREATABLE, IS CAUSED BY MUTATIONS IN SEVERAL
DIFFERENT GENES: ~15% OF CASES OF LCA ARE DUE TO A MUTATION IN A GENE, RPE65,
ENCODING A RETINAL PIGMENT EPITHELIAL PROTEIN. IN DOGS WITH A NULL MU-
TATION IN RPE65, SIGHT HAS BEEN RESTORED AFTER SUBRETINAL INJECTION OF AN
AAV VECTOR EXPRESSING RPE65. TRANSGENE EXPRESSION APPEARS TO BE STA-
BLE, WITH THE FIRST ANIMALS TREATED >5 YEARS AGO CONTINUING TO MANIFEST
ELECTROPHYSIOLOGIC AND BEHAVIORAL EVIDENCE OF VISUAL FUNCTION. AS IS
THE CASE FOR X-LINKED SCID, GENE TRANSFER MUST OCCUR RELATIVELY EARLY IN
LIFE TO ACHIEVE CORRECTION OF THE GENETIC DISEASE,ALTHOUGH THE EXACT
LIMITA-
TIONS IMPOSED BY AGE AWAIT CLINICAL STUDIES. AAV-RPE65 TRIALS HAVE
NOW BEEN APPROVED IN BOTH THE UNITED STATES AND GREAT BRITAIN. OTHER
INHERITED RETINAL DEGENERATIVE DISORDERS MAY ALSO BE AMENABLE TO COR-
RECTION BY GENE TRANSFER, AS ARE CERTAIN COMPLEX ACQUIRED DISORDERS
SUCH AS AGE-RELATED MACULAR DEGENERATION, WHICH AFFECTS SEVERAL
MILLION PEOPLE WORLDWIDE. THE NEOVASCULARIZATION THAT OCCURS IN AGE-RELATED
MACULAR DEGENERATION CAN BE INHIBITED BY EXPRESSION OF VASCULAR ENDO-
THELIAL GROWTH FACTOR (VEGF) INHIBITORS SUCH AS ANGIOSTATIN, OR
THROUGH THE USE OF RNAI-MEDIATED KNOCKDOWN OF VEGF. EARLY-PHASE
TRIALS OF SIRNAS THAT TARGET VEGF RNA ARE UNDERWAY, BUT THESE REQUIRE
REPEATED INTRAVITREAL INJECTION OF THE SIRNAS; AN AAV VECTOR-MEDIATED
APPROACH MIGHT ALLOW LONG-TERM KNOCKDOWN OF VEGF.

GENE THERAPY FOR CANCER

THE MAJORITY OF CLINICAL GENE TRANSFER EXPERIENCE HAS BEEN IN SUBJECTS
WITH CANCER (FIG. 65-1). AS A GENERAL RULE, A FEATURE THAT DISTINGUISHES
GENE THERAPIES FROM CONVENTIONAL CANCER THERAPEUTICS IS THAT THE
FORMER ARE LESS TOXIC, IN SOME CASES BECAUSE THEY ARE DELIVERED
LOCALLY (E.G., INTRATUMORAL INJECTIONS), AND IN OTHER CASES BECAUSE THEY ARE TAR-
GETED SPECIFICALLY TO ELEMENTS OF THE TUMOR (IMMUNOTHERAPIES, ANTI-
ANGIOGENIC APPROACHES).

CANCER GENE THERAPIES CAN BE DIVIDED INTO LOCAL AND SYSTEMIC AP-
PROACHES (TABLE 65-2). SOME OF THE EARLIEST CANCER GENE THERAPY TRIALS
FOCUSED ON LOCAL DELIVERY OF A PRODRUG OR A SUICIDE GENE THAT WOULD
IN-
CREASE SENSITIVITY OF TUMOR CELLS TO CYTOTOXIC DRUGS. A FREQUENTLY
USED
STRATEGY HAS BEEN INTRATUMORAL INJECTION OF AN ADENOVIRAL VECTOR EX-
PRESSING THE THYMIDINE KINASE (TK) GENE. CELLS THAT TAKE UP AND EX-
PRESS THE TK GENE CAN BE KILLED AFTER THE ADMINISTRATION OF
GANCYCLOVIR,
WHICH IS PHOSPHORYLATED TO A TOXIC NUCLEOSIDE BY TK. BECAUSE CELL DIVI-
SION IS REQUIRED FOR THE TOXIC NUCLEOSIDE TO AFFECT CELL VIABILITY, THIS
STRATEGY WAS INITIALLY USED IN AGGRESSIVE BRAIN TUMORS (GLIOBLASTOMA
MULTIFORME) WHERE THE CYCLING TUMOR CELLS WERE AFFECTED BUT THE NON-
DIVIDING NORMAL NEURONS WERE NOT. MORE RECENTLY, THIS APPROACH HAS
BEEN EXPLORED FOR LOCALLY RECURRENT PROSTATE, BREAST, AND COLON
TUMORS, AMONG OTHERS. ANOTHER LOCAL APPROACH USES ADENOVIRAL-MEDIATED EXPRESSION OF THE TUMOR SUPPRESSOR P53, WHICH IS MUTATED IN A WIDE VARIETY OF CANCERS. THIS STRATEGY HAS SHOWN COMPLETE AND PARTIAL RESPONSES IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK, ESOPHAGEAL CANCER, AND NON-SMALL CELL LUNG CANCER AFTER DIRECT INTRATUMORAL INJECTION OF THE VECTOR. RESPONSE RATES (~15%) ARE COMPARABLE TO THOSE OF OTHER SINGLE AGENTS. THE USE OF ONCOLYTIC VIRUSES THAT SELECTIVELY REPLICATE IN TUMOR CELLS BUT NOT IN NORMAL CELLS HAS ALSO SHOWN PROMISE IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK AND IN OTHER SOLID TUMORS. THIS APPROACH IS BASED ON THE OBSERVATION THAT DELETION OF CERTAIN VIRAL GENES ABOLISHES THEIR ABILITY TO REPLICATE IN NORMAL CELLS BUT NOT IN TUMOR CELLS. AN ADVANTAGE OF THIS STRATEGY IS THAT THE REPLICATING VECTOR CAN PROLIFERATE AND SPREAD WITHIN THE TUMOR, FACILITATING EVENTUAL TUMOR CLEARANCE. HOWEVER, PHYSICAL LIMITATIONS TO VIRAL SPREAD, INCLUDING FIBROSIS, INTERMIXED NORMAL CELLS, BASEMENT MEMBRANES, AND NECROTIC AREAS WITHIN THE TUMOR MAY REDUCE CLINICAL EFFICACY. ONCOLYTIC VIRUSES ARE LICENSED AND AVAILABLE IN SOME COUNTRIES, BUT NOT IN THE UNITED STATES.

BECAUSE METASTATIC DISEASE RATHER THAN UNCONTROLLED GROWTH OF THE PRIMARY TUMOR IS THE SOURCE OF MORTALITY FOR MOST CANCERS, THERE HAS BEEN CONSIDERABLE INTEREST IN DEVELOPING SYSTEMIC GENE THERAPY APPROACHES. ONE STRATEGY HAS BEEN TO PROMOTE MORE EFFICIENT RECOGNITION OF TUMOR CELLS BY THE IMMUNE SYSTEM. APPROACHES HAVE INCLUDED TRANSDUCTION OF TUMOR CELLS WITH IMMUNE-ENHANCING GENES ENCODING CYTOKINES, CHEMOKINES, OR CO-STIMULATORY MOLECULES. SUSTAINED CLINICAL RESPONSES PROVIDE EVIDENCE THAT THE TRANSDUCED CELLS CAN ACT AS A VACCINE. IN A RELATED APPROACH, PATIENT LYMPHOCYTES HAVE BEEN TRANS-
DUCED WITH GENES ENCODING A T CELL RECEPTOR-LIKE MOLECULE, WITH A TUMOR ANTIGEN-BINDING DOMAIN FUSED TO AN INTRACELLULAR SIGNALING DOMAIN TO ALLOW T CELL ACTIVATION, THEREBY CONVERTING NORMAL LYMPHOCYTES INTO CELLS CAPABLE OF RECOGNIZING AND DESTROYING TUMOR CELLS. A THIRD IMMUNOTHERAPY APPROACH RELIES ON EX VIVO MANIPULATION OF DENDRITIC CELLS TO ENHANCE THE PRESENTATION OF TUMOR ANTIGENS. THESE IMMUNOLOGIC APPROACHES MAY BE OF PARTICULAR VALUE IN TREATING MINIMAL RESIDUAL DISEASE AFTER OTHER ANTICANCER MODALITIES. GENE TRANSFER STRATEGIES HAVE ALSO BEEN DEVELOPED FOR INHIBITING TUMOR ANGIogenesis. THESE HAVE INCLUDED CONSTITUTIVE EXPRESSION OF ANGIogeneSIS INHIBITORS SUCH AS ANGIOSTATIN AND ENDOSTATIN; USE OF SIRNA TO REDUCE LEVELS OF VEGF OR VEGF RECEPTOR; AND COMBINED APPROACHES IN WHICH AUTOLOGOUS T CELLS ARE GENETICALLY MODIFIED TO RECOGNIZE ANTIGENS SPECIFIC TO TUMOR VAScULATURE. THESE STUDIES ARE STILL IN EARLY-PHASE TESTING. ANOTHER NOVEL SYSTEMIC APPROACH IS THE USE OF GENE TRANSFER TO PROTECT NORMAL CELLS FROM THE TOXICITIES OF CHEMOTHERAPY. THE MOST EXTENSIVELY STUDIED OF THESE APPROACHES HAS BEEN TRANSDUCTION OF HEMATOPOIETIC CELLS WITH GENES ENCODING RESISTANCE TO CHEMOTHERAPEUTIC AGENTS, INCLUDING THE MULTIDRUG RESISTANCE GENE MDRI OR THE GENE ENCODING 03-6-METHYLGuanINE DNA METHYLTRANSFERase (MGMT). EX VIVO TRANSDUCTION OF HEMATOPOIETIC CELLS, FOLLOWED BY AUTOLOGOUS TRANSPLANTATION, IS BEING INVESTIGATED AS A STRATEGY FOR ALLOWING ADMINISTRATION OF HIGHER DOSES OF CHEMOTHERAPY THAN WOULD OTHERWISE BE TOLERATED.

GENE THERAPy FOR VASCULAR DISEASE

THE THIRD MAJOR CATEGORY ADDRESSED BY GENE TRANSFER STUDIES IS CARDIOVASCULAR DISEASE. THE MOST EXTENSIVE EXPERIENCE HAS BEEN IN TRIALS DESIGNED TO INCREASE BLOOD FLOW TO EITHER SKELETAL (CRITICAL LIMB ISCHEMIA) OR CARDiac MUSCLE (ANGINA/MYOCARDIAL ISCHEMIA). INITIAL TREATMENT OPTIONS FOR BOTH OF THESE GROUPS INCLUDE MECHANICAL REVAScULIZATION OR MEDICAL MANAGEMENT, BUT A SUBSET OF PATIENTS ARE NOT CANDIDATES FOR, OR FAIL, THESE APPROACHES. THESE PATIENTS HAVE FORMED THE FIRST COHORTS FOR EVALUATION OF GENE TRANSFER TO ACHIEVE THERAPEUTIC ANGIogenesis. THE MAJOR TRANSGENE USED HAS BEEN VEGF, ATTRACTIVE BECAUSE OF ITS SPECIFICITY FOR ENDOTHELIAL CELLS; OTHER TRANSGENES HAVE INCLUDED FIBROBLAST GROWTH FACTOR (FGF) AND HYPOXIA-INDUCIBLE FACTOR 1, * SUBUNIT (HIF-1*). THE DESIGN OF MOST OF THE TRIALS HAS INCLUDED DIRECT IM (OR MYOCARDIAL) INJECTION OF EITHER A PLASMID OR AN ADENOVIRAL VECTOR EXPRESSING
THE TRANSGENE. BOTH OF THESE VECTORS ARE LIKELY TO RESULT IN ONLY SHORT-TERM EXPRESSION OF VEGF. THIS STRATEGY MAY BE ADEQUATE, HOWEVER, AS THERE IS NO NEED FOR CONTINUED TRANSGENE EXPRESSION ONCE THE NEW VESELS HAVE FORMED. DIRECT INJECTION FAVORS LOCAL EXPRESSION, WHICH SHOULD HELP TO AVOID SYSTEMIC EFFECTS SUCH AS RETINAL NEOVASCULARIZATION OR NEW VESSEL FORMATION IN A NASCENT TUMOR. INITIAL TRIALS OF ADENO-VEGF OR PLASMID-VEGF INJECTION HAVE RESULTED IN IMPROVEMENT OVER BASELINE IN TERMS OF FREQUENCY OF CLAUDICATION/ANGINA OR AMOUNTS OF NITROGLYCERIN CONSUMPTION. STUDY DESIGNS INCLUDING PLACEBO CONTROL GROUPS AND MORE OBJECTIVE ENDPOINTS (EXERCISE DURATION AT 3 OR 6 MONTHS, REST AND STRESS CARDIAC PERFUSION SCANS, AND REGIONAL WALL MOTION ASSESSED BY NONFLUOROSCOPIC ELECTROANATOMIC MAPPING) CONTINUE TO SUGGEST A BENEFICIAL EFFECT OF GENE TRANSFER, ALTHOUGH DEFINITIVE CONCLUSIONS WILL REQUIRE LARGER STUDIES. CONTINUING AREAS OF INVESTIGATION INCLUDE CHOICE OF THE OPTIMAL VECTOR (ADENOVIRAL VS. PLASMID), THE OPTIMAL TRANSGENE (VEGF, HIF-1*, FGF, ETC.), THE OPTIMAL METHOD OF DELIVERY IN CARDIAC INDICATIONS (INTRACORONARY VS. DIRECT MYOCARDIAL), IDEAL OBJECTIVE ENDPOINTS, AND WHETHER CONCURRENT ADMINISTRATION OF CYTOKINES TO MOBILIZE ENDOTHELIAL PROGENITOR CELLS WILL AUGMENT THE THERAPEUTIC EFFECT.

TABLE 65-3 TAKING HISTORY FROM SUBJECTS ENROLLED IN GENE TRANSFER STUDIES

ELEMENTS OF HISTORY FOR SUBJECTS ENROLLED IN GENE TRANSFER TRIALS

1. WHAT VECTOR WAS ADMINISTERED? IS IT PREDOMINANTLY INTEGRATING [RETROVIRAL, LENTIVIRAL, HERPESVIRUS (LATENCY AND REACTIVATION)], OR NON-INTEGRATING (PLASMID, ADENOVIRAL, AAV)?
2. WHAT WAS THE ROUTE OF ADMINISTRATION OF THE VECTOR?
3. WHAT WAS THE TARGET TISSUE?
4. WHAT GENE WAS TRANSFERRED IN? A DISEASE-RELATED GENE? A MARKER?
5. WERE THERE ANY ADVERSE EVENTS NOTED AFTER GENE TRANSFER?

SCREENING QUESTIONS FOR LONG-TERM FOLLOW-UP IN GENETRANSFER SUBJECTS###A

1. HAS A NEW MALIGNANCY BEEN DIAGNOSED?
2. HAS A NEW NEUROLOGIC/OPHTHALMOLOGIC DISORDER, OR EXACERBATION OF A PRE-EXISTING DISORDER, BEEN DIAGNOSED?
3. HAS A NEW AUTOIMMUNE OR RHEUMATOLOGIC DISORDER BEEN DIAGNOSED?
4. HAS A NEW HEMATOLOGIC DISORDER BEEN DIAGNOSED?

###AFACTORS INFLUENCING LONG-TERM RISK INCLUDE: INTEGRATION OF THE VECTOR INTO THE GENOME; VECTOR PERSISTENCE WITHOUT INTEGRATION, AND TRANSGENE-SPECIFIC EFFECTS.

OTHER DISEASES
THE POWER AND VERSATILITY OF GENE TRANSFER APPROACHES ARE SUCH THAT THERE ARE FEW SERIOUS DISEASE ENTITIES FOR WHICH GENE TRANSFER THERAPIES ARE NOT UNDER DEVELOPMENT. BESIDES THOSE ALREADY DISCUSSED, OTHER AREAS OF INTEREST INCLUDE GENE THERAPIES FOR HIV AND FOR NEURODEGENERATIVE DISORDERS. THE LATTER INCLUDE STUDIES IN PATIENTS WITH PARKINSON’S DISEASE, WHERE AAV VECTORS EXPRESSING ENZYMES REQUIRED FOR ENHANCED PRODUCTION OF DOPAMINE, OR OF THE INHIBITORY NEUROTRANSMITTER *-AMINOBUTYRIC ACID, HAVE BEEN INTRODUCED INTO AFFECTED AREAS OF THE BRAIN (STRIATUM, SUBTHALAMIC NUCLEUS) BY STEREOTACTIC NEUROSURGERY. IN ALZHEIMER’S DISEASE, AN EX VIVO APPROACH IN WHICH AUTOLOGOUS FIBROBLASTS ARE TRANSDUCED WITH A RETROVIRAL VECTOR EXPRESSING NERVE GROWTH FACTOR, THEN REIMPLANTED INTO THE BASAL FOREBRAIN, HAS SLOWED THE RATE OF COGNITIVE DECLINE IN A SMALL PHASE I STUDY.

SUMMARY

THE DEVELOPMENT OF NEW CLASSES OF THERAPEUTICS TYPICALLY TAKES TWO TO THREE DECADES; MONOCLONAL ANTIBODIES AND RECOMBINANT PROTEINS ARE RECENT EXAMPLES. GENE THERAPEUTICS, WHICH ENTERED CLINICAL TESTING IN THE EARLY 1990S, ARE WELL ALONG IN THE COURSE OF DEVELOPMENT, AND ARE LIKELY TO BECOME INCREASINGLY IMPORTANT AS A THERAPEUTIC MODALITY IN THE TWENTY-FIRST CENTURY. A CENTRAL QUESTION TO BE ADDRESSED IS THE LONG-TERM SAFETY OF GENE TRANSFER, AND REGULATORY AGENCIES HAVE MANDATED A 15-YEAR FOLLOW-UP FOR SUBJECTS ENROLLED IN GENE THERAPY TRIALS (TABLE 65-3). REALIZATION OF THE THERAPEUTIC BENEFITS OF THE HUMAN GENOME PROJECT, AND OF NEW DISCOVERIES SUCH AS RNAI, WILL DEPEND ON CONTINUED PROGRESS IN GENE TRANSFER TECHNOLOGY.

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FURTHER READINGS

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MANNO CS ET AL: SUCCESSFUL TRANSDUCTION OF LIVER IN HEMOPHILIA BY AAV-FACTOR IX AND LIMITATIONS IMPOSED BY THE HOST IMMUNE RESPONSE. NAT MED 12:342, 2006
CHAPTER 66 STEM CELL BIOLOGY

PART 4: REGENERATIVE MEDICINE

66 STEM CELL BIOLOGY
MINORU S. H. KO

STEM CELL BIOLOGY IS A RELATIVELY NEW FIELD THAT EXPLORES THE CHARACTERISTICS AND POSSIBLE CLINICAL APPLICATIONS OF THE DIFFERENT TYPES OF PLURIPOTENTIAL CELLS THAT SERVE AS THE PROGENITORS OF MORE DIFFERENTIATED CELL TYPES. IN ADDITION TO POTENTIAL THERAPEUTIC APPLICATIONS (CHAP. 67), PATIENT-DERIVED STEM CELLS CAN ALSO PROVIDE DISEASE MODELS AND A MEANS TO TEST DRUG EFFECTIVENESS.

IDENTIFICATION, ISOLATION, AND DERIVATION OF STEM CELLS

RESIDENT STEM CELLS  THE DEFINITION OF STEM CELLS REMAINS ELUSIVE. STEM CELLS WERE ORIGINALLY POSTULATED AS UNSPECIFIED OR UNDIFFERENTIATED CELLS THAT PROVIDE A SOURCE OF RENEWAL OF SKIN, INTESTINE, AND BLOOD CELLS THROUGHOUT THE LIFESPAN. THESE RESIDENT STEM CELLS ARE NOW IDENTIFIED IN A VARIETY OF ORGANS, I.E., EPITHELIA OF THE SKIN AND DIGESTIVE SYSTEM, BONE MARROW, BLOOD VESSELS, BRAIN, SKELETAL MUSCLE, LIVER, TESTIS, AND PANCREAS, BASED ON THEIR SPECIFIC LOCATIONS, MORPHOLOGY, AND BIOCHEMICAL MARKERS.

ISOLATED STEM CELLS  UNEQUIVOCAL IDENTIFICATION OF STEM CELLS REQUIRES THE SEPARATION AND PURIFICATION OF CELLS, USUALLY BASED ON A COMBINATION OF SPECIFIC CELL-SURFACE MARKERS. THESE ISOLATED STEM CELLS, E.G., HEMATOPOIETIC STEM (HS) CELLS, CAN BE STUDIED IN DETAIL AND USED IN CLINICAL APPLICATIONS, SUCH AS BONE MARROW TRANSPLANTATION (CHAP. 68). HOWEVER, THE LACK OF SPECIFIC CELL-SURFACE MARKERS FOR OTHER TYPES OF STEM CELLS HAS MADE IT DIFFICULT TO ISOLATE THEM IN LARGE QUANTITIES. THIS
CHALLENGE HAS BEEN PARTIALLY ADDRESSED IN ANIMAL MODELS BY GENETICALLY MARKING DIFFERENT CELL TYPES WITH GREEN FLUORESCENCE PROTEIN DRIVEN BY CELL-SPECIFIC PROMOTERS. ALTERNATIVELY, PUTATIVE STEM CELLS HAVE BEEN ISOLATED FROM A VARIETY OF TISSUES AS SIDE POPULATION (SP) CELLS USING FLUORESCENCE-ACTIVATED CELL SORTING AFTER STAINING WITH HOECHST 33342 DYE. HOWEVER, THE SP PHENOTYPE SHOULD BE USED WITH CAUTION AS IT MAY NOT BE FUNCTION FOR STEM CELLS.

CULTURED STEM CELLS IT IS DESIRABLE TO CULTURE AND EXPAND STEM CELLS IN VITRO TO OBTAIN A SUFFICIENT QUANTITY FOR ANALYSIS AND POTENTIAL THERAPEUTIC USE. ALTHOUGH THE DERIVATION OF STEM CELLS IN VITRO HAS BEEN A MAJOR OBSTACLE IN STEM CELL BIOLOGY, THE NUMBER AND TYPES OF CULTURED STEM CELLS HAVE INCREASED PROGRESSIVELY (TABLE 66-1). THE CULTURED STEM CELLS DERIVED FROM RESIDENT STEM CELLS ARE OFTEN CALLED ADULT STEM CELLS TO INDICATE THEIR ADULT ORIGINS AND TO DISTINGUISH THEM FROM EMBRYONIC STEM (ES) AND EMBRYONIC GERM (EG) CELLS. HOWEVER, CONSIDERING THE PRESENCE OF EMBRYO-DERIVED TISSUE-SPECIFIC STEM CELLS, E.G., TROPHOBLAST STEM (TS) CELLS, AND THE POSSIBLE DERIVATION OF SIMILAR CELLS FROM EMBRYO/FETUS, E.G., NEURAL STEM (NS) CELLS, IT IS MORE APPROPRIATE TO USE THE TERM, TISSUE STEM CELLS.

SUCCESSFUL DERIVATION OF CULTURED STEM CELLS (BOTH EMBRYONIC AND TISSUE STEM CELLS) OFTEN REQUIRES THE IDENTIFICATION OF NECESSARY GROWTH FACTORS AND CULTURE CONDITIONS, MIMICKING THE MICROENVIRONMENT OR NICHE OF THE RESIDENT STEM CELLS. FOR EXAMPLE, THE DERIVATION OF MOUSE TS CELLS, ONCE CONSIDERED IMPOSSIBLE, BECAME POSSIBLE BY USING FGF4, A LIGAND KNOWN TO BE EXPRESSED BY CELLS ADJACENT TO THE DEVELOPING TROPHOBLAST IN VIVO. THEREFORE, IT MAY BE POSSIBLE TO CULTURE OTHER RESIDENT STEM CELLS (E.G., INTESTINAL STEM CELLS) OR ISOLATED STEM CELLS (E.G., HS CELLS) BY STUDYING THE FACTORS THAT CONSTITUTE THEIR NORMAL NICHE.

SELF-RENEWAL AND PROLIFERATION OF STEM CELLS

SYMMETRIC ANDASYMMETRIC CELL DIVISION THE MOST WIDELY ACCEPTED STEM CELL DEFINITION IS A CELL WITH A UNIQUE CAPACITY TO PRODUCE UNALTERED DAUGHTER CELLS (SELF-RENEWAL) AND TO GENERATE SPECIALIZED CELL TYPES (POTENCY). SELF-RENEWAL CAN BE ACHIEVED IN TWO WAYS. ASYMMETRIC CELL DIVISION PRODUCES ONE DAUGHTER CELL THAT IS IDENTICAL TO THE PARENTAL CELL AND ONE DAUGHTER CELL THAT IS DIFFERENT FROM THE PARENTAL CELL AND IS A PROGENITOR OR DIFFERENTIATED CELL. ASYMMETRIC CELL DIVISION DOES NOT INCREASE THE NUMBER OF STEM CELLS. SYMMETRIC CELL DIVISION PRODUCES TWO IDENTICAL DAUGHTER CELLS. FOR STEM CELLS TO PROLIFERATE IN VITRO, THEY MUST DIVIDE SYMMETRICALLY. SELF-RENEWAL ALONE CANNOT DEFINE STEM CELLS,
BE-CAUSE ANY ESTABLISHED CELL LINE, E.G., HEla CELLS OR NIH3T3 CELLS, PROLIF-ERATE BY SYMMETRIC CELL DIVISION.

UNLIMITED EXPANSION IN VITRO RESIDENT STEM CELLS ARE OFTEN QUIESCENT AND DIVIDE INFREQUENTLY. HOWEVER, ONCE THE STEM CELLS ARE SUCCESSFULLY CULTURED IN VITRO, THEY OFTEN ACQUIRE THE CAPACITY TO DIVIDE CONTINUOUSLY AND THE ABILITY TO PROLIFERATE BEYOND THE NORMAL LIMIT OF PASSAGES TYPICAL OF PRIMARY CULTURED CELLS (SOMETIMES CALLED IMMORTALITY). THESE FEATURES ARE PRIMARILY SEEN IN ES CELLS, BUT HAVE ALSO BEEN DEMONSTRATED FOR NS CELLS, MS CELLS, MAPCs, MAGSCS (ADULT-DERIVED TISSUE STEM CELLS), AND USSCS (NEWBORN-DERIVED TISSUE STEM CELLS), THEREBY ENHANCING THE POTENTIAL OF THESE CELLS FOR THERAPEUTIC USE (TA- BLE 66-1).

STABILITY OF GENOTYPE AND PHENOTYPE THE CAPACITY TO ACTIVELY PROLIF-ERATE IS ASSOCIATED WITH THE POTENTIAL ACCUMULATION OF CHROMOSOMAL ABNORMALITIES AND MUTATIONS. MOUSE ES CELLS HAVE BEEN EXTENSIVELY USED TO PRODUCE GENE-TARGETED ANIMALS AND ARE KNOWN TO MAINTAIN THEIR EUPLOID KARYOTYPE AND GENOME INTEGRITY. IN CONTRAST, HUMAN ES CELLS APPEAR TO BE MORE SUSCEPTIBLE TO MUTATIONS AFTER LONG-TERM CUL-TURE. ANOTHER LIMITATION IS THE POSSIBLE FORMATION OF TUMORS AFTER TRANSPLANTING ACTIVELY DIVIDING STEM CELLS. MOUSE ES CELLS CAN FORM TER-ATOMAS WHEN INJECTED INTO IMMUNOSUPPRESSED ANIMALS.

POTENCY AND DIFFERENTIATION OF STEM CELLS

DEVELOPMENTAL POTENCY THE TERM POTENCY IS USED TO INDICATE A CELL’S ABILITY TO DIFFERENTIATE INTO SPECIALIZED CELL TYPES. THE CURRENT LACK OF KNOWLEDGE ABOUT THE MOLECULAR NATURE OF POTENCY REQUIRES THE EXPERIMEN-TAL MANIPULATION OF STEM CELLS TO DEMONSTRATE THEIR POTENCY. FOR EXAMPLE, IN VIVO TESTING CAN BE DONE BY INJECTING STEM CELLS INTO MOUSE BLASTOCYSTS OR IMMUNOSUPPRESSED ADULT MICE AND DETERMINING HOW MANY DIFFERENT CELL TYPES ARE FORMED FROM THE INJECTED CELLS. IN VITRO TESTING CAN BE DONE BY DIF-FERENTIATING CELLS IN VARIOUS CULTURE CONDITIONS TO DETERMINE HOW MANY DIFFERENT CELL TYPES ARE FORMED FROM THE CELLS. THE IN VIVO ASSAYS ARE NOT APPLICABLE TO HUMAN STEM CELLS. THE FORMAL DEMONSTRATION OF SELF-RENEWAL AND POTENCY IS PERFORMED BY DEMONSTRATING THAT A SINGLE CELL POSSESSES SUCH ABILITIES IN VITRO (CLONALITY). CULTURED STEM CELLS ARE TENTATIVELY GROUPED ACCORDING TO THEIR POTENCY (FIG.66-1).

FROM TOTIPOTENCY TO UNIPOTENCY TOTIPOTENT CELLS CAN FORM AN ENTIRE OR-
GANISM AUTONOMOUSLY. ONLY A FERTILIZED EGG (ZYGOTE) POSSESSES THIS FEATURE. PLURIPOTENT CELLS (E.G., ES CELLS) CAN FORM ALMOST ALL THE BODY’S CELL LINEAGES (ENDODERM, MESODERM, AND ECTODERM), INCLUDING GERM CELLS. MULTIPOTENT CELLS (E.G., HS CELLS) CAN FORM MULTIPLE CELL LINEAGES BUT CANNOT FORM ALL OF THE BODY’S CELL LINEAGES. OLIGOPOTENT CELLS (E.G., NS CELLS) CAN FORM MORE THAN ONE CELL LINEAGE BUT ARE MORE RESTRICTED THAN MULTIPOTENT CELLS. OLIGOPOTENT CELLS ARE SOMETIMES CALLED PROGENITOR CELLS OR PRECURSOR CELLS; HOWEVER, THESE TERMS ARE OFTEN MORE STRICTLY USED TO DESIGNATE PARTIALLY DIFFERENTIATED OR LINEAGE-COMMITTED CELLS (E.G., MYELOID PROGENITOR CELLS) THAT CAN DIVIDE INTO DIFFERENT CELL TYPES BUT LACK SELF-RENEWING CAPACITY. UNIPOTENT CELLS OR MONOPOTENT CELLS, E.G., SPERMATOGONIAL STEM (SS) CELLS, CAN FORM A SINGLE DIFFERENTIATED CELL LINEAGE. TERMINALLY DIFFERENTIATED CELLS, SUCH AS FIBROBLAST CELLS, ALSO HAVE A CAPACITY TO PROLIFERATE (WHICH MAY BE CALLED SELF-RENEWAL) BUT MAINTAIN THE SAME CELL TYPE (E.G., NO POTENCY TO FORM ANOTHER CELL TYPE) AND ARE NOT, THEREFORE, CONSIDERED UNIPOTENT CELLS.


426 PART 4: REGENERATIVE MEDICINE

TABLE 66-1 TYPES OF CULTURED STEM CELLS

NAME

EMBRYONIC STEM CELLS (ES, ESC)
**SOURCE, DERIVATION, MAINTENANCE, AND PROPERTIES**

ES CELLS CAN BE DERIVED BY CULTURING BLASTOCYSTS OR IMMUNO-SURGICALLY ISOLATED INNER CELL MASS (ICM) FROM BLASTOCYSTS ON A FEEDER LAYER OF MEFS WITH LIF (M) OR WITHOUT LIF (H). ES CELLS ARE TO ORIGINATE FROM THE EPIBLAST (M, H). ES CELLS GROW AS TIGHTLY ADHERENT MULTICELLULAR COLONIES WITH A POPULATION DOUBLING TIME OF ~ 12 H (M), MAINTAIN A STABLE EUPOID KARYOTYPE EVEN AFTER EXTENSIVE CULTURE AND MANIPULATION, CAN DIFFERENTIATE INTO A VARIETY OF CELL TYPES IN VITRO, AND CAN CONTRIBUTE TO ALL CELL TYPES, INCLUDING FUNCTIONAL SPERM AND OOCYTES, WHEN INJECTED INTO A BLASTOCYST (M). ES CELLS FORM RELATIVELY FLAT, COMPACT COLONIES WITH THE POPULATION DOUBLING TIME OF 35-40 H (H).
EG CELLS CAN BE DERIVED BY CULTURING PRIMORDIAL GERM CELLS (PGCS) FROM EMBRYOS AT E8.5-E12.5 ON A FEEDER LAYER OF MEFS WITH FGF2 AND LIF (M). EG CELLS CAN BE DERIVED BY CULTURING GONADAL TISSUES FROM 5-11 WEEK POST-FERTILIZATION EMBRYO /FETUS ON A FEEDER LAYER OF MEFS WITH FGF2, FORSKOLIN, AND LIF (H). EG CELLS SHOW ESSENTIALLY THE SAME PLURIPOTENCY AS ES CELLS WHEN INJECTED INTO MOUSE BLASTOCYSTS (M). THE ONLY KNOWN DIFFERENCE IS THE IMPRINTING STATUS OF SOME GENES (E.G., IGF2R): IMPRINTING IS NORMALLY ERASED DURING GERMLINE DEVELOPMENT, AND THUS, THE IMPRINTING STATUS OF IN EG CELLS IS DIFFERENT FROM THAT OF ES CELLS.

TS CELLS CAN BE DERIVED BY CULTURING TROPHECTODERM CELLS OF E3.5 BLASTOCYSTS, EXTRAEMBRYONIC ECTODERM OF E6.5 EMBRYOS, AND CHORIONIC ECTODERM OF E7.5 EMBRYOS ON A LAYER OF MEFS WITH FGF4 (M). TS CELLS CAN DIFFERENTIATE INTO TROPHOBLAST GIANT CELLS IN VITRO (M). TS CAN CONTRIBUTE EXCLUSIVELY TO ALL TROPHOBLAST SUBTYPES WHEN INJECTED INTO BLASTOCYSTS (M).

XENCELLS CAN BE DERIVED BY CULTURING THE ICM IN NON-ES CELL CULTURE CONDITION (M). XEN CELLS CAN CONTRIBUTE ONLY TO THE PARIETAL ENDODERM LINEAGE WHEN INJECTED INTO A BLASTOCYST (M).

EC CELLS CAN BE DERIVED FROM TERATOCARCINOMA-A TYPE OF CANCER THAT MOST COMMONLY DEVELOPS IN THE TESTES. EC CELLS RARELY SHOW PLURIPOTENCY IN VITRO, BUT THEY CAN CONTRIBUTE TO ALL CELL TYPES WHEN INJECTED INTO BLASTOCYSTS. EC CELLS OFTEN HAVE AN ANEUPLOID KARYOTYPE AND OTHER GENOME ALTERATIONS.

MS CELLS CAN BE DERIVED FROM BONE MARROW, MUSCLE, ADIPOSE TISSUE, PERIPHERAL BLOOD, AND UMBILICAL CORD BLOOD (M, H). MS CELLS CAN DIFFERENTIATE INTO MESENCHYMAL CELL TYPES, INCLUDING ADIPOCYTES, OSTEOCYTES, CHONDROCYTES, AND MYOCYTES (M, H).

MAPCS CAN BE DERIVED BY CULTURING BONE MARROW MONONUCLEAR CELLS, AFTER DEPLETING CD45#/# AND GLYA#/# CELLS, WITH FCS, EGF, AND PDGF-BB (H). MAPCS ARE VERY RARE CELLS THAT ARE PRESENT WITHIN MSC CULTURES FROM POSTNATAL BONE MARROW (M, H). MAPCS CAN ALSO BE ISOLATED FROM POSTNATAL MUSCLE AND BRAIN (M). MAPCS CAN BE CULTURED FOR >120 POPULATION DOUBLINGS. MAPCS CAN DIFFERENTIATE INTO ALL TISSUES IN VIVO WHEN INJECTED INTO A MOUSE BLASTOCYST, AND CAN DIFFERENTIATE INTO VARIOUS CELL LINEAGES OF MESODERMAL, ECTODERMAL, AND ENDODERMAL ORIGIN IN VITRO (M).

SS CELLS CAN BE DERIVED BY CULTURING NEWBORN TESTIS ON STS-FEEDER CELLS WITH GDNF (M). SS CELLS CAN RECONSTITUTE LONG-TERM SPERMATOGENESIS AFTER TRANSPLANTATION INTO RECIPIENT TESTES AND RESTORE FERTILITY.

GS CELLS CAN BE DERIVED FROM NEONATAL TESTIS (M). GS CELLS CAN DIFFERENTIATE INTO THREE GERMLAYERS IN VITRO AND CONTRIBUTE TO A VARIETY OF TISSUES, INCLUDING GERMLINE. WHEN INJECTED INTO BLASTOCYSTS.
MAGSC can be derived from adult testis (M). MAGSC can differentiate into three germlayers in vitro and can contribute to a variety of tissues, including germline, when injected into blastocysts.

NS cells can be derived from fetal and adult brain (subventricular zone, ven-tricular zone, and hippocampus) and cultured as a heterogeneous cell population of monolayer or floating cell clusters called neurospheres. NS cells can differentiate into neuron and glia in vivo and in vitro. Recently, the culture of pure population of symmetrically dividing adherent NS cells became possible in the presence of FGF2 and EGF.

USSCS are rare cells derived from newborn cord blood (H). USSCS can be derived by culturing the mononuclear fraction of cord blood in the presence of 30% FCS and 10^{-7} M dexamethasone. USSCS can differentiate into a variety of cell types in vitro and can contribute a variety of cell types in in vivo transplantation experiments in rat, mouse, and sheep (H). USSCS are CD45- adherent cells and can be expanded to 10^{15} cells without losing pluripotency (H).

**Note:** M, mouse; H, human, FGF, fibroblast growth factor; FCS, fetal calf serum, EGF, epidermal growth factor; PDGF, platelet-derived growth factor; GDNF, glial cell line-derived neurotrophic factor; LIF, leukemia inhibitory factor; MEF, mouse embryonic fibroblast.

Dington’s epigenetic landscape, this is analogous to a ball moving down a slope. The reversal of the terminally differentiated cells to totipotent or pluripotent cells (called nuclear reprogramming) can thus be seen as an uphill gradient that never occurs in normal conditions. However, nuclear reprogramming has been achieved using nuclear transplantation, or nuclear transfer (NT), procedures (often called “cloning”), where the nucleus of a differentiated cell is transferred into an enucleated oocyte. Although this is an error-prone procedure and the success rate is very low, live animals have been produced using adult somatic cells as donors in sheep, mouse, and other mammals. In mice, it has been demonstrated that ES cells derived from blastocysts made by somatic cell NT are indistinguishable from normal ES cells. NT can potentially be used to produce patient-specific ES cells carrying a genome identical to that of the patient. However, the successful implementation of this procedure has not been reported in humans. Setting aside technical and ethical issues, the limited supply of human oocytes will be a major
PROBLEM FOR CLINICAL APPLICATIONS OF NT. ALTERNATIVELY, SUCCESSFUL NUCLEAR REPROGRAMMING OF SOMATIC CELLS BY FUSING THEM WITH ES CELLS HAS BEEN DEMONSTRATED IN MOUSE AND HUMAN. HOWEVER, IT IS NOT YET CLEAR HOW ES-DERIVED DNA CAN BE REMOVED FROM HYBRID CELLS. MORE DIRECT NUCLEAR REPROGRAMMING OF SOMATIC CELLS BY TRANSFECTING SPECIFIC GENES OR BY EXPOSING THE CELLS TO ES CELL EXTRACTS IS THE SUBJECT OF CURRENT RESEARCH.

STEM CELL PLASTICITY OR TRANSDIFFERENTIATION THE PREVAILING PARADIGM IN DEVELOPMENTAL BIOLOGY IS THAT ONCE CELLS ARE DIFFERENTIATED, THEIR PHENOTYPES ARE STABLE. HOWEVER, A NUMBER OF REPORTS HAVE SHOWN THAT TISSUE STEM CELLS, WHICH ARE THOUGHT TO BE LINEAGE-COMMITTED MULTIPO-TENT CELLS, POSSESS THE CAPACITY TO DIFFERENTIATE INTO CELL TYPES OUTSIDE THEIR LINEAGE RESTRICTIONS (CALLED TRANSDIFFERENTIATION). FOR EXAMPLE, HS CELLS MAY BE CONVERTED INTO NEURONS AS WELL AS GERM CELLS. THIS FEATURE MAY PROVIDE A MEANS TO USE TISSUE STEM CELLS DERIVED DIRECTLY FROM A PATIENT FOR THERAPEUTIC PURPOSES, THEREBY ELIMINATING THE NEED TO USE EMBRYONIC STEM CELLS OR ELABORATE PROCEDURES SUCH AS NUCLEAR REPROGRAMMING A PATIENT’S SOMATIC CELLS. HOWEVER, MORE STRICT CRITERIA AND RIGOROUS VALIDATION ARE REQUIRED TO ESTABLISH TISSUE STEM CELL PLASTICITY. FOR EXAMPLE, OBSERVATIONS OF TRANSDIFFERENTIATION MAY REFLECT CELL FUSION, CONTAMINATION WITH PROGENITOR CELLS FROM OTHER CELL LINEAGES, OR PERSISTENCE OF PLURIPOTENT EMBRYONIC CELLS IN ADULT ORGANS. THEREFORE, THE ASSIGNMENT OF POTENCY TO EACH CULTURED STEM CELL IN FIG. 66-1 SHOULD BE TAKEN WITH CAUTION. WHETHER TRANSDIFFERENTIATION EXISTS AND CAN BE USED FOR THERAPEUTIC PURPOSES REMAINS TO BE DETERMINED CONCLUSIVELY.

DIRECTED DIFFERENTIATION OF STEM CELLS PLURIPOTENT STEM CELLS (E.G., ES CELLS) CAN DIFFERENTIATE INTO MULTIPLE CELL TYPES, BUT IN CULTURE THEY NORMALLY DIFFERENTIATE INTO HETEROGENEOUS CELL POPULATIONS IN A STOCHASTIC MANNER. HOWEVER, FOR THERAPEUTIC USES, IT IS DESIRABLE TO DIRECT STEM CELLS INTO SPECIFIC CELL TYPES (E.G., INSULIN-SECRETING BETA CELLS). THIS IS AN ACTIVE AREA OF STEM CELL RESEARCH, AND PROTOCOLS ARE BEING DEVELOPED TO ACHIEVE THIS GOAL. IN ANY OF THESE DIRECTED CELL DIFFERENTIATION SYSTEMS, THE CELL PHENOTYP MUST BE EVALUATED CRITICALLY. INTERESTINGLY, IT HAS BEEN REPORTED THAT MOUSE ES CELLS CAN DIFFERENTIATE IN VITRO INTO OOCYTES AS WELL AS SPERM, WHICH ARE CAPABLE OF FERTILIZING AN OOCYTE TO PRODUCE LIVE OFFSPRING.
427 CHAPTER 67 APPLICATIONS OF STEM CELL BIOLOGY IN CLINICAL MEDICINE

FIGURE 66-1 POTENCY AND SOURCE DEVELOPMENTAL STAGE OF CULTURED STEM CELLS. FOR ABBREVIATIONS OF STEM CELLS, SEE TABLE 66-1. NOTE THAT STEM CELLS ARE OFTEN ABBREVIATED WITH OR WITHOUT “CELLS” E.G., ES CELLS OR ESCS FOR EMBRYONIC STEM CELLS. M, MOUSE; H, HUMAN.

MOLECULAR CHARACTERIZATION OF STEM CELLS

GENOMICS AND PROTEOMICS IN ADDITION TO STANDARD MOLECULAR BIOLOGICAL APPROACHES, GENOMICS AND PROTEOMICS HAVE BEEN EXTENSIVELY APPLIED TO THE ANALYSIS OF STEM CELLS. FOR EXAMPLE, DNA MICROARRAY ANALYSES HAVE REVEALED THE EXPRESSION LEVELS OF ESSENTIALLY ALL GENES AND IDENTIFIED SPECIFIC MARKERS FOR SOME STEM CELLS. SIMILARLY, THE PROTEIN PROFILES OF STEM CELLS HAVE BEEN ASSESSED BY USING MASS SPECTROMETRY. THESE METHODOLOGIES ARE BEGINNING TO PROVIDE A NOVEL MEANS TO CHARACTERIZE AND CLASSIFY VARIOUS STEM CELLS AND THE MOLECULAR MECHANISMS THAT GIVE THEM THEIR UNIQUE CHARACTERISTICS.

STEMNESS THIS TERM HAS BEEN USED TO DESIGNATE THE ESSENTIAL MOLECULAR CHARACTERISTICS OF STEM CELLS. IT IS ALSO USED TO INDICATE COMMON GE-

67 APPLICATIONS OF STEM CELL BIOLOGY IN CLINICAL MEDICINE JOHN A. KESSLER

ORGAN DAMAGE AND THE RESULTANT INFLAMMATORY RESPONSES INITIATE A SERIES OF REPAIR PROCESSES, INCLUDING STEM CELL PROLIFERATION, MIGRATION, AND DIFFERENTIATION, OFTEN IN COMBINATION WITH ANGIOGENESIS AND REMODELING OF THE EXTRACELLULAR MATRIX. ENDOGENOUS STEM CELLS IN TISSUES SUCH AS LIVER AND SKIN HAVE A REMARKABLE ABILITY TO REGENERATE THE ORGANS, WHEREAS HEART AND BRAIN HAVE A MUCH MORE LIMITED CAPABILITY FOR SELF-REPAIR. UNDER RARE CIRCUMSTANCES, CIRCULATING STEM CELLS MAY CONTRIBUTE TO REGENERATIVE RESPONSES BY MIGRATING INTO A TISSUE AND DIFFERENTIATING INTO ORGAN-SPECIFIC CELL TYPES. THE GOAL OF STEM CELL THERAPIES IS TO PROMOTE CELL REPLACEMENT IN ORGANS THAT ARE DAMAGED BEYOND THEIR ABILITY FOR SELF-REPAIR.
DIFFERENT TYPES OF STEM CELLS INCLUDE EMBRYONIC STEM (ES) CELLS, UMBILICAL CORD BLOOD STEM CELLS, ORGAN-SPECIFIC SOMATIC STEM CELLS (E.G., NEURAL STEM CELLS FOR TREATMENT OF THE BRAIN), AND SOMATIC STEM CELLS CAPABLE OF GENERATING CELL TYPES SPECIFIC FOR THE TARGET RATHER THAN THE DONOR ORGAN (E.G., BONE MARROW MESENCHYMAL STEM CELLS FOR CARDIAC REPAIR) (CHAP. 66). ES CELLS SELF-RENEW ENDLESSLY SO THAT A SINGLE CELL LINE WITH CAREFULLY CHARACTERIZED TRAITS CAN GENERATE LARGE NUMBERS OF CELLS THAT CAN BE IMMUNOLOGICALLY MATCHED WITH POTENTIAL TRANSPLANT RECIPIENTS. HOWEVER, LITTLE IS CURRENTLY KNOWN ABOUT THE MECHANISMS THAT GOVERN DIFFERENTIATION OF THESE CELLS OR PROCESSES THAT LIMIT THEIR UNBROKEN PROLIFERATION. HUMAN ES CELLS ARE DIFFICULT TO CULTURE AND GROW.

NETIC PROGRAMS SHARED AMONG ES CELLS AND TISSUE STEM CELLS (HS AND NS CELLS). A NUMBER OF COMMON GENES, SUCH AS STRESS-RESPONSE GENES, HAVE BEEN IDENTIFIED, BUT THE LACK OF COMMONALITY AMONG DIFFERENT STUDIES RAISES CONCERNS ABOUT THE VALIDITY OF THIS CONCEPT.

PIVOTAL GENES INVOLVED IN ES CELL REGULATION RECENT WORK HAS BEGUN TO IDENTIFY GENES INVOLVED IN THE REGULATION OF STEM CELL FUNCTION. FOR EXAMPLE, THREE GENES-POU5F1 (OCT3/4), NANOG, AND SOX2-GOVERN KEY GENE REGULATORY PATHWAYS/NETWORKS FOR THE MAINTENANCE OF SELF-RENEWAL AND PLURIPOTENCY OF MOUSE AND HUMAN ES CELLS. SIMILARLY, IT HAS BEEN SHOWN THAT THE INTERACTION AND BALANCE AMONG THREE TRANSCRIPTION FACTORS-POU5F1, CDX2, AND GATA6-DETERMINE THE FATE OF MOUSE ES CELLS: UPREGULATION OF CDX2 DIFFERENTIATES ES CELLS INTO TROPHOBLAST CELLS, WHEREAS UPREGULATION OF GATA6 DIFFERENTIATES ES CELLS INTO PRIMITIVE ENDODERM. THESE TYPES OF ANALYSES SHOULD PROVIDE MOLECULAR CLUES ABOUT THE FUNCTION OF STEM CELLS AND LEAD TO A MORE EFFECTIVE MEANS TO MANIPULATE STEM CELLS FOR FUTURE THERAPEUTIC USE.

FURTHER READINGS


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ODORICO J ET AL (EDS): HUMAN EMBRYONIC STEM CELLS. NEW YORK, BIOS
SLOWLY. ES CELLS TEND TO DEVELOP ABNORMAL KARYOTYPES AND HAVE THE PO-
TENTIAL TO FORM TERATOMAS IF THEY ARE NOT COMMITTED TO THE DESIRED 
CELL TYPES BEFORE TRANSPLANTATION. THE STUDY OF HUMAN ES CELLS HAS BEEN 
CONTROVERSIAL, AND THEIR USE IN CLINICAL APPLICATIONS WOULD BE 
UNACCEPTABLE TO SOME PATIENTS AND PHYSICIANS DESPITE THEIR ENORMOUS POTENTIAL. 
SOMATIC CELL NUCLEAR TRANSFER ("THERAPEUTIC CLONING") REPRESENTS AN AL-
TERNATIVE METHOD FOR CREATING ES CELL LINES THAT ARE GENETICALLY IDENTICAL 
TO THE PATIENT. IT MAY ALSO BE POSSIBLE TO DERIVE PLURIPOTENT STEM CELLS 
FROM SPERMATOGONIA IN THE ADULT HUMAN TESTIS, PROVIDING ANOTHER 
STRATEGY FOR OBTAINING GENETICALLY IDENTICAL STEM CELLS. 
UMBILICAL CORD BLOOD STEM/PROGENITOR CELLS ARE ASSOCIATED WITH LESS 
GRAFT-VERSUS-HOST DISEASE COMPARED TO MARROW STEM CELLS. THEY HAVE 
LESS HLA RESTRICTION THAN ADULT MARROW STEM CELLS, AND THEY ARE LESS 
LIKELY TO BE CONTAMINATED WITH HERPESVIRUS. HOWEVER, IT IS UNCLEAR HOW 
MANY DIFFERENT CELL TYPES THESE CELLS CAN GENERATE, AND METHODS FOR 
DIFFERENTIATING THEM INTO NONHEMATOPOIETIC PHENOTYPES ARE LARGELY LACK-
ING. THE QUANTITY OF CELLS FROM ANY SINGLE SOURCE CAN ALSO BE LIMITING. 
ORGAN-SPECIFIC MULTIPOTENT STEM CELLS ARE ALREADY SOMEWHAT SPE-
CIALIZED AND MAY BE EASIER TO INDUCE INTO DESIRED CELL TYPES. THESE 
CELLS COULD POTENTIALLY BE OBTAINED FROM THE PATIENT AND AMPLIFIED IN CUL-
TURE, THEREBY CIRCUMVENTING THE PROBLEMS ASSOCIATED WITH IMMUNE RE-
JECION. MULTIPOTENT STEM CELLS ARE RELATIVELY EASY TO HARVEST FROM BONE 
MARROW (CHAP. 68) BUT ARE MORE DIFFICULT TO ISOLATE FROM OTHER TISSUES, 
SUCH AS HEART AND BRAIN. SUBSTANTIAL EFFORTS HAVE THEREFORE BEEN 
DEVOTED TO OBTAINING MORE PLURIPOTENT STEM CELL POPULATIONS, SUCH AS BONE 
MARROW MESENCHYMAL STEM CELLS (MSCS) OR ADIPOSE STEM CELLS, FOR USE IN REGENERATIVE STRATEGIES. TISSUE CULTURE EVIDENCE SUGGESTS THAT 
THESE STEM CELL POPULATIONS ARE ABLE TO GENERATE A VARIETY OF CELL TYPES, IN-
CLUDING MYOCYTES, CHONDROCYTES, TENDON CELLS, OSTEOSTRABLTS, 
CARDIOMYO-
CYTES, ADIPOCYTES, HEPATOCYTES, AND NEURONS, THROUGH A PROCESS KNOWN AS TRANSDIFFERENTIATION. HOWEVER, IT IS UNCLEAR HOW EFFECTIVELY THESE 
DIFFERENTIATED CELLS INTEGRATE INTO ORGANS, SURVIVE, AND FUNCTION AFTER
TRANSPLANTATION IN VIVO. EARLY STUDIES OF BONE MARROW-DERIVED STEM CELLS TRANSPLANTED INTO HEART, LIVER, AND OTHER ORGANS SUGGESTED THAT THE CELLS HAD DIFFERENTIATED INTO ORGAN-SPECIFIC CELL TYPES. SUBSEQUENT STUDIES, HOWEVER, REVEALED THAT THE STEM CELLS HAD FUSED WITH CELLS RESIDENT IN THE ORGANS. FURTHER STUDIES WILL BE NECESSARY TO DETERMINE WHETHER TRANSDIFFERENTIATION OF MSCS OR OTHER STEM CELL POPULATIONS OCCURS AT A HIGH ENOUGH FREQUENCY TO BE USEFUL FOR STEM CELL REPLACEMENT THERAPY.

REGARDLESS OF THE SOURCE OF THE STEM CELLS USED IN REGENERATIVE STRATEGIES, A NUMBER OF GENERIC PROBLEMS MUST BE OVERCOME FOR THE DEVELOPMENT OF SUCCESSFUL CLINICAL APPLICATIONS. THESE INCLUDE DEVELOPMENT OF METHODS FOR RELIABLY GENERATING LARGE NUMBERS OF SPECIFIC CELL TYPES, MINIMIZING THE RISK OF TUMOR FORMATION OR PROLIFERATION OF INAPPROPRIATE CELL TYPES, ENSURING THE VIABILITY AND FUNCTION OF THE ENGRAFTED CELLS, OVERCOMING IMMUNE REJECTION WHEN AUTOGRAFTS ARE NOT USED, AND FACILITATING REVASCULARIZATION OF THE REGENERATED TISSUE. EACH ORGAN SYSTEM WILL ALSO POSE TISSUE-SPECIFIC PROBLEMS FOR STEM CELL THERAPIES.

STRATEGIES FOR STEM CELL REPLACEMENT

STEM CELL TRANSPLANTATION IS NOT A NEW CONCEPT AND IT IS ALREADY PART OF ESTABLISHED MEDICAL PRACTICE. HEMATOPOIETIC STEM CELLS (HSCS) (CHAP. 68) ARE RESPONSIBLE FOR THE LONG-TERM REPOPULATION OF ALL BLOOD ELEMENTS IN BONE MARROW TRANSPLANT RECIPIENTS. HSC TRANSPLANTATION IS NOW THE GOLD STANDARD AGAINST WHICH OTHER STEM CELL TRANSPLANTATION THERAPIES WILL BE MEASURED. TRANSPLANTATION OF DIFFERENTIATED CELLS IS ALSO A CLINICAL REALITY, AS DONATED ORGANS (E.G., LIVER, KIDNEY) AND TISSUES (I.E., CORNEA, EYE, SKIN) ARE OFTEN USED TO REPLACE DAMAGED TISSUES. HOWEVER, THE CLINICAL NEED FOR TRANSPLANTABLE TISSUES AND ORGANS FAR OUTWEIGHS THE AVAILABLE SUPPLY, AND ORGAN TRANSPLANTATION HAS LIMITED POTENTIAL FOR SOME TISSUES SUCH AS THE BRAIN. STEM CELLS OFFER THE POSSIBILITY OF A RENEWABLE SOURCE OF CELL REPLACEMENT FOR VIRTUALLY ALL ORGANS.

AT LEAST THREE DIFFERENT THERAPEUTIC CONCEPTS FOR CELL REPLACEMENT HAVE BEEN CONSIDERED (FIG. 67-1): (1) INJECTION OF STEM CELLS DIRECTLY INTO THE DAMAGED ORGAN OR INTO THE CIRCULATION, ALLOWING THEM TO "HOME" INTO THE DAMAGED TISSUE; (2) IN VITRO DIFFERENTIATION OF STEM
CELLS FOLLOWED BY TRANSPLANTATION INTO A DAMAGED ORGAN—E.G., PANCREATIC ISLET CELLS COULD BE GENERATED FROM STEM CELLS PRIOR TO TRANSPLANTATION INTO PATIENTS WITH DIABETES, WHEREAS CARDIOMYOCYTES COULD BE GENERATED TO TREAT ISCHEMIC HEART DISEASE; AND (3) STIMULATION OF ENDOGENOUS STEM CELLS TO FACILITATE REPAIR—E.G., ADMINISTRATION OF APPROPRIATE GROWTH FACTORS TO AMPLIFY NUMBERS OF ENDOGENOUS STEM/PROGENITOR CELLS OR DIRECT THEM TO DIFFERENTIATE INTO THE DESIRED CELL TYPES. IN ADDITION TO THESE STRATEGIES FOR CELL REPLACEMENT, THE EX VIVO OR IN SITU GENERATION OF TISSUES PROVIDES AN ALTERNATIVE MEANS OF TISSUE ENGINEERING (CHAP. 69). STEM CELLS ARE ALSO EXCELLENT VEHICLES FOR CELLULAR GENE THERAPY (CHAP. 65).

DISEASE-SPECIFIC STEM CELL APPROACHES
ISCHEMIC HEART DISEASE AND CARDIOMYOCYTE REGENERATION

BECAUSE OF THE HIGH PREVALENCE OF ISCHEMIC HEART DISEASE, EXTENSIVE EFFORTS HAVE BEEN DEVOTED TO CELL REPLACEMENT OF CARDIOMYOCYTES. HISTORICALLY, THE ADULT HEART HAS BEEN VIEWED AS A TERMINALLY DIFFERENTIATED ORGAN WITHOUT THE CAPACITY FOR REGENERATION. HOWEVER, THE HEART HAS THE ABILITY TO ACHIEVE LOW LEVELS OF CARDIOMYOCYTE REGENERATION AS WELL AS REVASCULARIZATION. THIS REGENERATION IS LIKELY ACCOMPLISHED BY CARDI-

FIGURE 67-1 STRATEGIES FOR TRANSPLANTATION OF STEM CELLS. 1. UNDIFFERENTIATED OR PARTIALLY DIFFERENTIATED STEM CELLS MAY BE INJECTED DIRECTLY IN THE TARGET ORGAN OR INTRAVENOUSLY. 2. STEM CELLS MAY BE DIFFERENTIATED EX VIVO PRIOR TO INJECTION INTO THE TARGET ORGAN. 3. GROWTH FACTORS OR OTHER DRUGS MAY BE INJECTED TO STIMULATE ENDOGENOUS STEM CELL POPULATIONS.

AC STEM CELLS RESIDENT IN THE HEART, AND POSSIBLY BY CELLS ORIGINATING IN THE BONE MARROW. IF SUCH CELLS COULD BE CHARACTERIZED, ISOLATED, AND AMPLIFIED EX VIVO, THEY MIGHT PROVIDE AN IDEAL SOURCE OF STEM CELLS FOR THERAPEUTIC USE. FOR EFFECTIVE MYOCARDIAL REPAIR, CELLS MUST BE DELIVERED EITHER SYSTEMICALLY OR LOCALLY, AND THE CELLS MUST SURVIVE, ENGRAFT, AND DIFFERENTIATE INTO FUNCTIONAL CARDIOMYOCYTES THAT COUPLE MECHANICALLY AND ELECTRICALLY WITH THE RECIPIENT MYOCARDIUM. THE OPTIMAL METHOD FOR CELL DELIVERY IS NOT YET CLEAR, AND VARIOUS EXPERIMENTAL STUDIES HAVE EMPLOYED INTRAMYOCARDIAL, TRANSENDOCARDIAL, INTRAVENOUS, AND INTRACORONARY INJECTIONS. IN EXPERIMENTAL MYOCARDIAL INFARCTION, FUNCTIONAL IMPROVEMENTS HAVE BEEN ACHIEVED AFTER TRANSPLANTATION OF A VARIETY OF DIFFERENT CELL TYPES, INCLUDING ES CELLS, BONE MARROW STEM CELLS, ENDOTHelial STEM CELLS, AND ADIPOSE STEM CELLS. BONE MARROW STEM CELLS IN
PARTICULAR HAVE BEEN EXAMINED IN CLINICAL TRIALS OF HUMAN ISCHEMIC HEART DISEASE. THESE HAVE LARGELY BEEN SMALL, NONRANDOMIZED STUDIES THAT TYPICALLY COMBINE CELL TREATMENT WITH CONVENTIONAL THERAPIES. ALTHOUGH THE FATE OF THE CELLS AND MECHANISMS BY WHICH THEY ALTERED CARDIAC FUNCTION ARE OPEN QUESTIONS, THESE STUDIES HAVE SHOWN SMALL BUT MEASURABLE IMPROVEMENT IN CARDIAC FUNCTION AND, IN SOME CASES, REDUCTION IN INFARCT SIZE. THE PREPONDERANCE OF EVIDENCE SUGGESTS THAT THE FUNCTIONAL BENEFITS ARE NOT DERIVED FROM DIRECT GENERATION OF CARDIOMYOCYTES BUT RATHER FROM INDIRECT EFFECTS OF THE STEM CELLS ON RESIDENT CELLS. THIS MAY REFLECT THE RELEASE OF SOLUBLE GROWTH FACTORS, INDUCTION OF ANGIOGENESIS, OR SOME OTHER MECHANISM.

DIABETES MELLITUS

THE SUCCESS OF ISLET CELL AND PANCREAS TRANSPLANTATION PROVIDES PROOF OF CONCEPT FOR A CELL-BASED APPROACH FOR TYPE I DIABETES. HOWEVER, THE DEMAND FOR DONOR PANCREATA FAR EXCEEDS THE NUMBER AVAILABLE, AND MAINTENANCE OF LONG-TERM GRAFT SURVIVAL REMAINS A PROBLEM. THE SEARCH FOR A RENEWABLE SOURCE OF STEM CELLS CAPABLE OF REGENERATING PANCREATIC ISLETS HAS THEREFORE BEEN INTENSIVE. PANCREATIC * CELL TURNOVER OCCURS IN THE NORMAL PANCREAS, ALTHOUGH THE SOURCE OF THE NEW * CELLS IS CONTROVERSIAL. ATTEMPTS TO PROMOTE ENDOGENOUS REGENERATIVE PROCESSES HAVE NOT YET BEEN SUCCESSFUL, BUT THIS REMAINS A POTENTIALLY VIABLE APPROACH. A NUMBER OF DIFFERENT CELL TYPES ARE CANDIDATES FOR USE IN STEM CELL REPLACEMENT, INCLUDING ES CELLS, HEPATIC PROGENITOR CELLS, PANCREATIC DUCTAL PROGENITOR CELLS, AND BONE MARROW STEM CELLS. SUCCESSFUL THERAPY WILL DEPEND ON DEVELOPING A SOURCE OF CELLS THAT CAN BE AMPLIFIED AND HAVE THE ABILITY TO SYNTHESIZE, STORE, AND RELEASE INSULIN WHEN IT IS REQUIRED, PRIMARILY IN RESPONSE TO CHANGES IN THE GLUCOSE LEVEL. THE PROLIFERATIVE CAPACITY OF THE REPLACEMENT CELLS MUST BE TIGHTLY REGULATED TO AVOID EXCESSIVE EXPANSION OF * CELL NUMBERS WITH THE CONSEQUENT DEVELOPMENT OF HYPERINSULINEMIA/HYPOGLYCEMIA, AND THE CELLS MUST AVOID IMMUNE REJECTION. ALTHOUGH
ES CELLS CAN BE DIFFERENTIATED INTO CELLS THAT PRODUCE INSULIN, THESE CELLS
HAVE RELATIVELY LOW INSULIN CONTENT AND A HIGH RATE OF APOPTOSIS, AND THEY GENERALLY LACK THE CAPACITY TO NORMALIZE BLOOD GLUCOSE IN DIABETIC ANIMALS. THUS, ES CELLS HAVE NOT YET BEEN USEFUL FOR THE LARGE-SCALE PRODUCTION OF DIFFERENTIATED ISLET CELLS.
DURING EMBRYOGENESIS, THE PANCREAS, LIVER, AND GASTROINTESTINAL TRACT ARE ALL DERIVED FROM THE ANTERIOR ENDODERM, AND TRANSDIFFERENTIATION OF THE PANCREAS TO LIVER AND VICE VERSA HAS BEEN OBSERVED IN CERTAIN PATHOLOGIC CONDITIONS. MULTIPOTENTIAL STEM CELLS ALSO RESIDE WITHIN GASTRIC GLANDS AND INTESTINAL CRYPTS. THUS, HEPATIC, PANCREATIC, AND/OR GASTROINTESTINAL PRECURSOR CELLS MAY BE CANDIDATES FOR CELL-BASED THERAPY OF DIABETES.

NERVOUS SYSTEM

NEURAL CELLS HAVE BEEN DIFFERENTIATED FROM A VARIETY OF STEM CELL POPULATIONS. HUMAN ES CELLS CAN BE INDUCED TO GENERATE NEURAL STEM CELLS, AND THESE CELLS CAN GIVE RISE TO NEURONS, OLIGODENDROGLIA, AND ASTROCYTES. THESE NEURAL STEM CELLS HAVE BEEN TRANSPLANTED INTO THE RODENT BRAIN WITH FORMATION OF APPROPRIATE CELL TYPES AND NO TUMOR FORMATION. MULTIPOTENT STEM CELLS PRESENT IN THE ADULT BRAIN CAN ALSO GENERATE ALL OF THE MAJOR NEURAL CELL TYPES, BUT HIGHLY INVASIVE PROCEDURES WOULD BE NECESSARY TO OBTAIN AUTOLOGOUS CELLS. FETAL NEURAL STEM CELLS DERIVED FROM MISCARRIAGES OR ABORTUSES ARE AN ALTERNATIVE, AND A CLINICAL TRIAL OF FETAL NEURAL STEM CELLS IN BATTEB DISEASE IS COMMENCING. TRANSDIFFERENTIATION OF BONE MARROW AND ADIPOSE STEM CELLS INTO NEURAL STEM CELLS, AND VICE VERSA, HAS BEEN REPORTED, AND CLINICAL TRIALS OF SUCH CELLS HAVE BEGUN FOR A NUMBER OF NEUROLOGIC DISORDERS. CLINICAL TRIALS OF A CONDITIONALLY IMMORTALIZED HUMAN CELL LINE AND OF HUMAN UMBILICAL CORD BLOOD CELLS IN STROKE ARE ALSO PLANNED. NEUROLOGIC DISORDERS THAT HAVE ALREADY BEEN TARGETED FOR STEM CELL THERAPIES INCLUDE SPINAL CORD INJURY, AMYOTROPHIC LATERAL SCLEROSIS, STROKE, TRAUMATIC BRAIN INJURY, BATTEN DISEASE, AND PARKINSON’S DISEASE. IN PARKINSON’S DISEASE, THE MAJOR MOTOR FEATURES RESULT FROM THE LOSS OF A SINGLE CELL POPULATION, DOPAMINERGIC NEURONS WITHIN THE SUBSTANTIA NIGRA PARS COMPACTA. TWO CLINICAL TRIALS OF FETAL NIGRAL TRANSPLANTATION FAILED TO MEET THEIR PRIMARY ENDPOINT AND WERE COMPLICATED BY THE DEVELOPMENT OF DYSKINESIA. TRANSPLANTATION OF STEM CELL-DERIVED DOPAMINE-PRODUCING CELLS OFFERS A NUMBER OF POTENTIAL ADVANTAGES OVER FETAL TRANSPLANTS, INCLUDING THE ABILITY OF STEM CELLS TO MIGRATE AND DISPERSE WITHIN TISSUE, THE POTENTIAL FOR ENGINEERING REGULATABLE RELEASE OF DOPAMINE, AND THE ABILITY TO ENGINEER CELLS TO PRODUCE FACTORS THAT WILL ENHANCE CELL SURVIVAL. NEVERTHELESS, THE EXPERIENCE WITH FETAL TRANSPLANTS POINTS OUT THE DIFFICULTIES THAT MAY BE ENCOUNTERED.
AT LEAST SOME OF THE NEUROLOGIC DYSFUNCTION AFTER SPINAL CORD INJURY REFLECTS DEMYELINATION, AND BOTH ES CELLS AND MARROW-DERIVED STEM CELLS ARE ABLE TO FACILITATE REMYELINATION AFTER EXPERIMENTAL SPINAL CORD INJURY. CLINICAL TRIALS OF MARROW-DERIVED STEM CELLS HAVE ALREADY BEGUN, AND THIS MAY BE THE FIRST DISEASE TARGETED FOR THE CLINICAL USE OF ES CELLS. MARROW-DERIVED STEM CELLS ARE ALSO BEING USED IN THE TREATMENT OF STROKE, TRAUMATIC BRAIN INJURY, AND AMYOTROPHIC LATERAL SCLEROSIS (ALS), WHERE POSSIBLE BENEFITS ARE MORE LIKELY TO BE INDIRECT TROPHIC EFFECTS OR REMYELINATION RATHER THAN NEURON REPLACEMENT. AT PRESENT, NO POPULATION OF TRANSPLANTED STEM CELLS HAS BEEN SHOWN TO GENERATE NEURONS THAT EXTEND AXONS OVER LONG DISTANCES TO FORM SYNAPTIC CONNECTIONS (SUCH AS WOULD BE NECESSARY FOR REPLACEMENT OF UPPER MOTOR NEURONS IN ALS, STROKE, OR OTHER DISORDERS).

**LIVER**

TRANSPLANTATION IS CURRENTLY THE ONLY SUCCESSFUL TREATMENT FOR END-STAGE LIVER DISEASES, BUT THIS APPROACH IS LIMITED BY THE SHORTAGE OF LIVER GRAFTS. CLINICAL TRIALS OF HEPATOCYTE TRANSPLANTATION DEMONSTRATE THAT IT CAN POTENTIALLY SUBSTITUTE FOR ORGAN TRANSPLANTATION, BUT THE PAUCITY OF AVAILABLE CELLS ALSO LIMITS THIS STRATEGY. POTENTIAL SOURCES OF STEM CELLS INCLUDE ENDOGENOUS LIVER STEM CELLS (SUCH AS OVAL CELLS), ES CELLS, BONE MARROW CELLS, AND UMBILICAL CORD BLOOD CELLS. ALTHOUGH A SERIES OF STUDIES IN HUMANS AS WELL AS ANIMALS SUGGESTED THAT TRANSPLANTED BONE MARROW STEM CELLS CAN GENERATE HEPATOCYTES, THIS PHENOMENON LARGELY REFLECTS THE FUSION OF THE TRANSPLANTED CELLS WITH ENDOGENOUS LIVER CELLS, GIVING THE ERRONEOUS APPEARANCE OF NEW HEPATOCYTES. ES CELLS HAVE BEEN DIFFERENTIATED INTO HEPATOCYTES AND TRANSPLANTED IN ANIMAL MODELS OF LIVER FAILURE WITHOUT FORMATION OF TERATOMAS.

**OTHER ORGAN SYSTEM AND THE FUTURE**

THE USE OF STEM CELLS IN REGENERATIVE MEDICINE HAS BEEN STUDIED FOR MANY OTHER ORGAN SYSTEMS AND CELL TYPES, INCLUDING SKIN, EYE, CARTILAGE, BONE, KIDNEY, LUNG, ENDOMETRIUM, VASCULAR ENDOTHELIUM, SMOOTH MUSCLE, STRIATED MUSCLE, AND OTHERS. IN FACT, THE POTENTIAL FOR STEM CELL REGENERATION OF DAMAGED ORGANS AND TISSUES IS VIRTUALLY LIMITLESS. HOWEVER, NUMEROUS OBSTACLES MUST BE OVERCOME BEFORE STEM CELL THERAPIES CAN BECOME A WIDESPREAD CLINICAL REALITY. ONLY HSCS HAVE BEEN ADEQUATELY CHARACTERIZED BY SURFACE MARKERS TO ALLOW UNAMBIGUOUS
IDENTIFICATION, A PREREQUISITE FOR RELIABLE CLINICAL APPLICATIONS. THE PATHWAYS FOR DIFFERENTIATING STEM CELLS INTO SPECIFIC CELLULAR PHENOTYPES ARE STILL UNKNOWN, THE MIGRATION OF TRANSPLANTED CELLS IS UNCONTROLLED, AND THE RESPONSE OF THE CELLS TO THE ENVIRONMENT OF DISEASED ORGANS IS UNPREDICTABLE. FUTURE STRATEGIES MAY EMPLOY THE COADMINISTRATION OF SCAFFOLDING, ARTIFICIAL EXTRACELLULAR MATRIX, AND/OR GROWTH FACTORS TO ORCHESTRATE DIFFERENTIATION OF STEM CELLS AND THEIR ORGANIZATION INTO APPROPRIATE CONSTITUENTS OF THE ORGAN. IMAGING TECHNIQUES ARE NEEDED TO VISUALIZE STEM CELLS IN VIVO AFTER TRANSPLANTATION INTO HUMANS. FORTUNATELY, STEM CELLS CAN BE ENGINEERED BEFORE TRANSPLANTATION TO CONTAIN CONTRAST AGENTS THAT MAY MAKE THIS FEASIBLE. THE POTENTIAL FOR TUMOR FORMATION AND THE PROBLEMS ASSOCIATED WITH IMMUNE REJECTION ARE SIGNIFICANT IMPEDIMENTS. MANY STRATEGIES FOR CELL REPLACEMENT ALREADY INCLUDE VASOACTIVE ENDOTHELIAL GROWTH FACTOR (VEGF) COADMINISTRATION TO FOSTER VASCULARIZATION, WHICH IS REQUIRED FOR SURVIVAL AND FUNCTION OF THE TRANSPLANT. SOME STEM CELLS HAVE BEEN ENGINEERED TO HAVE AN INDUCIBLE SUICIDE GENE SO THAT THE CELLS CAN BE ERADICATED IN THE EVENT OF TUMOR FORMATION OR SOME OTHER COMPLICATION. THE POTENTIAL FOR STEM CELL THERAPIES TO REVOLUTIONIZE MEDICAL CARE IS EXTRAORDINARY, AND DISORDERS SUCH AS MYOCARDIAL INFARCTION, DIABETES, PARKINSON’S DISEASE AND MANY OTHERS ARE ATTRACTIVE TARGETS. HOWEVER, SUCH STEM CELL-BASED THERAPIES ARE AT A VERY EARLY STAGE OF DEVELOPMENT, AND PERFECTION OF TECHNIQUES FOR CLINICAL TRANSPLANTATION OF PREDICTABLE, WELL-CHARACTERIZED CELLS WILL BE A DIFFICULT AND LENGTHY UNDERTAKING.

ETHICAL ISSUES

STEM CELL THERAPIES RAISE CONTENTIOUS ETHICAL ISSUES THAT MUST BE ADDRESSED IN PARALLEL WITH THE SCIENTIFIC AND MEDICAL OPPORTUNITIES. OUR SOCIETY HAS GREAT DIVERSITY IN RELIGIOUS BELIEFS, CONCEPTS OF INDIVIDUAL RIGHTS, TOLERANCE FOR UNCERTAINTY AND RISK, AND BOUNDARIES FOR HOW SCIENTIFIC INTERVENTIONS SHOULD BE USED TO ALTER THE OUTCOME OF DISEASE. IN THE UNITED STATES, THE FEDERAL GOVERNMENT HAS AUTHORIZED RESEARCH USING HUMAN ES LINES IN EXISTENCE BEFORE AUGUST 2001 BUT HAS RESTRICTED THE USE OF FEDERAL FUNDS FOR DEVELOPING NEW HUMAN ES LINES. HOWEVER, THESE EXISTING LINES DEVELOP ABNORMALITIES WITH TIME IN CULTURE AND ARE CONTAMINATED WITH MOUSE PROTEINS. THESE FINDINGS HAVE SPARKED RENEWED DEBATE ABOUT THE NEED TO DEVELOP NEW HUMAN ES CELL LINES. IN CONSIDERING ETHICAL ISSUES ASSOCIATED WITH THE USE OF STEM CELLS, IT
IS HELPFUL TO DRAW FROM EXPERIENCE WITH OTHER SCIENTIFIC ADVANCES, SUCH AS ORGAN TRANSPLANTATION, RECOMBINANT DNA TECHNOLOGY, IMPLANTATION OF MECHANICAL DEVICES, NEUROSCIENCE AND COGNITIVE RESEARCH, IN VITRO FERTILIZATION, AND PRENATAL GENETIC TESTING. FROM THESE AND OTHER PRECE- DENTS, WE LEARN THE IMPORTANCE OF UNDERSTANDING AND TESTING FUNDAMENTAL BIOLOGY IN THE LABORATORY SETTING AND IN ANIMAL MODELS BEFORE APPLYING NEW TECHNIQUES IN CAREFULLY CONTROLLED CLINICAL TRIALS. WHEN THESE TRIALS OCCUR, THEY MUST INCLUDE FULL INFORMED CONSENT AND HAVE CAREFUL OVERSIGHT BY EXTERNAL REVIEW GROUPS. ULTIMATELY, MEDICAL INTERVENTIONS WILL BE SCIENTIFICALLY FEASIBLE BUT ETHICALLY OR SOCIALLY UNACCEPTABLE TO SOME MEMBERS OF A SOCIETY. STEM CELL RESEARCH RAISES QUESTIONS ABOUT THE DEFINITION OF HUMAN LIFE, AND IT HAS RAISED DEEP FEARS ABOUT OUR ABILITY TO BALANCE ISSUES OF JUSTICE AND SAFETY WITH THE NEEDS OF CRITICALLY ILL PATIENTS. HEALTH CARE PROVIDERS AND EXPERTS WITH BACKGROUNDS IN ETHICS, LAW, AND SOCIOLOGY MUST HELP GUARD AGAINST THE PREMATURE OR INAPPROPRIATE APPLICATION STEM CELL THERAPIES, AND THE INAPPROPRIATE USE OF VULNERABLE POPULATION GROUPS. ON THE OTHER HAND, THESE THERAPIES OFFER IMPORTANT NEW STRATEGIES FOR THE TREATMENT OF OTHERWISE IRREVERSIBLE DISORDERS. AN OPEN DIALOGUE BETWEEN THE SCIENTIFIC COMMUNITY, PHYSICIANS, PATIENTS, AND THEIR ADVOCATES, LAWMAKERS, AND THE LAY POPULATION IS IMPORTANT TO RAISE AND ADDRESS ETHICAL ISSUES AND TO BALANCE THE BENEFITS AND RISKS ASSOCIATED WITH STEM CELL TRANSFER.

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68 HEMATOPOIETIC STEM CELLS
DAVID T. SCADDEN, DAN L. LONGO

ALL OF THE CELL TYPES IN THE PERIPHERAL BLOOD AND SOME CELLS IN EVERY TISSUE OF THE BODY ARE DERIVED FROM HEMATOPOIETIC (HEMO: BLOOD; POIESIS: CRE-
ATION) STEM CELLS. IF THE HEMATOPOIETIC STEM CELL IS DAMAGED AND CAN NO
LONGER FUNCTION (E.G., DUE TO THE NUCLEAR ACCIDENT AT CHERNOBYL), A PER-
SON WOULD SURVIVE 2-4 WEEKS IN THE ABSENCE OF EXTRAORDINARY SUPPORT
MEASURES. WITH THE CLINICAL USE OF HEMATOPOIETIC STEM CELLS, TENS OF THOU-
SANDS OF LIVES ARE SAVED EACH YEAR (CHAP. 108). STEM CELLS PRODUCE TENS OF
BILLIONS OF BLOOD CELLS DAILY FROM A STEM CELL POOL THAT IS ESTIMATED TO BE
ONLY IN THE HUNDREDS OF THOUSANDS. HOW STEM CELLS DO THIS, HOW THEY
PERSIST FOR MANY DECADES DESPITE THE PRODUCTION DEMANDS, AND HOW THEY
MAY BE BETTER USED IN CLINICAL CARE ARE IMPORTANT ISSUES IN MEDICINE.
The study of blood cell production has become a paradigm for how other tissues may be organized and regulated. Basic research in hematopoiesis that includes defining stepwise molecular changes accompanying functional changes in maturing cells, aggregating cells into functional subgroups, and demonstrating hematopoietic stem cell regulation by a specialized microenvironment are concepts worked out in hematology, but they offer models for other tissues. Moreover, these concepts may not be restricted to normal tissue function but extend to malignancy. Stem cells are rare cells among a heterogeneous population of cell types, and their behavior is assessed mainly in experimental animal models involving reconstitution of hematopoiesis. Thus, much of what we know about stem cells is imprecise and based on inferences from genetically manipulated animals.

CARDINAL FUNCTIONS OF HEMATOPOIETIC STEM CELLS

ALL STEM CELL TYPES HAVE TWO CARDINAL FUNCTIONS: SELF-RENEWAL AND DIFFER-
ENTIATION (FIG. 68-1). STEM CELLS EXIST TO GENERATE, MAINTAIN, AND REPAIR
TISSUES. THEY FUNCTION SUCCESSFULLY IF THEY CAN REPLACE A WIDE VARIETY OF
SHORTER-LIVED MATURE CELLS OVER PROLONGED PERIODS. THE PROCESS OF SELF-
RENEWAL (SEE BELOW) ASSURES THAT A STEM CELL POPULATION CAN BE SUSTAINED
OVER TIME. WITHOUT SELF-RENEWAL, THE STEM CELL POOL COULD EXHAUST OVER
TIME AND TISSUE MAINTENANCE WOULD NOT BE POSSIBLE. THE PROCESS OF DIFFER-
ENTIATION PROVIDES THE EFFECTORS OF TISSUE FUNCTION: MATURE CELLS. WITH-
OUT PROPER DIFFERENTIATION, THE INTEGRITY OF TISSUE FUNCTION WOULD BE COMPROMISED AND ORGAN FAILURE WOULD ENSUE.
IN THE BLOOD, MATURE CELLS HAVE VARIABLE AVERAGE LIFE SPANS, RANGING FROM 7 H FOR MATURE NEUTROPHILS TO A FEW MONTHS FOR RED BLOOD CELLS TO
MANY YEARS FOR MEMORY LYMPHOCYTES. HOWEVER, THE STEM CELL POOL IS THE
FURTHER READINGS

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FIGURE 6-1 SIGNATURE CHARACTERISTICS OF THE STEM CELL. STEM CELLS HAVE TWO ESSENTIAL FEATURES: THE CAPACITY TO DIFFERENTIATE INTO A VARIETY OF MATURE CELL TYPES AND THE CAPACITY FOR SELF-RENEWAL. INTRINSIC FACTORS ASSOCIATED WITH SELF-RENEWAL INCLUDE EXPRESSION OF BMI-1, GFI-1, PTEN, STAT5, TEL/ATV6, P21, P18, MCL-1, MEL-18, RAE28, AND HOXB4. EXTRINSIC SIGNALS FOR SELF-RENEWAL INCLUDE NOTCH, WNT, SHH, AND TIE2/ANG-1. BASED MAINLY ON MURINE STUDIES, HEMATOPOIETIC STEM CELLS EXPRESS THE FOLLOWING CELL SURFACE MOLECULES: CD34, THY-1 (CD90), C-KIT RECEPTOR (CD117), CD133, CD164, AND C-MPL (CD110, ALSO KNOWN AS THE THROMBOPOIETIN RECEPTOR).

CENTRAL, DURABLE SOURCE OF ALL BLOOD AND IMMUNE CELLS, MAINTAINING A CAPACITY TO PRODUCE A BROAD RANGE OF CELLS FROM A SINGLE CELL SOURCE AND YET KEEPING ITSELF VIGOROUS OVER DECADES OF LIFE. AS AN INDIVIDUAL STEM CELL DIVIDES, IT HAS THE CAPACITY TO ACCOMPLISH ONE OF THREE DIVISION OUTCOMES: TWO STEM CELLS, TWO CELLS DESTINED FOR DIFFERENTIATION, OR ONE STEM CELL AND ONE DIFFERENTIATING CELL. THE FORMER TWO OUTCOMES ARE THE RESULT OF SYMMETRIC CELL DIVISION, WHEREAS THE LATTER INDICATES A DIFFERENT OUTCOME FOR THE TWO DAUGHTER CELLS-AN EVENT TERMED ASYMMETRIC CELL DIVISION. THE RELATIVE BALANCE FOR THESE TYPES OF OUTCOMES MAY CHANGE DURING DEVELOPMENT AND UNDER PARTICULAR KINDS OF DEMANDS ON THE STEM CELL POOL.

DEVELOPMENTAL BIOLOGY OF HEMATOPOIETIC STEM CELLS

DURING DEVELOPMENT, BLOOD CELLS ARE PRODUCED AT DIFFERENT SITES.
INITIALLY, THE YOLK SAC PROVIDES OXYGEN-CARRYING RED BLOOD CELLS, AND THEN SEVERAL SITES OF INTRAEMBRYONIC BLOOD CELL PRODUCTION BECOME INVOLVED. THESE INTRAEMBRYONIC SITES ENGAGE IN SEQUENTIAL ORDER, MOVING FROM THE GENITAL RIDGE AT A SITE WHERE THE AORTA, GONADAL TISSUE, AND MESONEPHROS ARE EMERGING TO THE FETAL LIVER AND THEN, IN THE SECOND TRIMESTER, TO THE BONE MARROW AND SPLEEN. AS THE LOCATION OF STEM CELLS CHANGES, THE RELATIVE ABUNDANCE OF CELLS THEY PRODUCE ALSO CHANGES, PROGRESSIVELY INCREASING.

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431 CHAPTER 68 HEMATOPOIETIC STEM CELLS

IN THE COMPLEXITY OF CELL TYPES FROM THOSE SIMPLY CARRYING OXYGEN TO PLATELETS SUPPORTING A MORE COMPLEX VASCULATURE TO THE CELLS OF INNATE IMMUNITY AND FINALLY TO THE CELLS OF ADAPTIVE IMMUNITY. STEM CELL PROLIFERATION REMAINS HIGH, EVEN IN THE BONE MARROW, UNTIL SHORTLY AFTER BIRTH, WHEN IT APPEARS TO DRAMATICALLY DECLINE. THE CELLS IN THE BONE MARROW ARE THOUGHT TO ARRIVE BY THE BLOODBORNE TRANSIT OF CELLS FROM THE FETAL LIVER AFTER CALCIFICATION OF THE LONG BONES HAS BEGUN. THE PRESENCE OF STEM CELLS IN THE CIRCULATION IS NOT UNIQUE TO A TIME WINDOW IN DEVELOPMENT. RATHER, HEMATOPOIETIC STEM CELLS APPEAR TO CIRCULATE THROUGHOUT LIFE. THE TIME THAT CELLS SPEND FREELY CIRCULATING APPEARS TO BE BRIEF (MEASURED IN MINUTES IN THE MOUSE), BUT THE CELLS THAT DO CIRCULATE ARE FUNCTIONAL AND CAN BE USED FOR TRANSPLANTATION. THE NUMBER OF STEM CELLS THAT CIRCULATE CAN BE INCREASED IN A NUMBER OF WAYS TO FACILITATE HARVEST AND TRANSFER TO THE SAME OR A DIFFERENT HOST.

MOBILITY OF HEMATOPOIETIC STEM CELLS

CELLS ENTERING AND EXITING THE BONE MARROW DO SO THROUGH A SERIES OF MOLECULAR INTERACTIONS. CIRCULATING STEM CELLS (THROUGH CD 162 AND CD44) ENGAGE THE LECTINS P- AND E-SELECTIN ON THE ENDOTHELIAL SURFACE TO SLOW THE MOVEMENT OF THE CELLS TO A ROLLING PHENOTYPE. STEM CELL INTEGRINS ARE THEN ACTIVATED AND ACCOMPLISH FIRM ADHESION BETWEEN THE STEM CELL AND VESSEL WALL, WITH A PARTICULARLY IMPORTANT ROLE FOR
STEM CELL VCAM-1 ENGAGING ENDOTHELIAL VLA-4. THE CHEMOKINE CXCL12 (SDF1) INTERACTING WITH STEM CELL CXCR4 RECEPTORS ALSO APPEARS TO BE IMPORTANT IN THE PROCESS OF STEM CELLS GETTING FROM THE CIRCULATION TO WHERE THEY ENGRAFT IN THE BONE MARROW. THIS IS PARTICULARLY TRUE IN THE DEVELOPMENTAL MOVE FROM FETAL LIVER TO BONE MARROW; HOWEVER, THE ROLE FOR THIS MOLECULE IN ADULTS APPEARS TO BE MORE RELATED TO RETENTION OF STEM CELLS IN THE BONE MARROW RATHER THE PROCESS OF GETTING THEM THERE. INTERRUPTING THAT RETENTION PROCESS THROUGH EITHER SPECIFIC MOLECULAR BLOCKERS OF THE CXCR4/CXCL12 INTERACTION, CLEAVAGE OF CXCL12, OR DOWNREGULATION OF THE RECEPTOR CAN ALL RESULT IN THE RELEASE OF STEM CELLS INTO THE CIRCULATION. THIS PROCESS IS AN INCREASINGLY IMPORTANT ASPECT OF RECOVERING STEM CELLS FOR THERAPEUTIC USE AS IT HAS PERMITTED THE HARVESTING PROCESS TO BE DONE BY LEUKAPHERESIS RATHER THAN BONE MARROW PUNCTURES IN THE OPERATING ROOM. REFINING OUR KNOWLEDGE OF HOW STEM CELLS GET INTO AND OUT OF THE BONE MARROW MAY IMPROVE OUR ABILITY TO OBTAIN STEM CELLS AND MAKE THEM MORE EFFICIENT AT FINDING THEIR WAY TO THE SPECIFIC SITES FOR BLOOD CELL PRODUCTION, THE SO-CALLED STEM CELL NICHE.

HEMATOPOIETIC STEM CELL MICROENVIRONMENT

THE CONCEPT OF A SPECIALIZED MICROENVIRONMENT, OR STEM CELL NICHE, WAS FIRST PROPOSED TO EXPLAIN WHY CELLS DERIVED FROM THE BONE MARROW OF ONE ANIMAL COULD BE USED IN TRANSPLANTATION AND AGAIN BE FOUND IN THE BONE MARROW OF THE RECIPIENT. THIS NICHE IS MORE THAN JUST A HOUSING SITE FOR STEM CELLS, HOWEVER. IT IS AN ANATOMIC LOCATION WHERE REGULATORY SIGNALS ARE PROVIDED THAT ALLOW THE STEM CELLS TO THRIVE, TO EXPAND IF NEEDED, AND TO PROVIDE VARYING AMOUNTS OF DESCENDANT DAUGHTER CELLS. IN ADDITION, UNREGULATED GROWTH OF STEM CELLS MAY BE PROBLEMATIC BASED ON THEIR UNDIFFERENTIATED STATE AND SELF-RENEWAL CAPACITY. THUS, THE NICHE MUST ALSO REGULATE THE NUMBER OF STEM CELLS PRODUCED. IN THIS MANNER, THE NICHE HAS THE DUAL FUNCTIONS OF SERVING AS A SITE OF NURTURE BUT IMPOSING LIMITS FOR STEM CELLS; IN EFFECT, ACTING AS BOTH A NEST AND A CAGE. THE NICHE FOR BLOOD STEM CELLS CHANGES WITH EACH OF THE SITES OF BLOOD PRODUCTION DURING DEVELOPMENT, BUT FOR MOST OF HUMAN LIFE IT IS LOCATED IN THE BONE MARROW. WITHIN THE BONE MARROW, AT LEAST TWO NICHE SITES HAVE BEEN PROPOSED: ON TRABECULAR BONE SURFACES AND IN THE PERIVASCULAR SPACE. STEM CELLS MAY BE FOUND IN BOTH PLACES BY HISTOLOGIC ANALYSIS, AND FUNCTIONAL REGULATION HAS BEEN SHOWN AT THE BONE SURFACE. SPECIFICALLY, BONE-FORMING MESENCHYMAL CELLS, OSTEOBLASTS,
PARTICIPATE IN HEMATOPOIETIC STEM CELL FUNCTION, AFFECTING THEIR LOCATION, PROLIFERATION, AND NUMBER. THE BASIS FOR THIS INTERACTION IS THROUGH A NUMBER OF MOLECULES MEDIATING LOCATION, SUCH AS THE CHEMOKINE CXCL12 (SDF1) AND N-CADHERIN, THROUGH PROLIFERATION SIGNALS MEDIATED BY ANGIOPOIETIN 1, AND SIGNALING TO MODULATE SELF-RENEWAL OR SURVIVAL BY FACTORS SUCH AS NOTCH LIGANDS, KIT LIGAND, AND WNTS. OTHER BONE COMPONENTS, SUCH AS THE EXTRACELLULAR MATRIX GLYCOPROTEIN, OSTEOPONTIN, AND THE HIGH IONIC CALCIUM FOUND AT TRABECULAR SURFACES, CONTRIBUTE TO THE UNIQUE MICROENVIRONMENT, OR STEM CELL NICHE, ON TRABECULAR BONE. THIS PHYSIOLOGY HAS PRACTICAL APPLICATIONS. FIRST, MEDICATIONS ALTERING NICHE COMPONENTS MAY HAVE AN EFFECT ON STEM CELL FUNCTION. THIS HAS NOW BEEN SHOWN FOR A NUMBER OF COMPOUNDS, AND SOME ARE BEING CLINICALLY TESTED. SECOND, IT IS NOW POSSIBLE TO ASSESS WHETHER THE NICHE PARTICIPATES IN DISEASE STATES AND TO EXAMINE WHETHER TARGETING THE NICHE WITH MEDICATIONS MAY ALTER THE OUTCOME OF CERTAIN DISEASES.

EXCESS CAPACITY OF HEMATOPOIETIC STEM CELLS

IN THE ABSENCE OF DISEASE, ONE NEVER RUNS OUT OF HEMATOPOIETIC STEM CELLS. INDEED, SERIAL TRANSPLANTATION STUDIES IN MICE SUGGEST THAT SUFFICIENT STEM CELLS ARE PRESENT TO RECONSTITUTE SEVERAL ANIMALS IN SUCCESSION, WITH EACH ANIMAL HAVING NORMAL BLOOD CELL PRODUCTION. THE FACT THAT ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS ALSO NEVER RUN OUT OF BLOOD CELLS IN THEIR LIFE SPAN, WHICH CAN EXTEND FOR DECADES, ARGUES THAT EVEN THE LIMITING NUMBERS OF STEM CELLS PROVIDED TO THEM ARE SUFFICIENT. HOW STEM CELLS RESPOND TO DIFFERENT CONDITIONS TO INCREASE OR DECREASE THEIR MATURE CELL PRODUCTION REMAINS POORLY UNDERSTOOD. CLEARLY, NEGATIVE FEEDBACK MECHANISMS AFFECT THE LEVEL OF PRODUCTION OF MOST OF THE CELLS, LEADING TO THE NORMAL TIGHTLY REGULATED BLOOD CELL COUNTS. HOWEVER, MANY OF THE REGULATORY MECHANISMS THAT GOVERN PRODUCTION OF MORE MATURE PROGENITOR CELLS DO NOT APPLY OR APPLY DIFFERENTLY TO STEM CELLS. SIMILARLY, MOST OF THE MOLECULES SHOWN TO BE ABLE TO CHANGE THE SIZE OF THE STEM CELL POOL HAVE LITTLE EFFECT ON MORE MATURE BLOOD CELLS. FOR EXAMPLE, THE GROWTH FACTOR ERYTHROPOIETIN, WHICH STIMULATES RED BLOOD CELL PRODUCTION FROM MORE MATURE PRECURSOR CELLS, HAS NO EFFECT ON STEM CELLS. SIMILARLY, GRANULOCYTE COLONY-STIMULATING FACTOR DRIVES THE RAPID PROLIFERATION OF GRANULOCYTE PRECURSORS BUT DOES NOT AFFECT CELL CYCLING OF STEM CELLS. RATHER, IT CHANGES THE LOCATION OF STEM CELLS BY INDIRECT MEANS, ALTERING MOLECULES SUCH AS CXCL12 THAT TETHER STEM CELLS TO THEIR NICHE. MOLECULES SHOWN TO BE IMPORTANT FOR ALTERING THE PROLIFERATION OF STEM CELLS, SUCH AS THE CYCLIN-DEPENDENT KINASE INHIBITOR P21CIP1, HAVE LITTLE OR NO EFFECT ON
HEMATOPOIETIC STEM CELLS HAVE GOVERNING MECHANISMS THAT ARE DISTINCT FROM THE CELLS THEY GENERATE.

HEMATOPOIETIC STEM CELL DIFFERENTIATION

HEMATOPOIETIC STEM CELLS SIT AT THE BASE OF A BRANCHING HIERARCHY OF CELLS CULMINATING IN THE MANY MATURE CELL TYPES THAT COMPOSE THE BLOOD AND IMMUNE SYSTEM (FIG. 68-2). THE MATURATION STEPS LEADING TO TERMINALLY DIFFERENTIATED AND FUNCTIONAL BLOOD CELLS TAKE PLACE BOTH AS A CONSEQUENCE OF INTRINSIC CHANGES IN GENE EXPRESSION AND NICHE-DIRECTED AND CYTOKINE-DIRECTED CHANGES IN THE CELLS. OUR KNOWLEDGE OF THE DETAILS REMAINS INCOMPLETE (SEE HTTP://STEMCELL.PRINCETON.EDU/ FOR A COMPREHENSIVE LISTING OF GENE EXPRESSION IN STEM CELLS). AS STEM CELLS MATURE TO PROGENITORS, PRECURSORS, AND, FINALLY, MATURE EFFECTOR CELLS, THEY UNDERGO A SERIES OF FUNCTIONAL CHANGES. THESE INCLUDE THE OBVIOUS ACQUISITION OF FUNCTIONS DEFINING MATURE BLOOD CELLS, SUCH AS PHAGOCYTIC CAPACITY OR HEMOGLOBINIZATION. THEY ALSO INCLUDE THE PROGRESSIVE LOSS OF PLASTICITY, I.E., THE ABILITY TO BECOME OTHER CELL TYPES. FOR EXAMPLE, THE MYELOID PROGENITOR CAN MAKE ALL CELLS IN THE MYELOID SERIES BUT NONE IN THE LYMPHOID SERIES. AS COMMON MYELOID PROGENITORS MATURE, THEY BECOME PRECURSORS FOR EITHER MONOCYTES AND GRANULOCYTES OR ERYTHROCYTES AND MEGAKARYOCYTES, BUT NOT BOTH. SOME AMOUNT OF REVERSIBILITY OF THIS PROCESS MAY EXIST EARLY IN THE DIFFERENTIATION CASCADE, BUT THAT IS LOST BEYOND A DISTINCT STAGE. AS CELLS DIFFERENTIATE, THEY MAY ALSO LOSE PROLIFERATIVE CAPACITY (FIG.68-3). MATURE GRANULOCYTES ARE INCAPABLE OF PROLIFERATION AND ONLY INCREASE IN NUMBER BY INCREASED PRODUCTION FROM PRECURSORS. LYMPHOID CELLS RETAIN THE CAPACITY TO PROLIFERATE BUT HAVE LINKED THEIR PROLIFERATION TO THE RECOGNITION OF PARTICULAR PROTEINS OR PEPTIDES BY SPECIFIC ANTIGEN RECEPTORS ON THEIR SURFACE. IN MOST TISSUES THE PROLIFERATIVE CELL POPULATION IS A MORE IMMATURE PROGENITOR POPULATION. IN GENERAL, CELLS WITHIN THE HIGHLY PROLIFERATIVE PROGENITOR CELL COMPARTMENT ARE ALSO RELATIVELY SHORT-LIVED, MAKING THEIR WAY THROUGH THE DIFFERENTIATION PROCESS IN A DEFINED MOLECULAR PROGRAM INVOLVING THE SEQUENTIAL ACTIVATION OF PARTICULAR SETS.

432 PART 4: REGENERATIVE MEDICINE

FIGURE 68-2 HIERARCHY OF HEMATOPOIETIC DIFFERENTIATION. STEM CELLS ARE MULTIPOTENT CELLS THAT ARE THE SOURCE OF ALL DESCENDANT CELLS.
AND HAVE THE CAPACITY TO PROVIDE EITHER LONG-TERM (MEASURED IN YEARS) OR SHORT-TERM (MEASURED IN MONTHS) CELL PRODUCTION. PROGENITOR CELLS HAVE A MORE LIMITED SPECTRUM OF CELLS THEY CAN PRODUCE AND ARE GENERALLY A SHORT-LIVED, HIGHLY PROLIFERATIVE POPULATION ALSO KNOWN AS TRANSIENT AMPLIFYING CELLS. PRECURSOR CELLS ARE CELLS COMMITTED TO A SINGLE BLOOD CELL LINEAGE BUT WITH A CONTINUED ABILITY TO PROLIFERATE; THEY DO NOT HAVE ALL THE FEATURES OF A FULLY MATURE CELL. MATURE CELLS ARE THE TERMINALLY DIFFERENTIATED PRODUCT OF THE DIFFERENTIATION PROCESS AND ARE THE EFFECTOR CELLS OF SPECIFIC ACTIVITIES OF THE BLOOD AND IMMUNE SYSTEM. PROGRESS THROUGH THE PATHWAYS IS MEDIATED BY ALTERATIONS IN GENE EXPRESSION. THE REGULATION OF THE DIFFERENTIATION BY SOLUBLE FACTORS AND CELL-CELL COMMUNICATIONS WITHIN THE BONE MARROW NICHE ARE STILL BEING DEFINED. THE TRANSCRIPTION FACTORS THAT CHARACTERIZE PARTICULAR CELL TRANSITIONS ARE ILLUSTRATED ON THE ARROWS; THE SOLUBLE FACTORS THAT CONTRIBUTE TO THE DIFFERENTIATION PROCESS ARE IN BLUE. SCF, STEM CELL FACTOR; EPO, ERYTHROPOIETIN, TPO, THROMBOPOIETIN.

OF GENES. FOR ANY PARTICULAR CELL TYPE, THE DIFFERENTIATION PROGRAM IS DIFFICULT TO SPEED UP. THE TIME IT TAKES FOR HEMATOPOIETIC PROGENITORS TO BECOME MATURE CELLS IS ~ 10-14 DAYS IN HUMANS, EVIDENT CLINICALLY BY THE INTERVAL BETWEEN CYTOTOXIC CHEMOTHERAPY AND BLOOD COUNT RECOVERY IN PATIENTS.

SELF-RENEWAL

THE HEMATOPOIETIC STEM CELL MUST BALANCE ITS THREE POTENTIAL FATES: APOPTOSIS, SELF-RENEWAL, AND DIFFERENTIATION. THE PROLIFERATION OF CELLS IS GENERALLY NOT ASSOCIATED WITH THE ABILITY TO UNDERGO A SELF-RENEWING DIVISION EXCEPT AMONG MEMORY T AND B CELLS AND AMONG STEM CELLS. SELF-RENEWAL CAPACITY GIVES WAY TO DIFFERENTIATION AS THE ONLY OPTION AFTER CELL DIVISION WHEN CELLS LEAVE THE STEM CELL COMPARTMENT, UNTIL THEY HAVE THE OPPORTUNITY TO BECOME MEMORY LYMPHOCYTES. IN ADDITION TO THIS SELF-RENEWING CAPACITY, STEM CELLS HAVE AN ADDITIONAL FEATURE CHARACTERIZING THEIR PROLIFERATION MACHINERY. STEM CELLS IN MOST MATURE ADULT TISSUES ARE DEEPLY QUIESCENT. IN THE HEMATOPOIETIC SYSTEM, STEM CELLS ARE ALSO HIGHLY CYTOKINE-RESISTANT, REMAINING DORMANT EVEN WHEN CYTOKINES DRIVE BONE MARROW PROGENITORS TO PROLIFERATION RATES MEASURED IN HOURS, NOT DAYS. STEM CELLS, IN CONTRAST, ARE THOUGHT TO DIVIDE AT INTERVALS MEASURED IN MONTHS TO YEARS, AT LEAST AS ESTIMATED IN NONHUMAN PRIMATES. THIS DEEP QUIESCENCE IS DIFFICULT TO OVERCOME IN VITRO, LIMITING THE ABILITY TO EFFECTIVELY EXPAND HUMAN HEMATOPOIETIC STEM CELLS. THE PROCESS MAY BE CONTROLLED BY PARTICULARLY HIGH LEVELS OF EXPRESSION OF CYCLIN-DEPENDENT KINASE INHIBITORS THAT RESTRICT ENTRY OF STEM CELLS INTO
CELL CYCLE, BLOCKING THE G1-S TRANSITION. MODIFYING THE LEVELS OF MOLECULES SUCH AS P21CIP1 AND P18INK4C IN THE LABORATORY HAS RESULTED IN INCREASED STEM CELL PROLIFERATION AND NUMBER IN MICE AND IN SOME LIMITED HUMAN CELL STUDIES. EXOGENOUS SIGNALS FROM THE NICHE ALSO APPEAR TO ENFORCE QUIESCENCE, INCLUDING THE ACTIVATION OF THE TYROSINE KINASE RECEPTOR TIE2 ON STEM CELLS BY ANGIOPOIETIN 1 ON OSTEOBLASTS.

THE REGULATION OF STEM CELL PROLIFERATION ALSO APPEARS TO CHANGE WITH AGE. IN MICE, THE CYCLIN-DEPENDENT KINASE INHIBITOR P16INK4A ACCUMULATES IN STEM CELLS IN OLDER ANIMALS AND IS ASSOCIATED WITH A CHANGE IN FIVE DIFFERENT STEM CELL FUNCTIONS, INCLUDING CELL CYCLING. LOWERING EXPRESSION OF P16INK4A IN OLDER ANIMALS IMPROVES STEM CELL CYCLING AND CAPACITY TO RECONSTITUTE HEMATOPOIESIS IN ADOPTIVE HOSTS, MAKING THEM SIMILAR TO YOUNGER ANIMALS. MATURE CELL NUMBERS ARE UNAFFECTED.

THEREFORE, MOLECULAR EVENTS GOVERNING THE SPECIFIC FUNCTIONS OF STEM CELLS ARE BEING GRADUALLY MADE CLEAR AND OFFER THE POTENTIAL OF NEW APPROACHES TO CHANGING STEM CELL FUNCTION FOR THERAPY. ONE CRITICAL STEM CELL FUNCTION THAT REMAINS POORLY DEFINED IS THE MOLECULAR REGULATION OF SELF-RENEWAL.

FOR MEDICINE, SELF-RENEWAL IS PERHAPS THE MOST IMPORTANT FUNCTION OF STEM CELLS BECAUSE IT IS CRITICAL IN REGULATING THE NUMBER OF STEM CELLS. STEM CELL NUMBER IS A KEY LIMITING PARAMETER FOR BOTH AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION.

WERE WE TO HAVE THE ABILITY TO USE FEWER STEM CELLS OR EXPAND LIMITED NUMBERS OF STEM CELLS EX VIVO, IT MIGHT BE POSSIBLE TO REDUCE THE MORBIDITY AND EXPENSE OF STEM CELL HARVESTS AND ENABLE USE OF OTHER STEM CELL SOURCES. SPECIFICALLY, UMBILICAL CORD BLOOD IS A RICH SOURCE OF STEM CELLS. HOWEVER, THE VOLUME OF CORD BLOOD UNITS IS EXTREMELY SMALL, AND THEREFORE THE TOTAL NUMBER OF HEMATOPOIETIC STEM CELLS THAT CAN BE OBTAINED IS GENERALLY ONLY SUFFICIENT TO TRANSPLANT AN INDIVIDUAL OF <40 KG. THIS LIMITATION RESTRICTS WHAT WOULD OTHERWISE BE AN EXTREMELY PROMISING SOURCE OF STEM CELLS.

TWO FEATURES OF CORD BLOOD STEM CELLS ARE PARTICULARLY...
IMPORTANT.
(1) THEY ARE DERIVED FROM A DIVERSITY OF INDIVIDUALS THAT FAR EXCEEDS
ADULT DONOR POOL AND THEREFORE CAN OVERCOME THE MAJORITY OF IMMU-
NOLOGIC CROSS-MATCHING OBSTACLES. (2) CORD BLOOD STEM CELLS HAVE A
LARGE NUMBER OF T CELLS ASSOCIATED WITH THEM, BUT (PARADOXICALLY) THEY
APPEAR TO BE ASSOCIATED WITH A LOWER INCIDENCE OF GRAFT-VERSUS-HOST
DIS-EASE WHEN COMPARED WITH SIMILARLY MISMATCHED STEM CELLS FROM OTHER
SOURCES. IF STEM CELL EXPANSION BY SELF-RENEWAL COULD BE ACHIEVED, THE
NUMBER OF CELLS AVAILABLE MIGHT BE SUFFICIENT FOR USE IN LARGER ADULTS.
AN ALTERNATIVE APPROACH TO THIS PROBLEM IS TO IMPROVE THE EFFICIENCY OF
ENGRAFTMENT OF DONOR STEM CELLS. GRAFT ENGINEERING IS EXPLORING METH-
ODS OF ADDING CELL COMPONENTS THAT MAY ENHANCE ENGRAFTMENT. FUR-
THERMORE, AT LEAST SOME DATA SUGGEST THAT DEPLETION OF HOST NK
(NATURAL KILLER) CELLS MAY LOWER THE NUMBER OF STEM CELLS NECESSARY TO
RECONSTITUTE HEMATOPOIESIS.
SOME LIMITED UNDERSTANDING OF SELF-RENEWAL EXISTS AND, INTRIGUINGLY,
IMPlicates gene products that are associated with the chromatin
state,
a high-order organization of chromosomal DNA that influences trans-
cription. These include members of the polycomb family, a group of
zinc finger-containing transcriptional regulators that interact with the
chromatin structure, contributing to the accessibility of groups of
genes for transcription. Certain members, including BMI-1 and GFI-1,
are important in enabling hematopoietic stem cell self-renewal
through modification of cell cycle regulators such as the cyclin-
dependent kinase inhibitors. In the absence of either of these genes, hematopoietic stem cells decline in number and function. In contrast,
dysregulation of BMI-1 has been associated with leukemia; it may pro-
mote leukemic stem cell self-renewal when it is overexpressed. Other transcription regulators have also been associated with self-
renewal, particularly homeobox, or “HOX,” genes. These transcription factors
are named for their ability to govern large numbers of genes,
including
those determining body patterning in invertebrates. HOXB4 is capable
of inducing extensive self-renewal of stem cells through its DNA-bind-
ing motif. Other members of the HOX family of genes have been noted
to affect normal stem cells, but they are also associated with leukemia.
External signals that may influence the relative self-renewal versus dif-
ferentiation outcomes of stem cell cycling include the notch ligands
and specific Wnt ligands. Intracellular signal transducing intermedi-
ates are also implicated in regulating self-renewal but, interestingly,
are
not usually associated with the pathways activated by notch or Wnt
receptors. They include PTEN, an inhibitor of the AKT pathway, and
STAT5, both of which are usually downstream of activated growth fac-
TOR RECEPTORS AND NECESSARY FOR NORMAL STEM CELL FUNCTIONS, INCLUDING SELF-RENEWAL, AT LEAST IN MOUSE MODELS. THE CONNECTIONS BETWEEN THESE MOLECULES REMAIN TO BE DEFINED, AND THEIR ROLE IN PHYSIOLOGIC REGULATION OF STEM CELL SELF-RENEWAL IS STILL POORLY UNDERSTOOD.

CANCER IS SIMILAR TO AN ORGAN WITH SELF-RENEWING CAPACITY

THE RELATIONSHIP OF STEM CELLS TO CANCER IS AN IMPORTANT EVOLVING DIMENSION OF ADULT STEM CELL BIOLOGY. CANCER MAY SHARE PRINCIPLES OF ORGANIZATION WITH NORMAL TISSUES. CANCER MIGHT HAVE THE SAME HIERARCHICAL ORGANIZATION OF CELLS WITH A BASE OF STEM-LIKE CELLS CAPABLE OF THE SIGNATURE STEM-CELL FEATURES, SELF-RENEWAL AND DIFFERENTIATION. THESE STEM-LIKE CELLS MIGHT BE THE BASIS FOR PERPETUATION OF THE TUMOR AND REPRESENT A SLOWLY DIVIDING, RARE POPULATION WITH DISTINCT REGULATORY MECHANISMS, INCLUDING A RELATIONSHIP WITH A SPECIALIZED MICROENVIRONMENT. A SUBPOPULATION OF SELF-RENEWING CELLS IN CANCER HAS BEEN DEFINED. A MORE SOPHISTICATED UNDERSTANDING OF THE STEM-CELL ORGANIZATION OF CANCERS MAY LEAD TO IMPROVED STRATEGIES FOR ATTACKING THE MANY COMMON AND DIFFICULT-TO-TREAT TYPES OF MALIGNANCIES THAT HAVE BEEN RELATIVELY REFRACTORY TO INTERVENTIONS AIMED AT DIVIDING CELLS.

DOES THE CONCEPT OF CANCER STEM CELLS PROVIDE INSIGHT INTO THE CELLULAR ORIGIN OF CANCER? THE FACT THAT SOME CELLS WITHIN A CANCER HAVE STEM CELL-LIKE PROPERTIES DOES NOT NECESSARILY MEAN THAT THE CANCER AROSE IN THE STEM CELL ITSELF. RATHER, MORE MATURE CELLS COULD HAVE ACQUIRED THE SELF-RENEWAL CHARACTERISTICS OF STEM CELLS. ANY SINGLE GENETIC EVENT IS UNLIKELY TO BE SUFFICIENT TO ENABLE FULL TRANSFORMATION OF A NORMAL CELL TO A FRANKLY MALIGNANT ONE. RATHER, CANCER IS A MULTISTEP PROCESS, AND FOR THE MULTIPLE STEPS TO ACCUMULATE, THE CELL OF ORIGIN MUST BE ABLE TO PERSIST FOR PROLONGED PERIODS. IT MUST ALSO BE ABLE TO GENERATE LARGE NUMBERS OF DAUGHTER CELLS. THE NORMAL STEM CELL HAS THESE PROPERTIES AND, BY VIRTUE OF ITS HAVING INTRINSIC SELF-RENEWAL CAPABILITY, MAY BE MORE READILY CONVERTED TO A MALIGNANT PHENOTYPE. THIS HYPOTHESIS HAS BEEN TESTED EXPERIMENTALLY IN THE HEMATOPOIETIC SYSTEM. TAKING ADVANTAGE OF THE CELL-SURFACE MARKERS THAT DISTINGUISH HEMATOPOIETIC CELLS OF VARYING MATURITY, STEM CELLS, PROGENITORS, PRECURSORS, AND MATURE CELLS CAN BE ISOLATED. POWERFUL TRANSFORMING GENE CONSTRUCTS WERE PLACED IN THESE CELLS, AND IT WAS FOUND THAT THE CELL WITH THE GREATEST POTENTIAL TO PRODUCE A MALIGNANCY WAS INDEED THE STEM CELL. THIS DOES NOT PROVE THAT STEM CELLS GIVE RISE TO ALL TUMORS, BUT IT DOES SUGGEST THAT STEM CELLS MAY BE SUSCEPTIBLE TO MALIGNANT CONVERSION AND MAY BE THE
POPULATION OF GREATEST INTEREST IN DEVELOPING STRATEGIES TO PROTECT AGAINST, MONITOR, OR TREAT NASCENT MALIGNANCY.

WHAT ELSE CAN HEMATOPOIETIC STEM CELLS DO?

SOME EXPERIMENTAL DATA HAVE SUGGESTED THAT HEMATOPOIETIC STEM CELLS OR OTHER CELLS MOBILIZED INTO THE CIRCULATION BY THE SAME FACTORS THAT MOBILIZE HEMATOPOIETIC STEM CELLS ARE CAPABLE OF PLAYING A ROLE IN HEALING THE VASCULAR AND TISSUE DAMAGE ASSOCIATED WITH STROKE AND MYOCARDIAL INFarCTION. THESE DATA ARE CONTROVERSIAL, AND THE APPLICABILITY OF A STEM-CELL APPROACH TO NONHEMATOPOIETIC CONDITIONS REMAINS EXPERIMENTAL. HOWEVER, THE APPLICATION OF THE EVOLVING KNOWLEDGE OF HEMATOPOIETIC STEM CELL BIOLOGY MAY LEAD TO WIDE-RANGING CLINICAL USES.

PAGE NO. 78

434 PART 4: REGENERATIVE MEDICINE

THE STEM CELL THEREFORE REPRESENTS A TRUE DUAL-EDGED SWORD. IT HAS TREMENDOUS HEALING CAPACITY AND IS ESSENTIAL FOR LIFE. UNCONTROLLED, IT CAN THREATEN THE LIFE IT MAINTAINS. UNDERSTANDING HOW STEM CELLS FUNCTION, THE SIGNALS THAT MODIFY THEIR BEHAVIOR, AND THE TISSUE NICHES THAT MODULATE STEM CELL RESPONSES TO INJURY AND DISEASE ARE CRITICAL FOR MORE EFFECTIVELY DEVELOPING STEM CELL-BASED MEDICINE. THAT ASPECT OF MEDICINE WILL INCLUDE THE USE OF THE STEM CELLS AND THE USE OF DRUGS TO TARGET STEM CELLS TO ENHANCE REPAIR OF DAMAGED TISSUES. IT WILL ALSO INCLUDE THE CAREFUL BALANCE OF INTERVENTIONS TO CONTROL STEM CELLS WHERE THEY MAY BE DYSFUNCTIONAL OR MALIGNANT.

69 TISSUE ENGINEERING
JENNIFER ANDERSON, JOSEPH P. VACANTI

THE ORIGINS OF TISSUE ENGINEERING DATE TO THE SIXTEENTH CENTURY WHEN COMPLEX SKIN FLAPS WERE USED TO REPLACE THE NOSE. MODERN TISSUE ENGINEERING COMBINES THE DISCIPLINES OF MATERIALS SCIENCES AND LIFE SCIENCES TO REPLACE A DISEASED OR DAMAGED ORGAN WITH A LIVING, FUNCTIONAL SUBSTITUTE. THE MOST COMMON TISSUE ENGINEERING APPROACH COMBINES CELLS AND MATRICES TO PRODUCE A LIVING STRUCTURE (FIG. 69-1). THESE STRATEGIES ALSO INCLUDE THE USE OF SCAFFOLDING, CELLS, AND GROWTH FACTORS TO SHAPE NEW TISSUES. THE TERM REGENERATIVE MEDICINE HAS EMERGED AS A CONCEPT
CELLULAR COMPONENTS OF TISSUE ENGINEERING

THE FOUNDATION OF TISSUE ENGINEERING IS THE COMBINATION OF A THREE-DIMENSIONAL SCAFFOLD WITH LIVE AND FUNCTIONAL CELLS. CELLS USED IN TISSUE ENGINEERING SHOULD BE EASILY ACCESSIBLE AND CAPABLE OF PROLIFERATION WHILE MAINTAINING THEIR DIFFERENTIATED FUNCTION. THERE ARE THREE POSSIBLE SOURCES FOR CELLS: AUTOLOGOUS, ALLOGENIC, AND XENOGENIC. AUTOLOGOUS CELLS ARE ISOLATED DIRECTLY FROM THE PATIENT. THEY HAVE THE ADVANTAGE OF AVOIDING IMMUNE-MEDIATED REJECTION. HOWEVER, A POTENTIAL LIMITATION IS THAT THEY MAY NOT BE AVAILABLE OR ABLE TO PROLIFERATE TO THE REQUIRED TISSUE MASS. ALLOGENIC CELLS ARE HARVESTED FROM A DONOR OTHER THAN THE PATIENT. THEY HAVE THE ADVANTAGE OF BEING MORE READILY AVAILABLE, BUT THE IMMUNE SYSTEM MUST BE MODULATED TO AVOID REJECTION. XENOGENIC CELLS, OR THOSE FROM A DIFFERENT SPECIES, MAY ALSO BE USED BUT ALSO RISK IMMUNE REJECTION OR TRANSMISSION OF ANIMAL PATHOGENS. ALTHOUGH CELLS SUCH AS FIBROBLASTS AND SMOOTH-MUSCLE CELLS PROLIFERATE RAPIDLY, OTHER CELLS PROLIFERATE SLOWLY OR LOSE THEIR TISSUE-SPECIFIC FUNCTION WHEN CULTURED, THEREBY LIMITING THEIR USE. IN ADDITION, CELLULAR CHARACTERISTICS MAY DEPEND ON THEIR LOCATION WITHIN THE BODY. FOR EXAMPLE, THE CELL-TO-CELL INTERACTIONS AND FUNCTION OF ENDOTHELIAL CELLS IN THE PULMONARY MICROVASCULATURE ARE DIFFERENT FROM THOSE IN THE BLOOD-BRAIN BARRIER. THE MICROENVIRONMENT OF THE CELL, INCLUDING THE PRESENCE OF OTHER CELL TYPES, SOLUBLE FACTORS, AND THE PRESENCE OF PHYSICAL OR MECHANICAL FORCES MAY ALSO ALTER THE FUNCTION OF A TRANSPLANTED STEM CELLS PROVIDE A PROMISING CELL SOURCE BECAUSE THEY ARE CAPABLE OF RAPID PROLIFERATION AND THEY CAN BE INDUCED TO DIFFERENTIATE INTO MULTIPLE CELL LINEAGES (CHAPS. 66 AND 67). HUMAN EMBRYONIC STEM CELLS ARE CAPABLE OF DIFFERENTIATING INTO ENDODERM, MESODERM, OR ECTODERM TISSUE TYPES. MULTIPOTENT ADULT STEM CELLS HAVE BEEN FOUND IN MULTIPLE MATURE TISSUES INCLUDING THE BONE MARROW, BRAIN, HEART, AND LIVER. IN ADDITION TO BEING ABLE TO DIFFERENTIATE INTO NUMEROUS LINEAGES, ADULT STEM CELLS GENERATE A RELATIVELY MUTED IMMUNE RESPONSE.

SCAFFOLDS

A SCAFFOLD PROVIDES A THREE-DIMENSIONAL FRAMEWORK TO SUPPORT THE TISSUE OR ORGAN-SPECIFIC CELLS. THE SCAFFOLD NOT ONLY PROVIDES MECHANICAL SUPPORT, BUT IT MUST ALSO SUPPLY CRITICAL NUTRIENTS AND TRANSPORT METABOLITES TO AND FROM THE DEVELOPING TISSUE. IMPORTANT SCAFFOLD PROPERTIES VARY DEPENDING ON THE TISSUE BUT TYPICALLY INCLUDE SPECIFIC
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FIGURE 69-1 SCHEMATIC OF BASIC PRINCIPLES OF TISSUE ENGINEERING. [FROM LANGER R, VACANTI J: TISSUE ENGINEERING. SCIENCE 260:1993 (FIG.1), WITH PERMISSION.]

BIOMECHANICAL PROPERTIES, POROSITY, BIOCOMPATIBILITY, AND APPROPRIATE SURFACE CHARACTERISTICS FOR CELL ADHESION AND DIFFERENTIATION. SCAFFOLDS CAN BE NATURAL MATERIALS OR SYNTHETIC POLYMERS AND ARE TYPICALLY BIODEGRADABLE. NATURAL MATERIALS SUCH AS COLLAGEN AND ALGINATES ARE BIOCOMPATIBLE. HOWEVER, IT IS DIFFICULT TO CONTROL THEIR MECHANICAL PROPERTIES, AND THEY MAY GENERATE AN IMMUNE REACTION. SYNTHETIC POLYMERS SUCH AS POLYGLYCOLIC ACID AND POLYETHYLENE, ON THE OTHER HAND, CAN BE TAILORED TO PROVIDE MORE ACCEPTABLE MECHANICAL PROPERTIES BUT ARE ASSOCIATED WITH A STRONG INFLAMMATORY RESPONSE. NONBIODEGRADABLE SYNTHETIC POLYMERS SUCH AS POLYTETRAFLUOROETHYLENE AND POLYETHYLENE PROVIDE WELL-DEFINED MECHANICAL AND STRUCTURAL PROPERTIES. HOWEVER, THEIR LONG-TERM PRESENCE IN THE BODY CAN LEAD TO A CHRONIC INFLAMMATORY RESPONSE, WHICH RESULTS IN POOR TISSUE QUALITY. POLYLACTIC ACID AND POLYGLYCOLIC ACID ARE EXAMPLES OF BIODEGRADABLE POLYMERS. ALTHOUGH THE DEGRADATION OF THESE MATERIALS CAN BE PARTIALLY

435 CHAPTER 69 TISSUE ENGINEERING

TABLE 69-1 FDA-APPROVED TISSUE-ENGINEERED PRODUCTS

NAME OF PRODUCT

ALLODERM (LIFECCELL)
APLIGRAPH (ORGANOGENSESIS)
CARTICEL
BRIEF DESCRIPTION

ACELLULAR DERMAL MATRIX FOR TISSUE REPAIR

LIVING SKIN EQUIVALENT APPROVED FOR THE
TREATMENT OF VENOUS LEG ULCERS AND
DIABETIC FOOT ULCERS
AUTOLOGOUS CHONDROCYTES APPROVED FOR
CARTILAGE REPAIR
LIVING SKIN EQUIVALENT APPROVED FOR FULL-
THICKNESS DIABETIC FOOT ULCERS
PORCINE SMALL-INTESTINE SUBMUCOSA FOR
REPLACEMENT OF DURA MATER
LIVING SKIN EQUIVALENT APPROVED FOR BURN
PATIENTS
LIVING SKIN EQUIVALENT APPROVED FOR BURN
PATIENTS
PORCINE SMALL-INTESTINE SUBMUCOSA FOR
DERMAL WOUNDS AND REINFORCEMENT OF
WEAKENED TISSUE

CONTROLLED, NONUNIFORM DEGRADATION AND VARYING DEGRADATION RATES
IN
DIFFERENT ANATOMIC LOCATIONS REPRESENT CHALLENGES.
THE SURFACE PROPERTIES OF THE MATERIALS USED FOR THE SCAFFOLD ARE IM-
PORTANT FOR ADHESION, MIGRATION, AND CELL DIFFERENTIATION. ONGOING RE-
SEARCH IS FOCUSED ON TETHERING GROWTH FACTORS OR PEPTIDE SEQUENCES TO
THE SURFACE OF THE SCAFFOLD TO IMPROVE ADHESION AND MIGRATION.

BIOREACTORS

INITIALLY, CELLS USED IN TISSUE ENGINEERING WERE CULTURED IN STATIC
CONDITIONS. IMPROVEMENTS IN BIOREACTOR TECHNOLOGY MORE CLOSELY
APPROXIMATE
PHYSIOLOGIC PARAMETERS FOR TISSUE GROWTH. BY MODULATING RATES OF
FLOW
AND MIXING, THE TRANSFER OF NUTRIENTS, GASES, METABOLITES, AND
REGULATORY
MOLECULES CAN BE MAXIMIZED. MECHANICAL STIMULI CAN ALSO IMPACT THE
NEWLY FORMING TISSUE. FOR EXAMPLE, TISSUE-ENGINEERED BLOOD VESSELS
EX-
POSED TO SHEAR STRESS IN A PULSATILE FLOW BIOREACTOR HAVE GREATER
BURST STRENGTH AND COLLAGEN CONTENT THAN THOSE NOT EXPOSED TO SHEAR STRESS.

TISSUE ENGINEERING SUCCESSES

SEVERAL TISSUE-ENGINEERED SKIN SUBSTITUTES HAVE BEEN APPROVED BY THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) AND IN USE FOR >10 YEARS (TABLE 69-1). ONE OF THESE PRODUCTS USES NEONATAL DERMAL FIBROBLASTS ISOLATED FROM HUMAN FORESKINS CULTURED ON A SCAFFOLD OF POLYLACTIDE COGlycolide. THE POLYMER SCAFFOLD GRADUALLY DEGRades IN THE PRESENCE OF WATER. A BILAYER SKIN SUBSTITUTE HAS ALSO BEEN DEVELOPED: DERMAL FIBROBLASTS ARE CULTURED IN A COLLAGEN SOLUTION AND THEN COATED WITH SEVERAL LAYERS OF KERATINOCYTES. CARTILAGE TISSUE ENGINEERING IS ALSO SHOWING PROMISE; AUTOLOGOUS CHONDROCYTES FROM A HEALTHY PORTION OF THE PATIENT’S JOINT ARE EXPANDED IN CULTURE AND THEN IMPLANTED INTO THE SITE OF INJURY. OTHER SCAFFOLD-BASED PRODUCTS ARE BASED ON PROCESSED ANIMAL SUBMUCOSA OR DURA. IN ADDITION TO THESE FDA-APPROVED PRODUCTS, NUMEROUS TISSUE-ENGINEERED PRODUCTS ARE CURRENTLY IN CLINICAL TRIALS (TABLE 69-2). ENGINEERED TISSUES BEING ACTIVELY INVESTIGATED INCLUDE BONE, MANDIBLE, TEETH, CARTILAGE, SKIN, CORNEA, BLADDER, URETHRA, SMALL-DIAMETER BLOOD VESSELS, AND THE PULMONARY ARTERY.

CHALLENGES TO TISSUE ENGINEERING

THE GREATEST SUCCESS IN TISSUE ENGINEERING TO DATE HAS BEEN IN TISSUES SUCH AS SKIN AND CARTILAGE WHERE THE REQUIREMENTS FOR NUTRIENTS AND OXYGEN ARE RELATIVELY LOW. DUE TO OXYGEN DIFFUSION LIMITATIONS, THE

**TABLE 69-2 TISSUE-ENGINEERED PRODUCTS IN CLINICAL TRIALS**

- TRC (AASTROM)
- LIVERX2000 (ALGENIX)
- ENCAPSULATED PROLIFERATED ISLET (AMCYTE)
- MYOCELL (BIOHEART)
- BIO SEED-C, BIO SEED-ORAL BONE (BIOTISSUE TECHNOLOGIES)
- E-MATRIX (ENCELLE)
- MARKLL (EXCORP)
- ICX-PRO, ICX-TRC (INTERCYTEX)
- HUCNS-SC (STEM CELL INC)
- NT-501 (NEUROTECH SA)
- PROCORD (PRONEURON)
- CHONDROCELECT (TIGENIX)
AUTOLOGOUS ADULT BONE MARROW CELLS FOR BONE GRAFTING
EXTRACORPOREAL LIVER ASSIST DEVICE
ENCAPSULATED ISLET CELLS

ENCAPSULATED CELLS FOR MYOCARDIAL INFARCTION
AUTOLOGOUS TISSUE REPAIR FOR BONE AND CARTILAGE
REPAIR OR REGENERATION OF DISEASED OR DAMAGED TISSUE
EXTRACORPOREAL LIVER ASSIST DEVICE
WOUND REPAIR AND HAIR REGENERATION
HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS
ENCAPSULATED CELL TECHNOLOGY FOR LONG-TERM DELIVERY OF THERAPEUTIC FACTORS TO RETINA
AUTOLOGOUS ACTIVATED MACROPHAGE THERAPY FOR PATIENTS WITH ACUTE COMPLETE SPINAL CORD INJURY
AUTOLOGOUS CHONDROCYTE IMPLANTATION
RETINAL PIGMENT EPITHELIAL CELLS IN MICRO-CARRIERS TO PROVIDE CONTINUOUS SOURCE OF DOPAMINE IN THE BRAIN
EXTRACORPOREAL LIVER ASSIST DEVICE

MAXIMAL THICKNESS OF AN ENGINEERED TISSUE IS 150-200 *M IF THERE IS NOT AN INTRINSIC CAPILLARY NETWORK. STRATEGIES USED TO OVERCOME THIS LIMITATION INCLUDE TRANSPLANTATION OF THE TISSUE DIRECTLY INTO THE PATIENT'S VASCULARATURE OR TRYING TO INDUCE ANGIGENESIS BY INCORPORATING GROWTH FACTORS SUCH AS VASCULAR ENDOTHELIAL CELL GROWTH FACTOR INTO THE SCAFFOLD. A MORE RECENT APPROACH INVOLVES THE CREATION OF AN INTRINSIC NETWORK OF VASCULAR CHANNELS IMMEDIATELY ADJACENT TO THE ENGINEERED TISSUE. A COMBINATION OF MICROELECTRO MECHANICAL SYSTEMS (MEMS) FABRICATION TECHNOLOGY AND COMPUTATIONAL MODELS OF FRACTAL BRANCHING ALLOWS THE CONSTRUCTION OF AN INTRINSIC MICROVASCULAR NETWORK SCAFFOLD WITHIN A BIOMATERIAL POLYMER. THIS PREFORMED CAPILLARY-LIKE NETWORK CAN BE SEEDED WITH CELLS AND ULTIMATELY SUSTAINS THE GROWTH AND FUNCTION OF COMPLEX THREE-DIMENSIONAL TISSUES. IMMUNE REJECTION OF ALLOGENIC CELLS IS ANOTHER MAJOR OBSTACLE. THE USE OF IMMUNOSUPPRESSIVE DRUGS IS NOT CONSIDERED AN OPTIMAL SOLU-
TION TO THIS PROBLEM. ONE POTENTIAL SOLUTION IS TO DEVELOP “UNIVERSAL DONOR” CELLS BY MASKING THE HISTOCOMPATIBILITY PROTEINS ON THE CELL SURFACE.
OFF-THE-SHELF AVAILABILITY WILL NEED TO BE ADDRESSED FOR TISSUE ENGINEERING PRODUCTS TO BE USED WIDELY. IDEALLY, PRODUCTS SHOULD BE REPRODUCIBLE AND AVAILABLE AT A WIDE VARIETY OF HOSPITALS, INCLUDING THOSE WITHOUT SOPHISTICATED FACILITIES FOR CELL CULTURE AND CELL PROLIFERATION.

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437 CHAPTER 70 NUTRITIONAL REQUIREMENTS AND DIETARY ASSESSMENT

PART 5: NUTRITION

70 NUTRITIONAL REQUIREMENTS AND DIETARY ASSESSMENT
JOHANNA DWYER

NUTRIENTS ARE SUBSTANCES THAT MUST BE SUPPLIED BY THE DIET BECAUSE THEY ARE NOT SYNTHESIZED IN THE BODY IN SUFFICIENT AMOUNTS. NUTRIENT REQUIREMENTS FOR GROUPS OF HEALTHY PERSONS HAVE BEEN DETERMINED EXPERIMENTALLY. FOR GOOD HEALTH WE REQUIRE ENERGY-PROVIDING NUTRIENTS (PROTEIN, FAT, AND CARBOHYDRATE), VITAMINS, MINERALS, AND WATER. SPECIFIC NUTRIENT REQUIREMENTS INCLUDE 9 ESSENTIAL AMINO ACIDS, SEVERAL FATTY ACIDS, 4 FAT-SOLUBLE VITAMINS, 10 WATER-SOLUBLE VITAMINS, AND CHOLINE. SEVERAL INORGANIC SUBSTANCES, INCLUDING 4 MINERALS, 7 TRACE MINERALS, 3 ELECTROLYTES, AND THE ULTRATRACE ELEMENTS, ALSO MUST BE SUPPLIED IN THE DIET. THE REQUIRED AMOUNTS OF THE ESSENTIAL NUTRIENTS DIFFER BY AGE AND PHYSIOLOGIC STATE. CONDITIONALLY ESSENTIAL NUTRIENTS ARE NOT REQUIRED
IN THE DIET BUT MUST BE SUPPLIED TO INDIVIDUALS WHO DO NOT SYNTHESIZE THEM IN ADEQUATE AMOUNTS, SUCH AS THOSE WITH GENETIC DEFECTS, THOSE HAVING PATHOLOGIC STATES WITH NUTRITIONAL IMPLICATIONS, AND DEVELOPMENTALLY IMMATURE INFANTS. MANY ORGANIC PHYTOCHEMICALS AND ZOOCHEMICALS PRESENT IN FOODS HAVE HEALTH EFFECTS. FOR EXAMPLE, DIETARY FIBER HAS BENEFICIAL EFFECTS ON GASTROINTESTINAL FUNCTION. OTHER BIOACTIVE FOOD CONSTITUENTS OR CONTAMINANTS SUCH AS LEAD MAY HAVE NEGATIVE HEALTH EFFECTS.

ESSENTIAL NUTRIENT REQUIREMENTS
ENERGY

FOR WEIGHT TO REMAIN STABLE, ENERGY INTAKE MUST MATCH ENERGY OUTPUT. THE MAJOR COMPONENTS OF ENERGY OUTPUT ARE RESTING ENERGY EXPENDITURE (REE) AND PHYSICAL ACTIVITY; MINOR SOURCES INCLUDE THE ENERGY COST OF METABOLIZING FOOD (THERMIC EFFECT OF FOOD OR SPECIFIC DYNAMIC ACTION) AND SHIVERING THERMOGENESIS (E.G., COLD-INDUCED THERMOGENESIS). THE AVERAGE ENERGY INTAKE IS ABOUT 2800 KCAL/D FOR AMERICAN MEN AND ABOUT 1800 KCAL/D FOR AMERICAN WOMEN, ALTHOUGH THESE ESTIMATES VARY WITH BODY SIZE AND ACTIVITY LEVEL. FORMULAS FOR ESTIMATING REE ARE USEFUL FOR ASSESSING THE ENERGY NEEDS OF AN INDIVIDUAL WhOSE WEIGHT IS STABLE. THUS, FOR MALES, REE = 900 + 10W, AND FOR FEMALES, REE = 700 + 7W, WHERE W IS WEIGHT IN KILOGRAMS. THE CALCULATED REE IS THEN ADJUSTED FOR PHYSICAL ACTIVITY LEVEL BY MULTIPLYING BY 1.2 FOR SEDENTARY, 1.4 FOR MODERATELY ACTIVE, OR 1.8 FOR VERY ACTIVE INDIVIDUALS. THE FINAL FIGURE PROVIDES A ROUGH ESTIMATE OF TOTAL CALORIC NEEDS IN A STATE OF ENERGY BALANCE. FORMULAS TO PROVIDE MORE PRECISE ESTIMATES OF ENERGY REQUIREMENTS ARE PROVIDED BY THE FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE, NATIONAL ACADEMY OF SCIENCES IN RECENT REPORTS ON DIETARY REFERENCE INTAKES. FOR FURTHER DISCUSSION OF ENERGY BALANCE IN HEALTH AND DISEASE, SEE CHAP. 72.

PROTEIN

DIETARY PROTEIN CONSISTS OF BOTH ESSENTIAL AND OTHER AMINO ACIDS THAT ARE REQUIRED FOR PROTEIN SYNTHESIS. THE NINE ESSENTIAL AMINO ACIDS ARE HISTIDINE, ISOLEUCINE, LEUCINE, LYSINE, METHIONINE/CYSTINE, PHENYLALANINE/TYROSINE, THREONINE, TRYPTOPHAN, AND VALINE. ALL AMINO ACIDS CAN BE USED FOR ENERGY, AND CERTAIN AMINO ACIDS (E.G., ALANINE) CAN ALSO BE USED FOR GLUCONEOGENESIS. WHEN ENERGY INTAKE IS INADEQUATE, PROTEIN INTAKE MUST BE INCREASED, SINCE INGESTED AMINO ACIDS ARE DIVERTED INTO PATHWAYS OF GLUCOSE SYNTHESIS AND OXIDATION. IN EXTREME ENERGY DEPRIVATION, PROTEIN-CALORIE MALNUTRITION MAY ENSUE (CHAP. 72). FOR ADULTS, THE RECOMMENDED DIETARY ALLOWANCE (RDA) FOR PROTEIN IS
ABOUT 0.6 G/KG DESIRABLE BODY WEIGHT PER DAY, ASSUMING THAT ENERGY NEEDS ARE MET AND THAT THE PROTEIN IS OF RELATIVELY HIGH BIOLOGIC VALUE.

CURRENT RECOMMENDATIONS FOR A HEALTHY DIET CALL FOR AT LEAST 10-14% OF CALORIES FROM PROTEIN. BIOLOGIC VALUE TENDS TO BE HIGHEST FOR ANIMAL PROTEINS, FOLLOWED BY PROTEINS FROM LEGUMES (BEANS), CEREALS (RICE, WHEAT, CORN), AND ROOTS. COMBINATIONS OF PLANT PROTEINS THAT COMPLEMENT ONE ANOTHER IN BIOLOGIC VALUE OR COMBINATIONS OF ANIMAL AND PLANT PROTEINS CAN INCREASE BIOLOGIC VALUE AND LOWER TOTAL PROTEIN REQUIREMENTS.

PROTEIN NEEDS INCREASE DURING GROWTH, PREGNANCY, LACTATION, AND REHABILITATION AFTER MALNUTRITION. TOLERANCE TO NORMAL AMOUNTS OF DIETARY PROTEIN IS DECREASED IN RENAL INSUFFICIENCY AND LIVER FAILURE, PRECIPITATING ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS OF THE LIVER.

**FAT AND CARBOHYDRATE**

FATS ARE A CONCENTRATED SOURCE OF ENERGY AND CONSTITUTE ON AVERAGE 34% OF CALORIES IN U.S. DIETS. FOR OPTIMAL HEALTH, SATURATED FAT AND TRANS-FAT SHOULD BE LIMITED TO <10% OF CALORIES, AND POLYUNSATURATED FATS TO <10% OF CALORIES, WITH NIONOUNSATURATED FATS CONSTITUTING THE REMAINDER OF FAT INTAKE. AT LEAST 55% OF TOTAL CALORIES SHOULD BE DERIVED FROM CARBOHYDRATES. THE BRAIN REQUIRES ABOUT 100 G/D OF GLUCOSE FOR FUEL; OTHER TISSUES USE ABOUT 50 G/D. SOME TISSUES (E.G., BRAIN AND RED BLOOD CELLS) RELY ON GLUCOSE SUPPLIED EITHER EXOGENOUSLY OR FROM MUSCLE PROTEOLYSIS. OVER TIME, SOME ADAPTATIONS IN CARBOHYDRATE NEEDS ARE POSSIBLE IN OTHER TISSUES DURING HYPOCALORIC STATES (CHAP. 339).

**WATER**

FOR ADULTS, 1.0-1.5 ML WATER PER KCAL OF ENERGY EXPENDITURE IS SUFFICIENT UNDER USUAL CONDITIONS TO ALLOW FOR NORMAL VARIATIONS IN PHYSICAL ACTIVITY, SWEATING, AND SOLUTE LOAD OF THE DIET. WATER LOSSES INCLUDE 50-100 ML/D IN THE FECES, 500-1000 ML/D BY EVAPORATION OR EXHALATION, AND, DEPENDING ON THE RENAL SOLUTE LOAD, 1000 ML/D IN THE URINE. IF EXTERNAL LOSSES INCREASE, INTAKES MUST INCREASE ACCORDINGLY TO AVOID UNDEHYDRATION. FEVER INCREASES WATER LOSSES BY APPROXIMATELY 200 ML/D PER #ºC; DIARRHEAL LOSSES VARY BUT MAY BE AS GREAT AS 5 L/D WITH SEVERE DIARRHEA. HEAVY SWEATING AND VOMITING ALSO INCREASE WATER LOSSES. WHEN RENAL FUNCTION IS NORMAL AND SOLUTE INTAKES ARE ADEQUATE, THE KIDNEYS CAN ADJUST TO INCREASED WATER INTAKE BY EXCRETING UP TO 18 L/D OF EXCESS WATER (CHAP. 334). HOWEVER, OBLIGATORY URINE OUTPUTS CAN COMPROMISE HYDRATION STATUS WHEN THERE IS INADEQUATE INTAKE OR WHEN LOSSES INCREASE IN DISEASE OR KIDNEY DAMAGE.

INFANTS HAVE HIGH REQUIREMENTS FOR WATER BECAUSE OF THEIR LARGE RATIO OF SURFACE AREA TO VOLUME, THE LIMITED CAPACITY OF THE IMMATURE KIDNEY TO
HANDLE HIGH RENAL SOLUTE LOADS, AND THEIR INABILITY TO COMMUNICATE THEIR THIRST. DURING PREGNANCY, 30 ML/D ADDITIONAL WATER IS NEEDED. DURING LACTATION, MILK PRODUCTION INCREASES WATER REQUIREMENTS BY APPROXIMATELY 1000 ML/D, OR 1 ML FOR EACH ML OF MILK PRODUCED. SPECIAL ATTENTION MUST BE PAID TO THE WATER NEEDS OF THE ELDERLY, WHO HAVE REDUCED TOTAL BODY WATER AND BLUNTED THIRST SENSATION, AND MAY BE TAKING DIURETICS.

OTHER NUTRIENTS

SEE CHAP. 71 FOR A DETAILED DESCRIPTION OF VITAMINS AND TRACE MINERALS.

DIETARY REFERENCE INTAKES AND RECOMMENDED DIETARY ALLOWANCES


INFANTS
0-6 MO
7-12 MO
CHILDREN
1-3 Y
4-8 Y
MALES
9-13 Y
14-18 Y
19-30 Y
31-50 Y
51-70 Y
>70 Y
FEMALES
9-13 Y
14-18 Y
19-30 Y
31-50 Y
51-70 Y
>70 Y
PREGNANCY
*18 Y
19-30 Y
31-50 Y
LACTATION
*18 Y
19-30 Y
31-50 Y

VITAMIN, *G/D

A###A

400
500

300
400

600
900
900
900
900
900
900
900
600
700
700
700
700
700
750
770
C
40
50

15
25
45
75
90
90
90
90

45
65
75
75
75
75
75

80
85
85

115
120
120

D###B, C
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MG/D###E

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**BLOTIN,**  
*G/D*

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**CHOLINE,**  
*MG/D*##G

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### AAS Retinol Activity Equivalents (RAEs). 1 RAE = 1 *G Retinol, 12 *G *-Carotene, 24 *G *-Carotene, or 24 *G *-Cryptoxanthin. To calculate RAES from Retinol Equivalents (RES) of provitamin A carotenoids in foods, divide the RES by 2. For preformed vitamin A in foods or supplements and for provitamin A carotenoids in supplements, 1 RE = 1 RAE.

### DAS *-Tocopherol. *-Tocopherol includes *RRR*-*-Tocopherol the only form of *-Tocopherol that occurs naturally in foods, and the 2R-stereoisomeric forms of *-Tocopherol (*RRR-, *RRS-, *RSR-, and *RSS*-*-Tocopherol) that occur in fortified foods and supplements. It does not include the 2S-stereoisomeric forms of *-Tocopherol (*SRR-, *SSR-, *SRS-).
AND SSS-
*-TOCOPHEROL), ALSO FOUND IN FORTIFIED FOODS AND SUPPLEMENTS.
###EAS NIACIN EQUIVALENTS (NE). 1 MG OF NIACIN = 60 MG OF TRYPTOPHAN; 0-6 MONTHS = PRE-
FORMED NIACIN (NOT NE).
###*AS DIETARY FOLATE EQUIVALENTS (DFES). 1 DFE = 1 *G FOOD FOLATE = 0.6 *G OF FOLIC ACID FROM
FORTIFIED FOOD OR AS A SUPPLEMENT CONSUMED WITH FOOD = 0.5 *G OF A SUPPLEMENT TAKEN
ON AN EMPTY STOMACH.
###*ALTHOUGH ALS HAVE BEEN SET FOR CHOLINE, THERE ARE FEW DATA TO ASSESS WHETHER A DIETARY
SUPPLY OF CHOLINE IS NEEDED AT ALL STAGES OF THE LIFE CYCLE, AND IT MAY BE THAT THE CHOLINE
REQUIREMENT CAN BE MET BY ENDOGENOUS SYNTHESIS AT SOME OF THESE STAGES.
###*BECAUSE 10 TO 30% OF OLDER PEOPLE MAY MALABSORB FOOD-BOUND B###12, IT IS ADVISABLE FOR
THOSE ≥50 YEARS TO MEET THEIR RDA MAINLY BY CONSUMING FOODS FORTIFIED WITH B###12 OR A
SUPPLEMENT CONTAINING B###12.
###*IN VIEW OF EVIDENCE LINKING INADEQUATE FOLATE INTAKE WITH NEURAL TUBE
DEFECTS IN THE FE-
TUS. IT IS RECOMMENDED THAT ALL WOMEN CALLABLE OF BECOMING PREGNANT
CONSUME 400 *G
FROM SUPPLEMENTS OR FORTIFIED FOODS IN ADDITION TO INTAKE OF FOOD FOLATE
FROM A VARIED
DIET.
###*IT IS ASSUMED THAT WOMEN WILL CONTINUE CONSUMING 400 *G FROM
SUPPLEMENTS OR FORTI-
FIED FOOD UNTIL THEIR PREGNANCY IS CONFIRMED AND THEY ENTER PRENATAL CARE,
WHICH ORDI-
NARILY OCCURS AFTER THE END OF THE PERICONCEPTBNAL PERIOD-THE CRITICAL TIME FOR
FORMATION OF THE NEURAL TUBE.

SOURCE: FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE-NATIONAL ACADEMY
OF SCIENCES
DIETARYREFERENCE INTAKES 2000, 2002, REPRINTED WITH PERMISSION. COURTESY OF
THE NA-
TIONAL ACADEMY PRESS, WASHINGTON, DC. HTTP://WWW.NAP.EDU

ESTIMATED AVERAGE REQUIREMENT

WHEN FLORID MANIFESTATIONS OF THE CLASSIC DIETARY DEFICIENCY DISEASES
SUCH AS RICKETS, SCURVY, XEROPHTHALMIA, AND PROTEIN-CALORIE MALNUTRI-
TION WERE COMMON, NUTRIENT ADEQUACY WAS INFERRED FROM THE ABSENCE
OF THEIR CLINICAL SIGNS. LATER, IT WAS DETERMINED THAT BIOCHEMICAL AND
OTHER CHANGES WERE EVIDENT LONG BEFORE THE CLINICAL DEFICIENCY
BECAME
APPARENT. CONSEQUENTLY, CRITERIA OF NUTRIENT ADEQUACY ARE NOW BASED
ON BIOLOGIC MARKERS WHEN THEY ARE AVAILABLE. PRIORITY IS GIVEN TO
SENSI-
TIVE BIOCHEMICAL, PHYSIOLOGIC, OR BEHAVIORAL TESTS THAT REFLECT EARLY
CHANGES IN REGULATORY PROCESSES OR MAINTENANCE OF BODY STORES OF NU-
TRIENTS. CURRENT DEFINITIONS FOCUS ON THE AMOUNT OF A NUTRIENT THAT MINIMIZES THE RISK OF CHRONIC DEGENERATIVE DISEASES. THE EAR IS THE AMOUNT OF A NUTRIENT ESTIMATED TO BE ADEQUATE FOR HALF OF THE HEALTHY INDIVIDUALS OF A SPECIFIC AGE AND SEX. THE TYPES OF EVIDENCE AND CRITERIA USED TO ESTABLISH NUTRIENT REQUIREMENTS VARY BY NUTRIENT, AGE, AND PHYSIOLOGIC GROUP. THE EAR IS NOT USEFUL CLINICALLY FOR ESTIMATING NUTRIENT ADEQUACY IN INDIVIDUALS BECAUSE IT IS A MEDIAN REQUIREMENT FOR A GROUP; 50% OF INDIVIDUALS IN A GROUP FALL BELOW THE REQUIREMENT AND 50% FALL ABOVE IT. THUS, A PERSON WITH A USUAL INTAKE AT THE EAR HAS A 50% RISK OF AN INADEQUATE INTAKE. FOR THESE REASONS, OTHER STANDARDS, DESCRIBED BELOW, ARE MORE USEFUL FOR CLINICAL PURPOSES.

RECOMMENDED DIETARY ALLOWANCES


ADEQUATE INTAKE

IT IS NOT POSSIBLE TO SET AN RDA FOR SOME NUTRIENTS THAT DO NOT HAVE AN ESTABLISHED EAR. IN THIS CIRCUMSTANCE, THE AI IS BASED ON OBSERVED, OR EXPERIMENTALLY DETERMINED, APPROXIMATIONS OF NUTRIENT INTAKES IN HEALTHY PEOPLE. IN THE DRIS ESTABLISHED TO DATE, ALS RATHER THAN RDAS ARE PROPOSED FOR INFANTS UP TO AGE 1 YEAR, AS WELL AS FOR CALCIUM, CHROMIUM, VITAMIN D, FLUORIDE, MANGANESE, PANTOTHENIC ACID, BIOTIN, CHOLINE, SODIUM, CHLORIDE, POTASSIUM, AND WATER FOR PERSONS OF ALL AGES.
TOLERABLE UPPER LEVELS OF NUTRIENT INTAKE

Healthy individuals derive no established benefit from consuming nutrient levels above the RDA or AI. Excessive nutrient intake can disturb body functions and cause acute, progressive, or permanent disabilities. The tolerable UL is the highest level of chronic nutrient intake (usually daily) that is unlikely to pose a risk of adverse health effects for most of the population. Data on the adverse effects of large amounts of many nutrients are unavailable or too limited to establish a UL. Therefore, the lack of a UL does not mean that the risk of adverse effects from high intake is nonexistent. Individual nutrients in foods that most people eat rarely reach levels that exceed the UL. However, nutritional supplements provide more concentrated amounts of nutrients per dose and, as a result, pose a greater potential risk of toxicity. Nutrient supplements are labeled with “Supplement Facts” that express the amount of nutrient in absolute units or as the percent of the DV provided per recommended serving size. Total nutrient consumption, including both food and supplements, should not exceed RDA levels.

FACTORS ALTERING NUTRIENT NEEDS

The DRIs are affected by age, sex, rate of growth, pregnancy, lactation, physical activity, composition of diet, coexisting diseases, and drugs. When only slight differences exist between the requirements for nutrient sufficiency and excess, dietary planning becomes more difficult.

PHYSIOLOGIC FACTORS

Growth, strenuous physical activity, pregnancy, and lactation increase needs for energy and several essential nutrients, including water. Energy needs rise during pregnancy, due to the demands of fetal growth, and during lactation, because of the increased energy required for milk production. Energy needs decrease with loss of lean body mass, the major determinant of REE. Because both health and physical activity tend to decline with age, energy needs in older persons, especially those over 70, tend to be less than those of younger persons.

DIETARY COMPOSITION

Dietary composition affects the biologic availability and utilization of nutrients. For example, the absorption of iron may be impaired by
HIGH AMOUNTS OF CALCIUM OR LEAD; NON-HEME IRON UPTAKE MAY BE IMPAIRED BY THE LACK OF ASCORBIC ACID AND AMINO ACIDS IN THE MEAL. PROTEIN UTILIZATION BY THE BODY MAY BE DECREASED WHEN ESSENTIAL AMINO ACIDS ARE NOT PRESENT IN SUFFICIENT AMOUNTS. ANIMAL FOODS, SUCH AS

### TABLE 70-2 DIETARY REFERENCE INTAKES: RECOMMENDED INTAKES FOR INDIVIDUALS-ELEMENTS

#### LIFE-STAGE GROUP

**INFANTS**
- 0-6 MO
- 7-12 MO

**CHILDREN**
- 1-3 Y
- 4-8 Y

**MALES**
- 9-13 Y
- 14-18 Y
- 19-30Y
- 31-50Y
- 51-70Y
- >70 Y

**FEMALES**
- 9-13 Y
- 14-18 Y
- 19-30Y
- 31-50Y
- 51-70Y
- >70 Y

**PREGNANCY**
- *18 Y
- 19-30Y
- 31-50Y

**LACTATION**
- *18 Y
- 19-30Y
- 31-50Y

**CALCIUM, MG/D**

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<th>7-12 MOS</th>
<th>1-3 Y</th>
<th>4-8 Y</th>
<th>9-13 Y</th>
<th>14-18 Y</th>
<th>19-30 Y</th>
<th>31-50 Y</th>
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* Values for pregnancy and lactation are age-specific.
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| NOTE: THIS TABLE PRESENTS RECOMMENDED DIETARY ALLOWANCES (RDAS) IN **BOLD** TYPE AND ADEQUATE INTAKES (ALS) IN ORDINARY TYPE. RDAS AND ALS MAY BOTH BE USED AS GOALS FOR INDIVIDUAL INTAKE. RDAS ARE SET TO MEET THE NEEDS OF ALMOST ALL INDIVIDUALS (97 TO 98%) IN A GROUP. FOR HEALTHY BREASTFED INFANTS, THE AI IS THE MEAN INTAKE. THE AI FOR OTHER LIFE STAGE AND GENDERGROUPS IS BELIEVED TO COVER NEEDS OF ALL INDIVIDUALS IN THE GROUP, BUT LACK OF DATA OR UNCERTAINTY IN THE DATA PREVENT BEING ABLE TO SPECIFY WITH CONFIDENCE THE PERCENTAGE OF INDIVIDUALS COVERED BY THIS INTAKE. 

**SOURCE:** FOOD AND NUTRITION BOARD, INSTITUTE OF MEDICINE-NATIONAL ACADEMY OF SCIENCES DIETARY REFERENCE INTAKES, 2000, 2002, REPRINTED WITH PERMISSION. COURTESY OF THE NATIONAL ACADEMY PRESS, WASHINGTON, DC. **HTTP://WWW.NAP.EDU** |
MILK, EGGS, AND MEAT, HAVE HIGH BIOLOGIC VALUES WITH MOST OF THE NEEDED AMINO ACIDS PRESENT IN ADEQUATE AMOUNTS. PLANT PROTEINS IN CORN (MAIZE), SOY, AND WHEAT HAVE LOWER BIOLOGIC VALUES AND MUST BE COMBINED WITH OTHER PLANT OR ANIMAL PROTEINS TO ACHIEVE OPTIMAL UTILIZATION BY THE BODY.

ROUTE OF ADMINISTRATION

THE RDAS APPLY ONLY TO ORAL INTAKES. WHEN NUTRIENTS ARE ADMINISTERED PARENTERALLY, SIMILAR VALUES CAN SOMETIMES BE USED FOR AMINO ACIDS, CARBOHYDRATES, FATS, SODIUM, CHLORIDE, POTASSIUM, AND MOST OF THE VITAMINS, SINCE THEIR INTESTINAL ABSORPTION IS NEARLY 100%. HOWEVER, THE ORAL BIOAVAILABILITY OF MOST MINERAL ELEMENTS MAY BE ONLY HALF THAT OBTAINED BY PARENTERAL ADMINISTRATION. FOR SOME NUTRIENTS THAT ARE NOT READILY STORED IN THE BODY, OR CANNOT BE STORED IN LARGE AMOUNTS, TIMING OF ADMINISTRATION MAY ALSO BE IMPORTANT. FOR EXAMPLE, AMINO ACIDS CANNOT BE USED FOR PROTEIN SYNTHESIS IF THEY ARE NOT SUPPLIED TOGETHER; INSTEAD THEY WILL BE USED FOR ENERGY PRODUCTION.

DISEASE

SPECIFIC DIETARY DEFICIENCY DISEASES INCLUDE PROTEIN-CALORIE MALNUTRITION; IRON, IODINE, AND VITAMIN A DEFICIENCY; MEGALOBLASTIC ANEMIA DUE TO VITAMIN B12 OR FOLIC ACID DEFICIENCY; VITAMIN D-DEFICIENCY RICKETS; SCURVY DUE TO LACK OF ASCORBIC ACID; BERIBERI DUE TO LACK OF THIAMINE; AND PELLAGRA DUE TO LACK OF NIACIN AND PROTEIN (CHAPS. 71 AND 72). EACH DEFICIENCY DISEASE IS CHARACTERIZED BY IMBALANCES AT THE CELLULAR LEVEL BETWEEN THE SUPPLY OF NUTRIENTS OR ENERGY AND THE BODY’S NUTRITIONAL NEEDS FOR GROWTH, MAINTENANCE, AND OTHER FUNCTIONS. IMBALANCES IN NUTRIENT INTAKES ARE RECOGNIZED AS RISK FACTORS FOR CERTAIN CHRONIC DEGENERATIVE DISEASES, SUCH AS SATURATED AND TRANS-FAT AND CHOLESTEROL IN CORONARY ARTERY DISEASE; SODIUM IN HYPERTENSION; OBESITY IN HORMONE-DEPENDENT ENDOMETRIAL AND BREAST CANCERS; AND ETHANOL IN ALCOHOLISM. HOWEVER, THE ETIOLOGY AND PATHOGENESIS OF THESE DISORDERS ARE MULTIFACTORIAL, AND DIET IS ONLY ONE OF MANY RISK FACTORS.

OSTEOPOROSIS, FOR EXAMPLE, IS ASSOCIATED WITH CALCIUM DEFICIENCY AS WELL AS RISK FACTORS RELATED TO ENVIRONMENT (E.G., SMOKING, SEDENTARY LIFESTYLE), PHYSIOLOGY (E.G., ESTROGEN DEFICIENCY), GENETIC DETERMINANTS (E.G., DEFECTS IN COLLAGEN METABOLISM), AND DRUG USE (CHRONIC STEROIDS) (CHAP. 348).

DIETARY ASSESSMENT

IN CLINICAL SITUATIONS, NUTRITIONAL ASSESSMENT IS AN ITERATIVE PROCESS
That involves (1) screening for malnutrition; (2) assessing food and dietary supplement intake, and establishing the absence or presence of malnutrition and its possible causes; and (3) planning for the most appropriate nutritional therapy. Some disease states affect the bioavailability, requirements, utilization, or excretion of specific nutrients. In these circumstances, specific measurements of various nutrients may be required to ensure adequate replacement (Chap. 72).

Most health care facilities have a nutrition screening process in place for identifying possible malnutrition after hospital admission. Nutritional screening is required by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO), but there are no universally recognized or validated standards. The factors that are usually assessed include abnormal weight for height or body mass index (e.g., BMI <18.5 or >25); reported weight change (involuntary loss or gain of >5 kg in the past 6 months) (Chap. 41); diagnoses with known nutritional implications (metabolic disease, any disease affecting the gastrointestinal tract, alcoholism, and others); present therapeutic dietary prescription; chronic poor appetite; presence of chewing and swallowing problems or major food intolerances; need for assistance with preparing or shopping for food, eating, or other aspects of self care; and social isolation. Reassessment of nutrition status should occur periodically in hospitalized patients—at least once every week.

A more complete dietary assessment is indicated for patients who exhibit a high risk of malnutrition based on nutrition screening. The type of assessment varies with the clinical setting, severity of the patient’s illness, and stability of his or her condition.

**Acute Care Settings**

Acute care settings, anorexia, various diseases, test procedures, and medications can compromise dietary intake. Under such circumstances, the goal is to identify and avoid inadequate intake and ensure appropriate alimentation. Dietary assessment focuses on what patients are currently eating, whether they are able and willing to eat, and whether they experience any problems with eating. Dietary intake assessment is based on information from observed intakes; medical record; history; clinical examination; and anthropometric, biochemical, and functional status. The objective is to gather enough information to establish the likelihood of malnutrition due to poor dietary intake or other causes and to assess whether nutritional therapy is indicated. Simple observations may suffice to suggest inadequate oral intake.
THESE INCLUDE DIETITIANS’ AND NURSES’ NOTES, THE AMOUNT OF FOOD EATEN ON TRAYS, FREQUENT TESTS AND PROCEDURES THAT ARE LIKELY TO CAUSE MEALS TO BE SKIPPED, NUTRITIONALLY INADEQUATE DIET ORDERS SUCH AS CLEAR LIQUIDS OR FULL LIQUIDS FOR MORE THAN A FEW DAYS, FEVER, GASTROINTESTINAL DISTRESS, VOMITING, DIARRHEA, A COMATOSE STATE, AND DISEASES OR TREATMENTS THAT INVOLVE ANY PART OF THE ALIMENTARY TRACT. ACUTELY ILL PATIENTS WITH DIET-RELATED DIS- EASES SUCH AS DIABETES REQUIRE ASSESSMENT BECAUSE AN INAPPROPRIATE DIET MAY EXACERBATE THESE CONDITIONS AND ADVERSELY AFFECT OTHER THERAPIES.

ABNORMAL BIOCHEMICAL VALUES [SERUM ALBUMIN LEVELS <35 G/L (<3.5 MG/DL); SERUM CHOLESTEROL LEVELS <3.9 MMOL/L (<150 MG/DL)] ARE NONSPECIFIC BUT MAY ALSO INDICATE A NEED FOR FURTHER NUTRITIONAL ASSESSMENT. MOST THERAPEUTIC DIETS OFFERED IN HOSPITALS ARE CALCULATED TO MEET INDIVIDUAL NUTRIENT REQUIREMENTS AND THE RDA. HOWEVER, THERE ARE EXCEPTIONS INCLUDING CLEAR LIQUIDS, SOME FULL LIQUID DIETS, AND TEST DIETS, WHICH ARE INADEQUATE FOR SEVERAL NUTRIENTS AND SHOULD NOT BE USED, IF POSSIBLE, FOR MORE THAN 24 H. AS MUCH AS HALF OF THE FOOD SERVED TO HOSPITALIZED PATIENTS IS NOT EATEN, SO IT CANNOT BE ASSUMED THAT THE INTAKES OF HOSPITALIZED PATIENTS ARE ADEQUATE. DIETARY ASSESSMENT SHOULD COMPARE HOW MUCH AND WHAT FOOD THE PATIENT HAS CONSUMED WITH THE DIET THAT HAS BEEN PROVIDED. MAJOR DEVIATIONS IN INTAKES OF ENERGY, PROTEIN, FLUIDS, OR OTHER NUTRIENTS OF SPECIAL CONCERN FOR THE PATIENT’S ILLNESS SHOULD BE NOTED AND CORRECTED. NUTRITIONAL MONITORING IS ESPECIALLY IMPORTANT FOR PATIENTS WHO ARE VERY ILL AND WHO HAVE EXTENDED LENGTHS OF STAY. PATIENTS WHO ARE FED BY SPECIAL ENTERAL AND PARENTERAL ROUTES ALSO REQUIRE SPECIAL NUTRITIONAL AS- SESSMENT AND MONITORING BY PHYSICIANS WITH TRAINING IN NUTRITION SUPPORT AND/OR DIETITIANS WITH CERTIFICATION IN NUTRITION SUPPORT (CHAP. 73).

AMBULATORY SETTINGS

THE AIM OF DIETARY ASSESSMENT IN THE OUTPATIENT SETTING IS TO DETERMINE WHETHER THE PATIENT’S USUAL DIET IS A HEALTH RISK IN ITSELF OR IF IT CONTRIBUTES TO EXISTING CHRONIC DISEASE-RELATED PROBLEMS. DIETARY ASSESSMENT ALSO PROVIDES THE BASIS FOR PLANNING A DIET THAT FULFILLS THERAPEUTIC GOALS WHILE ENSURING PATIENT ADHERENCE. THE OUTPATIENT DIETARY ASSESSMENT
SHOULD REVIEW THE ADEQUACY OF PRESENT AND USUAL FOOD INTAKES, INCLUDING VITAMIN AND MINERAL SUPPLEMENTS, MEDICATIONS, AND ALCOHOL, AS ALL OF THESE MAY AFFECT THE PATIENT’S NUTRITIONAL STATUS. THE ASSESSMENT SHOULD FOCUS ON THE DIETARY CONSTITUENTS THAT ARE MOST LIKELY TO BE INVOLVED OR COMPROMISED BY A SPECIFIC DIAGNOSIS, AS WELL AS ANY COMORBIDITIES THAT ARE PRESENT. MORE THAN ONE DAY’S INTAKE SHOULD BE REVIEWED TO PROVIDE A BETTER REPRESENTATION OF THE USUAL DIET. THERE ARE MANY WAYS TO ASSESS THE ADEQUACY OF THE PATIENT’S HABITUAL DIET. THESE INCLUDE A FOOD GUIDE, A FOOD EXCHANGE LIST, A DIET HISTORY, OR A FOOD FREQUENCY QUESTIONNAIRE. A COMMONLY USED FOOD GUIDE FOR HEALTHY PERSONS IS THE USDA’S FOOD PYRAMID, WHICH IS USEFUL AS A BASIS FOR IDENTIFYING INADEQUATE INTAKES OF ESSENTIAL NUTRIENTS, AS WELL AS LIKELY EXCESSES IN FAT, SATURATED FAT, SODIUM, SUGAR, AND ALCOHOL (TABLE 70-3). THE GUIDE IS AVAILABLE ONLINE (WWW.MYPYRAMID.GOV) AND CAN BE TAILORED TO THE NEEDS OF PERSONS OF DIFFERENT AGES AND LIFE STAGES BY VARYING THE NUMBER OF SERVINGS. THE PROCESS OF REVIEWING THE GUIDE WITH PATIENTS HELPS TO IDENTIFY FOOD GROUPS EATEN IN EXCESS OF RECOMMENDATIONS OR IN INSUFFICIENT QUANTITIES AND HELPS THEM TO TRANSITION TO HEALTHIER DIETARY PATTERNS. FOR THOSE PRESCRIBED THERAPEUTIC DIETS, ASSESSMENT AGAINST PRESCRIPTIONS STATED AS FOOD EXCHANGE LISTS MAY BE USEFUL. THESE INCLUDE, FOR EXAMPLE, THE AMERICAN DIABETES ASSOCIATION FOOD EXCHANGE LISTS FOR DIABETES, OR THE AMERICAN DIETETIC ASSOCIATION FOOD EXCHANGE LISTS FOR RENAL DISEASE.

441 CHAPTER 71 VITAMIN AND TRACE MINERAL DEFICIENCY AND EXCESS

TABLE 70-3 MY PYRAMID: THE USDA FOOD GUIDE PYRAMID FOR HEALTHY PERSONS

SERVINGS AND EXAMPLES OF STANDARD PORTION SIZES

FRUITS, CUPS
VEGETABLES, CUPS
GRAINS, OZ EQ
(1 SLICE BREAD, 1 CUP READY TO EAT CEREAL, 0.5 CUP COOKED RICE, PASTA, COOKED CEREAL)
MEAT AND BEANS, OZ EQ
(1 OZ LEAN MEAT, POULTRY, OR FISH; 
1 EGG, 1 TBSP. PEANUT BUTTER, 
0.25 CUP COOKED DRY BEANS, OR 
0.5 OZ NUTS OR SEEDS) 
MILK, CUPS 
(1 CUP MILK OR YOGURT, 1.5 OZ 
NATURAL OR 2 OZ PROCESSED 
CHEESE) 
OILS, TSP 
DISCRETIONARY CALORIE ALLOWANCE, 
KCAL (REMAINING CALORIES AFTER 
ACCOUNTING FOR ALL OF THE ABOVE)

LOWER: 
1600 KCAL

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132 

MODERATE: 
2200 KCAL

2 
3 
7 

6 
3 
6 
290 

HIGHER: 
2800 KCAL

2.5 
3.5 
10 

7 
3 
8 
426
VITAMINS AND TRACE MINERALS ARE REQUIRED CONSTITUENTS OF THE HUMAN DIET SINCE THEY ARE EITHER INADEQUATELY SYNTHESIZED OR NOT SYNTHESIZED IN THE HUMAN BODY. ONLY SMALL AMOUNTS OF THESE SUBSTANCES ARE NEEDED FOR CARRYING OUT ESSENTIAL BIOCHEMICAL REACTIONS (E.G., ACTING AS COENZYMES OR PROSTHETIC GROUPS). OVERT VITAMIN OR TRACE MINERAL DEFICIENCIES ARE RARE IN WESTERN COUNTRIES DUE TO A PLENTIFUL, VARIED, AND INEXPENSIVE FOOD SUPPLY; HOWEVER, MULTIPLE NUTRIENT DEFICIENCIES MAY APPEAR TOGETHER IN PERSONS WHO ARE CHRONICALLY ILL OR ALCOHOLIC. MOREOVER, SUBCLINICAL VITAMIN AND TRACE MINERAL DEFICIENCIES, AS DIAGNOSED BY LABORATORY TESTING, ARE QUITE COMMON IN THE NORMAL POPULATION—ESPECIALLY IN THE GERIATRIC AGE GROUP. FAMINE, EMERGENCY-AFFECTED AND DISPLACED POPULATIONS, AND REFUGEES ARE AT INCREASED RISK FOR PROTEIN-ENERGY MALNUTRITION AND CLASSIC MICRONUTRIENT DEFICIENCIES (VITAMIN A, IRON, IODINE), AS WELL AS FOR THIAMINE (BERIBERI), RIBOFLAVIN, VITAMIN C (SCURVY), AND NIACIN (PELLAGRA) OVERT DEFICIENCIES. BODY STORES OF VITAMINS AND MINERALS VARY TREMENDOUSLY. FOR EXAMPLE, VITAMIN B12 AND VITAMIN A STORES ARE LARGE, AND AN ADULT MAY NOT BECOME DEFICIENT FOR 1 OR MORE YEARS AFTER BEING ON A DEPLETED DIET. HOWEVER, FOLATE AND THIAMINE MAY BECOME DEPLETED WITHIN WEEKS WHEN EATING A DEFICIENT DIET. THERAPEUTIC MODALITIES CAN DEPLETE ESSENTIAL NUTRIENTS FROM THE BODY, FOR EXAMPLE, HEMODIALYSIS REMOVES WATER-SOLUBLE VITAMINS, WHICH MUST BE REPLACED BY SUPPLEMENTATION. THERE ARE SEVERAL ROLES FOR VITAMINS AND TRACE MINERALS IN DISEASES: (1) DEFICIENCIES OF VITAMINS AND MINERALS MAY BE CAUSED BY DISEASE STATES SUCH AS MALABSORPTION; (2) BOTH DEFICIENCY AND EXCESS OF VITAMINS AND MINERALS CAN CAUSE DISEASE IN AND OF THEMSELVES (E.G., VITAMIN A INTOXICATION AND LIVER DISEASE); AND (3) VITAMINS AND MINERALS IN HIGH DOSES MAY BE USED AS DRUGS (E.G., NIACIN FOR HYPERCHOLESTEROLEMIA). THE HEMATOLOGIC-RELATED VITAMINS AND MINERALS (CHAPS. 98, 100) ARE CONSIDERED ONLY BRIEFLY IN THIS CHAPTER, AS ARE THE BONE-RELATED VITAMINS AND MINERALS (VITAMIN D, CALCIUM, PHOSPHORUS; CHAP. 346), SINCE THEY ARE COVERED ELSEWHERE (TABLES 71-1, 71-2, AND FIG. 71-1).
NUTRITIONAL STATUS ASSESSMENT

FULL NUTRITIONAL STATUS ASSESSMENT IS RESERVED FOR SERIOUSLY ILL PATIENTS AND THOSE AT VERY HIGH NUTRITIONAL RISK WHEN THE CAUSE OF MALNUTRITION IS STILL UNCERTAIN AFTER INITIAL CLINICAL EVALUATION AND DIETARY ASSESSMENT. IT INVOLVES MULTIPLE DIMENSIONS, INCLUDING DOCUMENTATION OF DIETARY INTAKE, ANTHROPOMETRIC MEASUREMENTS, BIOCHEMICAL MEASUREMENTS OF BLOOD AND URINE, CLINICAL EXAMINATION, HEALTH HISTORY, AND FUNCTIONAL STATUS.

FOR FURTHER DISCUSSION OF NUTRITIONAL ASSESSMENT, SEE CHAP. 72.

GLOBAL CONSIDERATIONS

NEW NUTRIENT-BASED TERMINOLOGIES WITH DIETARY REFERENCE INTAKES HAVE BEEN DEVELOPED NOT ONLY IN NORTH AMERICA, BUT IN THE UNITED KINGDOM AND EUROPE, AND BY THE WORLD HEALTH ORGANIZATION/FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS (WHO/FAO). THESE DIFFERENT STANDARDS HAVE MANY SIMILARITIES IN THEIR BASIC CONCEPTS, DEFINITIONS, AND LEVELS OF NUTRIENTS RECOMMENDED, BUT THERE ARE SOME DIFFERENCES, OWING TO ASSUMPTIONS MADE, FUNCTIONAL CRITERIA CHOSEN, THE TIMELINESS OF THE EVIDENCE REVIEWED, AND EXPERT JUDGMENT.

FURTHER READINGS

GIBSON RS: PRINCIPLES OF NUTRITIONAL ASSESSMENT, 2D ED. OXFORD UNIVERSITY PRESS, LONDON, 2005
SHILS ME ET AL (EDS): MODERN NUTRITION IN HEALTH AND DISEASE, 10TH ED. PHILADELPHIA, LIPPINCOTT WILLIAMS AND WILKINS, 2005

VITAMINS

THIAMINE (VITAMIN B###1)

THIAMINE WAS THE FIRST B VITAMIN TO BE IDENTIFIED AND IS THEREFORE ALSO REFERRED TO AS VITAMIN B###1. THIAMINE FUNCTIONS IN THE DECARBOXYLATION OF KETOACIDS, SUCH AS PYRUVATE -KETOGLUTARATE, AND BRANCHED-CHAIN AMINO ACIDS AND THUS IS A SOURCE OF ENERGY GENERATION. IN ADDITION, THIAMINE PYROPHOSPHATE ACTS AS A CO ENZYME FOR A TRANSKETOLASE REACTION THAT MEDIATES THE CONVERSION OF HEXOSE AND PENTOSE PHOSPHATES. IT HAS ALSO BEEN POSTULATED THAT THIAMINE PLAYS A ROLE IN PERIPHERAL NERVE CONDUCTION, ALTHOUGH THE EXACT CHEMICAL REACTIONS UNDERLYING THIS FUNCTION ARE UNKNOWN.
FOOD SOURCES  THE MEDIAN INTAKE OF THIAMINE IN THE UNITED STATES FROM FOOD ALONE IS 2 MG/D. PRIMARY FOOD SOURCES FOR THIAMINE INCLUDE YEAST, ORGAN MEAT, PORK, LEGUMES, BEEF, WHOLE GRAINS, AND NUTS. MILLED RICE OR GRAINS CONTAIN LITTLE THIAMINE, IF ANY. THIAMINE DEFICIENCY IS THEREFORE MORE COMMON IN CULTURES THAT RELY HEAVILY ON A RICE-BASED DIET. TEA, COFFEE (REGULAR AND DECAFFEINATED), RAW FISH, AND SHELLFISH CONTAIN THIAMINASES, WHICH CAN DESTROY THE VITAMIN. THUS, DRINKING LARGE AMOUNTS OF TEA OR COFFEE CAN THEORETICALLY LOWER THIAMINE BODY STORES.

DEFICIENCY  MOST DIETARY DEFICIENCY OF THIAMINE WORLDWIDE IS THE RESULT OF POOR DIETARY INTAKE. IN WESTERN COUNTRIES, THE PRIMARY CAUSES OF THIAMINE DEFICIENCY ARE ALCOHOLISM AND CHRONIC ILLNESS, SUCH AS CANCER. ALCOHOL INTERFERES DIRECTLY WITH THE ABSORPTION OF THIAMINE AND WITH THE SYNTHESIS OF THIAMINE PYROPHOSPHATE. THIAMINE SHOULD ALWAYS BE REPLENISHED WHEN REFEEDING A PATIENT WITH ALCOHOLISM, AS CARBOHYDRATE REPLETION WITHOUT ADEQUATE THIAMINE CAN PRECIPITATE ACUTE THIAMINE DEFICIENCY. OTHER AT-RISK POPULATIONS ARE WOMEN WITH PROLONGED HYPEREMESIS GRAVIDARUM AND ANOREXIA, PATIENTS WITH AN OVERALL POOR NUTRITIONAL STATUS ON PARENTERAL GLUCOSE, AND PATIENTS ON CHRONIC DIURETIC THERAPY DUE TO INCREASED URINARY THIAMINE LOSSES. MATERNAL THIAMINE DEFICIENCY CAN LEAD TO INFANTILE BERIBERI IN BREAST-FED CHILDREN. THIAMINE DEFICIENCY SHOULD ALSO BE CONSIDERED IN THE SETTING OF MOTOR VEHICLE ACCIDENTS ASSOCIATED WITH HEAD INJURY. THIAMINE DEFICIENCY IN ITS EARLY STAGE INDUCES ANOREXIA AND NONSPECIFIC SYMPTOMS (E.G., IRRITABILITY, DECREASE IN SHORT-TERM MEMORY). PROLONGED THIAMINE DEFICIENCY CAUSES BERIBERI, WHICH IS CLASSICALLY CATEGORIZED AS WET OR DRY, ALTHOUGH THERE IS CONSIDERABLE OVERLAP. IN EITHER

PAGE NO. 85

442 PART 5: NUTRITION

TABLE 71-1 PRINCIPAL CLINICAL FINDINGS OF VITAMIN MALNUTRITION

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th></th>
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<tbody>
<tr>
<td>THIAMINE</td>
<td></td>
</tr>
<tr>
<td>RIBOFLAVIN</td>
<td></td>
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<tr>
<td>NIACIN</td>
<td></td>
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<tr>
<td>VITAMIN B###6</td>
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<tr>
<td>FOLATE</td>
<td></td>
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</tbody>
</table>
VITAMIN B##12
VITAMIN C
VITAMIN A
VITAMIN D
VITAMIN E
VITAMIN K

CLINICAL FINDING

BERIBERI: NEUROPATHY, MUSCLE WEAKNESS AND WASTING, CARDIOMEGALY, EDEMA, OPHTHALMOPLEGIA, CONFABULATION MAGENTA TONGUE, ANGULAR STOMATITIS, SEBORRHEA, CHEILOSIS PELLAGRA: PIGMENTED RASH OF SUN-EXPOSED AREAS, BRIGHT RED TONGUE, DIARRHEA, APATHY, MEMORY LOSS, DISORIENTATION SEBORRHEA, GLOSSITIS CONVULSIONS, NEUROPATHY, DEPRESSION, CONFUSION, MICROCYTIC ANEMIA MEGALOBLASTIC ANEMIA, ATROPHIC GLOSSITIS, DEPRESSION.
* HOMOCYSTEINE MEGALOBLASTIC ANEMIA, LOSS OF VIBRATORY AND POSITION SENSE, ABNORMAL GAiT, DEMENTIA, IMPOTENCE, LOSS OF BLADDER AND BOWEL CONTROL, * HOMOCYSTEINE, * METHYLMALONIC ACID

SCURVY: PETECHIAE, ECCHYMOSIS, COILED HAIRS, INFLAMED AND BLEEDING GUMS, JOINT EFFUSION, POOR WOUND HEALING, FATIGUE XEROPHTHALMIA, NIGHTBLINDNESS, BITOT'S SPOTS, FOLLICULAR HYPERKERATOSIS, IMPAIRED EMBRYONIC DEVELOPMENT, IMMUNE DYSFUNCTION RICKETS: SKELETAL DEFORMATION, RACHITIC ROSARY, BOWED LEGS; OSTEOMALACIA PERIPHERAL NEUROPATHY, SPINOCEREBELLAR ATAXIA, SKELETAL MUSCLE ATROPHY, RETINOPATHY

ELEVATED PROTHROMBIN TIME, BLEEDING

DIETARY LEVEL PER DAY ASSOCIATED WITH OVERT DEFICIENCY IN ADULTS

<0.3 MG/1000 KCAL
<0.6 MG
<9.0 NIACIN EQUIVALENTS
<0.2 MG
<100 *G/D
<1.0 *G/D
CONTRIBUTING FACTORS TO DEFICIENCY

ALCOHOLISM, CHRONIC DIURETIC USE, HYPEREMESIS

ALCOHOLISM, VITAMIN B###6 DEFICIENCY, RIBOFLAVIN DEFICIENCY, TRYPTOPHAN DEFICIENCY

ALCOHOLISM, ISONIAZID

ALCOHOLISM SULFASALAZINE, PYRIMETHAMINE, TRIAMTERENE

GASTRIC ATROPHY (PERNICIOUS ANEMIA), TERMINAL ILEAL DISEASE, STRICT VEGETARIANISM, ACID REDUCING DRUGS (EG, H###2 BLOCKERS)

SMOKING, ALCOHOLISM

FAT MALABSORPTION, INFECTION, MEASLES, ALCOHOLISM, PROTEIN-ENERGY MALNUTRITION

AGING, LACK OF SUNLIGHT EXPOSURE, FAT MALABSORPTION, DEEPLY PIGMENTED SKIN

OCCURS ONLY WITH FAT MALABSORPTION, OR GENETIC ABNORMALITIES OF VITAMIN E METABOLISM/TRANSPORT

FAT MALABSORPTION, LIVER DISEASE, ANTIBIOTIC USE

FORM OF BERIBERI, PATIENTS MAY COMPLAIN OF PAIN AND PARESTHESIA. WET BERIBERI PRESENTS PRIMARILY WITH CARDIOVASCULAR SYMPTOMS, DUE TO IMPAIRED MYOCARDIAL ENERGY METABOLISM AND DYSAUTONOMIA, AND CAN OCCUR AFTER 3 MONTHS OF A THIAMINE-DEFICIENT DIET. PATIENTS PRESENT WITH AN ENLARGED HEART, TACHYCARDIA, HIGH-OUTPUT CONGESTIVE HEART FAILURE, PERIPHERAL EDEMA, AND PERIPHERAL NEURITIS. PATIENTS WITH DRY BERIBERI PRESENT WITH A SYMMETRIC PERIPHERAL NEUROPATHY OF THE MOTOR AND SENSORY SYSTEMS WITH DIMINISHED REFLEXES. THE NEUROPATHY AFFECTS THE LEGS MOST MARKEDLY, AND PATIENTS HAVE DIFFICULTY RISING FROM A SQUATTING POSITION.

ALCOHOLIC PATIENTS WITH CHRONIC THIAMINE DEFICIENCY MAY ALSO HAVE CENTRAL NERVOUS SYSTEM (CNS) MANIFESTATIONS KNOWN AS WERNICKE'S ENCEPHALOPATHY, CONSISTING OF HORIZONTAL NYSTAGMUS, OPHTHALMOPLEGIA (DUE TO WEAKNESS OF ONE OR MORE EXTRAOCULAR MUSCLES), CEREBELLAR ATAXIA, AND MENTAL IMPAIRMENT (CHAP. 387). WHEN THERE IS AN ADDITIONAL LOSS OF MEMORY AND A CONFABULATORY PSYCHOSIS, THE
SYNDROME IS KNOWN AS WERNICKE-KORSAKOFF SYNDROME. DESPITE THE TYPICAL CLINICAL PICTURE AND HISTORY, WERNICKE-KORSAKOFF SYNDROME IS UNDERDIAGNOSED. THE LABORATORY DIAGNOSIS OF THIAMINE DEFICIENCY IS USUALLY MADE BY A FUNCTIONAL ENZYMATIC ASSAY OF TRANSKETOLASE ACTIVITY MEASURED BEFORE AND AFTER THE ADDITION OF THIAMINE PYROPHOSPHATE. A >25% STIMULATION BY THE ADDITION OF THIAMINE PYROPHOSPHATE (AN ACTIVITY COEFFICIENT OF 1.25) IS TAKEN AS ABNORMAL. THIAMINE OR THE PHOSPHORYLATED ESTERS OF THIAMINE IN SERUM OR BLOOD CAN ALSO BE MEASURED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) TO DETECT DEFICIENCY.

THIAMINE DEFICIENCY

IN ACUTE THIAMINE DEFICIENCY WITH EITHER CARDIOVASCULAR OR NEUROLOGIC SIGNS, 100 MG/D OF THIAMINE SHOULD BE GIVEN PARENTERALLY FOR 7 DAYS, FOLLOWED BY 10 MG/D ORALLY UNTIL THERE IS COMPLETE RECOVERY. CARDIOVASCULAR IMPROVEMENT OCCURS WITHIN 24 H, AND OPHTHALMOPLEGIC IMPROVEMENT OCCURS WITHIN 24 H OTHER MANIFESTATIONS GRADUALLY CLEAR, ALTHOUGH PSYCHOSIS IN WERNICKE-KORSAKOFF SYNDROME MAY BE PERMANENT OR PERSIST FOR SEVERAL MONTHS.

TOXICITY ALTHOUGH ANAPHYLAXIS HAS BEEN REPORTED AFTER HIGH DOSES OF THIAMINE, NO ADVERSE EFFECTS HAVE BEEN RECORDED FROM EITHER FOOD OR SUPPLEMENTS AT HIGH DOSES. THIAMINE SUPPLEMENTS MAY BE BOUGHT OVER THE COUNTER IN DOSES OF UP TO 50 MG/D.

RIBOFLAVIN (VITAMIN B2)


DEFICIENCY AND EXCESS RIBOFLAVIN DEFICIENCY IS ALMOST ALWAYS DUE TO DIETARY DEFICIENCY. MILK, OTHER DAIRY PRODUCTS, AND ENRICHED BREADS AND CEREALS ARE THE MOST IMPORTANT DIETARY SOURCES OF RIBOFLAVIN IN THE UNITED STATES, ALTHOUGH LEAN MEAT, FISH, EGGS, BROCCOLI, AND LEGUMES ARE
ALSO GOOD SOURCES. RIBOFLAVIN IS EXTREMELY SENSITIVE TO LIGHT, AND MILK SHOULD BE STORED IN CONTAINERS THAT PROTECT AGAINST PHOTODEGRADATION. LABORATORY DIAGNOSIS OF RIBOFLAVIN DEFICIENCY CAN BE MADE BY MEASUREMENT OF RED BLOOD CELL OR URINARY RIBOFLAVIN CONCENTRATIONS OR BY MEASUREMENT OF ERYTHROCYTE GLUTATHIONE REDUCTASE ACTIVITY, WITH AND WITHOUT ADDED FAD. BECAUSE THE CAPACITY OF THE GASTROINTESTINAL TRACT TO ABSORB RIBOFLAVIN IS LIMITED (~20 MG IF GIVEN IN ONE ORAL DOSE), RIBOFLAVIN TOXICITY HAS NOT BEEN DESCRIBED.

NIACIN (VITAMIN B###3)

THE TERM Niacin REFERS TO NICOTINIC ACID AND NICOTINAMIDE AND THEIR BIOLOGICALLY ACTIVE DERIVATIVES. NICOTINIC ACID AND NICOTINAMIDE SERVE AS PRECURSORS OF TWO COENZYMES, NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) AND NAD PHOSPHATE (NADP), WHICH ARE IMPORTANT IN NUMEROUS OXIDATION AND REDUCTION REACTIONS IN THE BODY. IN ADDITION, NAD AND NADP ARE ACTIVE IN ADENINE DIPHOSPHATE-RIBOSE TRANSFER REACTIONS INVOLVED IN DNA REPAIR AND CALCIUM MOBILIZATION.

METABOLISM AND REQUIREMENTS Niacin Bioavailability is high from beans, milk, meat, and eggs. Bioavailability from cereal grains is lower. Since flour is enriched with the “free” niacin (i.e., non-coenzyme form), bioavailability is excellent. Median intakes of niacin in the United States considerably exceed the recommended dietary allowance (RDA). The amino acid tryptophan can be converted to niacin with an efficiency of 60:1 by weight. Thus, the RDA for niacin is expressed in niacin equivalents. A lower conversion of tryptophan to niacin occurs in vitamin B###6 and/or riboflavin deficiencies, or in the presence of isoniazid. The urinary excretion products of niacin include 2-pyridone and 2-methyl nicotinamide, measurements of which are used in diagnosis of niacin deficiency.

DEFICIENCY Niacin deficiency causes pellagra, which is mostly found among people eating corn-based diets in parts of China, Africa, and India. Pellagra in North America is found mainly among alcoholics; in patients with congenital defects of intestinal and kidney absorption of
TRYPTOPHAN (HARTNUP DISEASE; CHAP. 358); AND IN PATIENTS WITH CARCINOID SYNDROME (CHAP. 344), WHERE THERE IS INCREASED CONVERSION OF TRYPTOPHAN TO SEROTONIN. IN THE SETTING OF FAMINE OR POPULATION DISPLACEMENT, THE OCCURRENCE OF PELLAGRA RESULTS FROM THE ABSOLUTE LACK OF NIACIN BUT ALSO THE DEFICIENCY OF MICRONUTRIENTS REQUIRED FOR THE CONVERSION OF TRYPTOPHAN TO NIACIN (E.G., IRON, RIBOFLAVIN, AND PYRIDOXINE). THE EARLY SYMPTOMS OF PELLAGRA Include LOSS OF APPETITE, GENERALIZED WEAKNESS AND IRRITABILITY, ABDOMINAL PAIN, AND VOMITING.

BRIGHT RED GLOSSITIS THEN ENSUES, FOLLOWED BY A CHARACTERISTIC SKIN RASH THAT IS PIGMENTED AND SCALING, PARTICULARLY IN SKIN AREAS EXPOSED TO SUNLIGHT. THIS RASH IS KNOWN AS CASAL’S NECKLACE BECAUSE IT FORMS A RING AROUND THE NECK; IT IS SEEN IN ADVANCED CASES. VAGINITIS AND ESOPHAGITIS MAY ALSO OCCUR. DIARRHEA (IN PART DUE TO PROCTITIS AND IN PART DUE TO MALABSORPTION), DEPRESSION, SEIZURES, AND DEMENTIA ARE ALSO PART OF THE PELLAGRA SYNDROME—THE FOUR DS: DERMATITIS, DIARRHEA, AND DEMENTIA LEADING TO DEATH.

PELLAGRA

TREATMENT OF PELLAGRA CONSISTS OF ORAL SUPPLEMENTATION OF 100-200 MG OF NICOTINAMIDE OR NICOTINIC ACID THREE TIMES DAILY FOR 5 DAYS. HIGH DOSES OF NICOTINIC ACID (2 G/D IN A TIME-RELEASE FORM) ARE USED FOR THE TREATMENT OF ELEVATED CHOLESTEROL AND TRIGLYCERIDE LEVELS AND/OR LOW HIGH-DENSITY LIPOPROTEIN (HDL) CHOLESTEROL LEVEL (CHAP. 350).

TOXICITY PROSTAGLANDIN-MEDIATED FLUSHING DUE TO BINDING OF THE VITAMIN TO A G PROTEIN-COUPLED RECEPTOR HAS BEEN OBSERVED AT DAILY DOSES AS LOW AS 50 MG OF NIACIN WHEN TAKEN AS A SUPPLEMENT OR AS THERAPY FOR DYSLIPIDEMIA. THERE IS NO EVIDENCE OF TOXICITY FROM NIACIN DERIVED FROM FOOD SOURCES. FLUSHING ALWAYS STARTS IN THE FACE AND MAY BE ACCOMPANIED BY SKIN DRYNESS, ITCHING, PARESTHESIA, AND HEADACHE. PRE-MEDICATION WITH ASPIRIN MAY ALLEVIATE THESE SYMPTOMS. FLUSHING IS SUBJECT TO TACHYPHYLAXIS AND OFTEN IMPROVES WITH TIME. NAUSEA, VOMITING, AND ABDOMINAL PAIN ALSO OCCUR AT SIMILAR DOSES OF NIACIN. HEPATIC TOXICITY IS THE MOST SERIOUS TOXIC REACTION DUE TO NIACIN AND MAY PRESENT AS JAUNDICE WITH ELEVATED ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT) LEVELS. A FEW CASES OF FULMINANT HEPATITIS REQUIRING LIVER TRANSPLANTATION HAVE BEEN REPORTED AT DOSES OF

TABLE 71-2 DEFICIENCIES AND TOXICITIES OF METALS

ELEMENT

BORON
CALCIUM
COPPER
CHROMIUM
FLUORIDE
IODINE
IRON
MANGANESE
MOLYBDENUM
SELENIUM
PHOSPHOROUS
ZINC

DEFICIENCY

NO BIOLOGIC FUNCTION DETERMINED

REDUCED BONE MASS, OSTEOPOROSIS
ANEMIA, GROWTH RETARDATION, DEFECTIVE KERATINIZATION AND PIGMENTATION OF HAIR, HYPOTHERMIA, DEGENERATIVE CHANGES IN AORTIC ELASTIN, OSTEOGENIA, MENTAL DETERIORATION IMPAIRED GLUCOSE TOLERANCE
* DENTAL CARIES
THYROID ENLARGEMENT, *T##4 CRETINISM
MUSCLE ABNORMALITIES, KILONYCHIA, PICA, ANEMIA, * WORK PERFORMANCE, IMPAIRED COGNITIVE DEVELOPMENT, PREMATURE LABOR,
* PERINATAL MATERNAL MORTALITY
IMPAIRED GROWTH AND SKELETAL DEVELOPMENT,
REPRODUCTION, LIPID AND CARBOHYDRATE METABOLISM; UPPER BODY RASH
SEVERE NEUROLOGIC ABNORMALITIES

CARDIOMYOPATHY, HEART FAILURE, STRIATED MUSE LE DEGENERATION

RICKETS (OSTEOMALACIA), PROXIMAL MUSCLE WEAKNESS, RHABDOMYOLYSIS, PARESTHESIA, ATAXIA, SEIZURE, CONFUSION, HEART FAILURE, HEMOLYSIS, ACIDOSIS
GROWTH RETARDATION, * TASTE AND SMELL, ALOPECIA, DERMATITIS, DIARRHEA, IMMUNE DYSFUNCTION, FAILURE TO THRIVE, GONADAL ATROPHY, CONGENITAL MALFORMATIONS

TOXICITY

DEVELOPMENTAL DEFECTS, MALE STERILITY, TESTICULAR ATROPHY
RENAL INSUFFICIENCY (MILK-ALKALAI SYNDROME),
NEPHROLITHIASIS, IMPAIRED IRON ABSORPTION
NAUSEA VOMITING, DIARRHEA, HEPATIC FAILURE, TREMOR,
MENTAL DETERIORATION, HEMOLYTIC ANEMIA, RENAL
DYSFUNCTION

OCCUPATIONAL: RENAL FAILURE, DERMATITIS, PULMONARY CANCER
DENTAL AND SKELETAL FLUOROSIS, OSTEOSCLEROSIS
THYROID DYSFUNCTION, ACNE-LIKE ERUPTIONS
GASTROINTESTINAL EFFECTS (NAUSEA, VOMITING, DIARRHEA,
CONSTIPATION), IRON OVERLOAD WITH ORGAN DAMAGE,
ACUTE SYSTEMIC TOXICITY

GENERAL: NEUROTOXICITY, PARKINSON-LIKE SYMPTOMS
OCCUPATIONAL: ENCEPHALITIS-LIKE SYNDROME, PARKINSON-
LIKE SYNDROME, PSYCHOSIS, PNEUMOCONIOSIS
REPRODUCTIVE AND FETAL ABNORMALITIES

GENERAL: ALOPECIA, NAUSEA, VOMITING, ABNORMAL NAILS,
EMOTIONAL LABILITY, PERIPHERAL NEUROPATHY, LASSITUDE,
GARLIC ODOR TO BREATH, DERMATITIS
OCCUPATIONAL: LUNG AND NASAL CARCINOMAS, LIVER
NECROSIS, PULMONARY INFLAMMATION
HYPERPHOSPHATEMIA

GENERAL: REDUCED COPPERABSORPTION, GASTRITIS, SWEATING,
FEVER, NAUSEA, VOMITING
OCCUPATIONAL: RESPIRATORY DISTRESS, PULMONARY FIBROSIS

TOLERABLE UPPER (DIETARY)
INTAKE LEVEL

20 MG/D (EXTRAPOLATED FROM
ANIMAL DATA)
2500 MG/D (MILK-ALKALAI)

10 MG/D (LIVER TOXICITY)

ND
10 MG/D (FLUOROSIS)

1100 *G/D (THYROID DYSFUNCTION)
45 MG/D OF ELEMENTAL IRON (GI
SIDE EFFECTS)

11 MG/D (NEUROTOXICITY)

2 MG/D EXTRAPOLATED FROM
ANIMAL DATA
400 *G/D (HAIR, NAIL CHANGES)

4000 MG/D

40 MG/D (IMPAIRED COPPER
METABOLISM)
444 PART 5: NUTRITION

3-9 G/D. OTHER TOXIC REACTIONS INCLUDE GLUCOSE INTOLERANCE, HYPERURICEMIA, MACULAR EDEMA, AND MACULAR CYSTS. THE UPPER LIMIT FOR DAILY NIACIN INTAKE HAS BEEN SET AT 35 MG. HOWEVER, THIS UPPER LIMIT DOES NOT PERTAIN TO THE THERAPEUTIC USE OF NIACIN.

PYRIDOXINE (VITAMIN B###6)

VITAMIN B###6 REFERS TO A FAMILY OF COMPOUNDS INCLUDING PYRIDOXINE, PYRIDOXAL, PYRIDOXAMINE, AND THEIR 5'-PHOSPHATE DERIVATIVES. 5'-PYRIDOXAL PHOSPHATE (PLP) IS A COFACTOR FOR MORE THAN 100 ENZYMES INVOLVED IN AMINO ACID METABOLISM. VITAMIN B###6 IS ALSO INVOLVED IN HEME AND NEUROTRANSMITTER SYNTHESIS AND IN THE METABOLISM OF GLYCOGEN, LIPIDS, STEROIDS, SPHINGOID BASES, AND SEVERAL VITAMINS, INCLUDING THE CONVERSION OF TRYPTOPHAN TO NIACIN.

DIETARY SOURCES  PLANTS CONTAIN VITAMIN B###6 IN THE FORM OF PYRIDOXINE, WHEREAS ANIMAL TISSUES CONTAIN PLP AND PYRIDOXAMINE PHOSPHATE. THE VITAMIN B###6 CONTAINED IN PLANTS IS LESS BIOAVAILABLE THAN THAT FROM ANIMAL TISSUES. RICH FOOD SOURCES OF VITAMIN B###6 INCLUDE LEGUMES, NUTS, WHEAT BRAN, AND MEAT, ALTHOUGH IT IS PRESENT IN ALL FOOD GROUPS.

DEFICIENCY SYMPTOMS OF VITAMIN B###6 DEFICIENCY INCLUDE EPITHELIAL CHANGES, AS SEEN FREQUENTLY WITH OTHER B VITAMIN DEFICIENCIES. IN ADDITION, SEVERE VITAMIN B###6 DEFICIENCY CAN LEAD TO PERIPHERAL NEUROPATHY, ABNORMAL ELECTROENCEPHALOGRAMS, AND PERSONALITY CHANGES INCLUDING DEPRESSION AND CONFUSION. IN INFANTS, DIARRHEA, SEIZURES, AND ANEMIA HAVE BEEN REPORTED. MICROCYTIC, HYPOCHROMIC ANEMIA IS DUE TO DIMINISHED HEMOGLOBIN SYNTHESIS, SINCE THE FIRST ENZYME INVOLVED
IN HEME BIOSYNTHESIS (AMINOLEVULINATE SYNTHASE) REQUIRES PLP AS A COFACTOR (CHAP. 98). IN SOME CASE REPORTS, PLATELET DYSFUNCTION HAS ALSO BEEN REPORTED. SINCE VITAMIN B###6 IS NECESSARY FOR THE CONVERSION OF HOMOCYSTEINE TO QSTATHIONINE, IT IS POSSIBLE THAT CHRONIC LOW-GRADE VITAMIN B###6 DEFICIENCY MAY RESULT IN HYPERHOMOCYSTEINEMIA AND INCREASED RISK OF CARDIOVASCULAR DISEASE (CHAPS. 235, 358). INDEPENDENT OF HOMOCYSTEINE, LOW LEVELS OF CIRCULATING VITAMIN B###6 HAVE ALSO BEEN ASSOCIATED WITH INFLAMMATION AND ELEVATED C-REACTIVE PROTEIN LEVELS. CERTAIN MEDICATIONS SUCH AS ISONIAZID, L-DOPA, PENICILLAMINE, AND CYCLOSERINE INTERACT WITH PLP DUE TO A REACTION WITH CARBONYL GROUPS. PYRIDOXINE SHOULD BE GIVEN CONCURRENTLY WITH ISONIAZID TO AVOID NEUROPATHY. THE INCREASED RATIO OF AST (OR SGOT) TO ALT (OR SGPT) SEEN IN ALCOHOLIC LIVER DISEASE REFLECTS THE RELATIVE VITAMIN B###6 DEPENDENCE OF ALT. VITAMIN B###6 DEPENDENCY SYNDROMES THAT REQUIRE PHARMACOLOGIC DOSES OF VITAMIN B###6 ARE RARE; THEY INCLUDE CYSTATHIONINE *-SYNTHASE DEFICIENCY, PYRIDOXINE-RESPONSIVE (PRIMARILY SIDEROBLASTIC) ANEMIAS, AND GYRATE ATROPHY WITH CHORIoretINAL DEGENERATION DUE TO DECREASED ACTIVITY OF THE MITOCHONDRIAL ENZYME ORNITHINE AMINOTRANSFERASE. IN THESE SITUATIONS, 100-200 MG/D OF ORAL VITAMIN B###6 IS REQUIRED FOR TREATMENT. HIGH DOSES OF VITAMIN B###6, HAVE BEEN USED TO TREAT CARPAL TUNNEL SYNDROME, PREMENSTRUAL SYNDROME, SCHIZOPHRENIA, AUTISM, AND DIABETIC NEUROPATHY BUT HAVE NOT BEEN FOUND TO BE EFFECTIVE.

FIGURE 71-1 THE STRUCTURES AND PRINCIPAL FUNCTIONS OF VITAMINS ASSOCIATED WITH HUMAN DISORDERS.
SHOULD NOT BE GIVEN WITH L-DOPA, SINCE THE VITAMIN INTERFERES WITH THE ACTION OF THIS DRUG.

TOXICITY  THE SAFE UPPER LIMIT FOR VITAMIN B###6 HAS BEEN SET AT 100 MG/D, ALTHOUGH NO ADVERSE EFFECTS HAVE BEEN ASSOCIATED WITH HIGH INTAKES OF VITAMIN B###6 FROM FOOD SOURCES ONLY.  WHEN TOXICITY OCCURS, IT CAUSES A SEVERE SENSORY NEUROPATHY, LEAVING PATIENTS UN ABLE TO WALK.  SOME CASES OF PHOTOSENSITIVITY AND DERMATITIS HAVE ALSO BEEN REPORTED.

FOLATE, VITAMIN B###12  
SEE CHAP. 90.

VITAMIN C

BOTH ASCORBIC ACID AND ITS OXIDIZED PRODUCT DEHYDROASCORBIC ACID ARE BIOLOGICALLY ACTIVE.  ACTIONS OF VITAMIN C INCLUDE ANTIOXIDANT ACTIVITY, PROMOTION OF NONHEME IRON ABSORPTION, CARNITINE BIOSYNTHESIS, THE CONVERSION OF DOPAMINE TO NOREPINEPHRINE, AND THE SYNTHESIS OF MANY PEPTIDE HORMONES.  VITAMIN C IS ALSO IMPORTANT FOR CONNECTIVE TISSUE METABOLISM AND CROSS-LINKING (PROLINE HYDROXYLATION), AND IT IS A COMPONENT OF MANY DRUG-METABOLIZING ENZYME SYSTEMS, PARTICULARLY THE MIXED-FUNCTION OXIDASE SYSTEMS.

ABSORPTION AND DIETARY SOURCES  ALMOST COMPLETE ABSORPTION OF VITAMIN C OCCURS IF <100 MG IS ADMINISTERED IN A SINGLE DOSE; HOWEVER, ONLY 50% OR LESS IS ABSORBED AT DOSES >1 G.  ENHANCED DEGRADATION AND FECAL AND URINARY EXCRETION OF VITAMIN C OCCUR AT HIGHER INTAKE LEVELS.  GOOD DIETARY SOURCES OF VITAMIN C INCLUDE CITRUS FRUITS, GREEN VEGETABLES (ESPECIALLY BROCCOLI), TOMATOES, AND POTATOES.  CONSUMPTION OF FIVE SERVINGS OF FRUITS AND VEGETABLES A DAY PROVIDES VITAMIN C IN EXCESS OF THE RDA, 90 MG/D FOR MALES AND 75 MG/D FOR FEMALES.  IN ADDITION, APPROXIMATELY 40% OF THE U.S. POPULATION CONSUMES VITAMIN C AS A DIETARY SUPPLEMENT IN WHICH “NATURAL FORMS” OF VITAMIN C ARE NO MORE BIOAVAILABLE THAN SYNTHETIC FORMS.  SMOKING, HEMODIALYSIS, PREGNANCY, AND STRESS (E.G., INFECTION, TRAUMA) APPEAR TO INCREASE VITAMIN C REQUIREMENTS.

DEFICIENCY  VITAMIN C DEFICIENCY CAUSES SCURVY.  IN THE UNITED STATES, THIS IS SEEN PRIMARILY AMONG THE POOR AND ELDERLY, IN ALCOHOLICS WHO CONSUME <10 MG/D OF VITAMIN C, AND ALSO IN INDIVIDUALS CONSUMING MACROBIOTIC DIETS.  IN
ADDITION TO GENERALIZED FATIGUE, SYMPTOMS OF SCURVY PRIMARILY REFLECT IMPAIRED FORMATION OF MATURE CONNECTIVE TISSUE AND INCLUDE BLEEDING INTO SKIN (PETECHIAE, ECCHYMOSES, PERIFOLLICULAR HEMORRHAGES); INFLAMED AND BLEEDING GUMS; AND MANIFESTATIONS OF BLEEDING INTO JOINTS, THE PERITONEAL CAVITY, PERICARDIUM, AND THE ADRENAL GLANDS. IN CHILDREN, VITAMIN C DEFICIENCY MAY CAUSE IMPAIRED BONE GROWTH. LABORATORY DIAGNOSIS OF VITAMIN C DEFICIENCY IS MADE ON THE BASIS OF LOW PLASMA OR LEUKOCYTE LEVELS.

ADMINISTRATION OF VITAMIN C (200 MG/D) IMPROVES THE SYMPTOMS OF SCURVY WITHIN A MATTER OF SEVERAL DAYS. HIGH-DOSE VITAMIN C SUPPLEMENTATION (E.G., 1-2 G/D) MIGHT SLIGHTLY DECREASE THE SYMPTOMS AND DURATION OF UPPER RESPIRATORY TRACT INFECTIONS. VITAMIN C SUPPLEMENTATION HAS ALSO BEEN REPORTED TO BE USEFUL IN CHEDIAK-HIGASHI SYNDROME (CHAP. 61) AND OSTEOGENESIS IMPERFECTA (CHAP. 357). DIETS HIGH IN VITAMIN C HAVE BEEN CLAIMED TO LOWER THE INCIDENCE OF CERTAIN CANCERS, PARTICULARLY ESOPHAGEAL AND GASTRIC CANCERS. IF PROVED, THIS EFFECT MAY BE DUE TO THE FACT THAT VITAMIN C CAN PREVENT THE CONVERSION OF NITRITES AND SECONDARY AMINES TO CARCINOGENIC NITROSAMINES. HOWEVER, ONE INTERVENTION STUDY FROM CHINA DID NOT SHOW VITAMIN C TO BE PROTECTIVE.

TOXICITY TAKING >2 G OF VITAMIN C IN A SINGLE DOSE MAY RESULT IN ABDOMINAL PAIN, DIARRHEA, AND NAUSEA. SINCE VITAMIN C MAY BE METABOLIZED TO OXALATE, IT IS FEARED THAT CHRONIC, HIGH-DOSE VITAMIN C SUPPLEMENTATION COULD RESULT IN AN INCREASED PREVALENCE OF KIDNEY STONES. HOWEVER, THIS HAS NOT BEEN BORNE OUT IN SEVERAL TRIALS, EXCEPT IN PATIENTS WITH PREEXISTING RENAL DISEASE. THUS, IT IS REASONABLE TO ADVISE PATIENTS WITH A PAST HISTORY OF KIDNEY STONES TO NOT TAKE LARGE DOSES OF VITAMIN C. THERE IS ALSO AN UNPROVEN BUT POSSIBLE RISK THAT CHRONIC HIGH DOSES OF VITAMIN C COULD PROMOTE IRON OVERLOAD IN PATIENTS TAKING SUPPLEMENTAL IRON. HIGH DOSES OF VITAMIN C CAN INDUCE HEMOLYSIS IN PATIENTS WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY, AND DOSES >1 G/D CAN CAUSE FALSE-NEGATIVE GUAIAC REACTIONS AS WELL AS INTERFERE WITH TESTS FOR URINARY GLUCOSE.
BIOTIN IS A WATER-SOLUBLE VITAMIN THAT PLAYS A ROLE IN GENE EXPRESSION, GLUCONEOGENESIS, AND FATTY ACID SYNTHESIS AND SERVES AS A CO2 CARRIER
ON THE SURFACE OF BOTH CYTOSOLIC AND MITOCHONDRIAL CARBOXYLASE ENZYMES. THE VITAMIN ALSO FUNCTIONS IN THE CATABOLISM OF SPECIFIC AMINO ACIDS (E.G., LEUCINE). EXCELLENT FOOD SOURCES OF BIOTIN INCLUDE ORGAN MEAT SUCH AS LIVER OR KIDNEY, SOY, BEANS, YEAST, AND EGG YOLKS; HOWEVER, EGG WHITE CONTAINS THE PROTEIN AVIDIN, WHICH STRONGLY BINDS THE VITAMIN AND REDUCES ITS BIOAVAILABILITY.

BIOTIN DEFICIENCY DUE TO LOW DIETARY INTAKE IS RARE; RATHER, DEFICIENCY IS DUE TO INBORN ERRORS OF METABOLISM. BIOTIN DEFICIENCY HAS BEEN INDUCED BY EXPERIMENTAL FEEDING OF EGG WHITE DIETS AND IN PATIENTS WITH SHORT BOWELS WHO RECEIVED BIOTIN-FREE PARENTERAL NUTRITION. IN THE ADULT, BIOTIN DEFICIENCY RESULTS IN MENTAL CHANGES (DEPRESSION, HALLUCINATIONS), PARESTHESIA, ANOREXIA, AND NAUSEA. A SCALING, SEBORRHEIC, AND ERYTHEMATOUS RASH MAY OCCUR AROUND THE EYES, NOSE, AND MOUTH AS WELL AS ON THE EXTREMITIES. IN INFANTS, BIOTIN DEFICIENCY PRESENTS AS HYPOTONIA, LETHARGY, AND APATHY. IN ADDITION, THE INFANT MAY DEVELOP ALOPECIA AND A CHARACTERISTIC RASH THAT INCLUDES THE EARS. THE LABORATORY DIAGNOSIS OF BIOTIN DEFICIENCY CAN BE ESTABLISHED BASED ON A DECREASED URINARY CONCENTRATION OR AN INCREASED URINARY EXCRETION OF 3-HYDROXYISovaleric ACID AFTER A LEUCINE CHALLENGE. TREATMENT REQUIRES PHARMACOLOGIC DOSES OF BIOTIN, USING UP TO 10 MG/D. NO TOXICITY IS KNOWN.

PANTOTHENIC ACID (VITAMIN B5)
PANTOTHENIC ACID IS A COMPONENT OF COENZYME A AND PHOSPHOPANTETHINE, WHICH ARE INVOLVED IN FATTY ACID METABOLISM AND THE SYNTHESIS OF CHOLESTEROL, STEROID HORMONES, AND ALL COMPOUNDS FORMED FROM ISOPRENOID UNITS. IN ADDITION, PANTOTHENIC ACID IS INVOLVED IN THE ACETYLATION OF PROTEINS. THE VITAMIN IS EXCRETED IN THE URINE, AND THE LABORATORY DIAGNOSIS OF DEFICIENCY IS MADE ON THE BASIS OF LOW URINARY VITAMIN LEVELS.

THE VITAMIN IS UBIQUITOUS IN THE FOOD SUPPLY. LIVER, YEAST, EGG YOLKS, WHOLE GRAINS, AND VEGETABLES ARE PARTICULARLY GOOD SOURCES. HUMAN PANTOTHENIC ACID DEFICIENCY HAS BEEN DEMONSTRATED ONLY IN EXPERIMENTAL FEEDING OF DIETS LOW IN PANTOTHENIC ACID OR BY GIVING A SPECIFIC PANTOTHENIC ACID ANTAGONIST. THE SYMPTOMS OF PANTOTHENIC ACID DEFICIENCY ARE NONSPECIFIC AND INCLUDE GASTROINTESTINAL DISTURBANCE, DEPRESSION, MUSCLE CRAMPS, PARESTHESIA, ATAXIA, AND HYPOGLYCEMIA.
PANTOTHENIC ACID DEFICIENCY IS BELIEVED TO HAVE CAUSED THE BURNING FEET SYNDROME SEEN IN PRISONERS OF WAR DURING WORLD WAR II. NO TOXICITY OF THIS VITAMIN HAS BEEN REPORTED.

CHOLINE
CHOLINE IS A PRECURSOR FOR ACETYLCHOLINE, PHOSPHOLIPIDS, AND BETAIN. CHOLINE IS NECESSARY FOR THE STRUCTURAL INTEGRITY OF CELL MEMBRANES, CHOLINERGIC NEUROTRANSMISSION, LIPID AND CHOLESTEROL METABOLISM, METHYL-GROUP METABOLISM, AND TRANSMEMBRANE SIGNALING. RECENTLY, A RECOMMENDED ADEQUATE INTAKE WAS SET AT 550 MG/D FOR ADULT MALES AND 425 MG/D FOR ADULT FEMALES, ALTHOUGH CERTAIN GENETIC POLYMORPHISMS CAN INCREASE AN INDIVIDUALS REQUIREMENT FOR CHOLINE. CHOLINE IS THOUGHT TO BE A “CONDITIONALLY ESSENTIAL” NUTRIENT, IN THAT DE NOVO SYNTHESIS OCCURS IN THE LIVER AND IS LESS THAN THE VITAMIN’S UTILIZATION ONLY UNDER CERTAIN STRESS CONDITIONS (E.G., ALCOHOLIC LIVER DISEASE). THE DIETARY REQUIREMENT OF CHOLINE DEPENDS ON THE STATUS OF OTHER METHYL-GROUP DONORS (FOLATE, VITAMIN B12, AND METHIONINE) AND THUS VARIES WIDELY. CHOLINE IS WIDELY DISTRIBUTED IN FOOD (E.G., EGG YOLK, WHEAT GERM, ORGAN MEAT, MILK) IN THE FORM OF LECITHIN (PHOSPHATIDYLCHOLINE). CHOLINE DEFICIENCY HAS OCCURRED IN PATIENTS RECEIVING PARENTERAL NUTRITION DEVOID OF CHOLINE. DEFICIENCY RESULTS IN FATTY LIVER, ELEVATED TRANSAMINASE LEVELS, AND SKELETAL MUSCLE DAMAGE WITH HIGH CREATINE PHOSPHOKINASE VALUES. THE DIAGNOSIS OF CHOLINE DEFICIENCY IS CURRENTLY MADE ON THE BASIS OF LOW PLASMA LEVELS, ALTHOUGH NONSPECIFIC CONDITIONS (E.G., HEAVY EXERCISE) MAY SUPPRESS PLASMA LEVELS. TOXICITY FROM CHOLINE RESULTS IN HYPOTENSION, CHOLINERGIC SWEATING, DIARRHEA, SALIVATION, AND A FISHY BODY ODOR. THE UPPER LIMIT FOR CHOLINE HAS BEEN SET AT 3.5 G/D. THERAPEUTICALLY, CHOLINE HAS BEEN SUGGESTED FOR PATIENTS WITH DEMENTIA AND FOR PATIENTS AT HIGH RISK OF CARDIOVASCULAR DISEASE, DUE TO ITS ABILITY TO LOWER CHOLESTEROL AND HOMOCYSTEINE LEVELS. HOWEVER, SUCH BENEFITS HAVE YET TO BE DOCUMENTED. CHOLINE- AND BETAINE-RESTRICTED DIETS ARE OF THERAPEUTIC VALUE IN TRIMETHYLAMINURIA (FISH ODOR SYNDROME).

FLAVONOIDS

FLAVONOIDs CONSTITUTE A LARGE FAMILY OF POLYPHENOLS THAT CONTRIBUTE TO THE AROMA, TASTE, AND COLOR OF FRUITS AND VEGETABLES. MAJOR GROUPS OF DIETARY FLAVONOIDs INCLUDE ANTHOCYANIDINS IN BERRIES; CATECHINS IN GREEN TEA AND CHOCOLATE; FLAVONOLS (E.G., QUERCITIN) IN BROCCOLI, KALE, LEEKS, ONION, AND THE SKINS OF GRAPES AND APPLES; AND ISOFLAVONES (E.G., GENISTEIN) IN LEGUMES. ISOFLAVONES HAVE A LOW BIOAVAILABILITY AND ARE PARTIALLY METABOLIZED BY THE INTESTINAL FLORA. THE DIETARY INTAKE OF FLAVONOIDs IS ESTIMATED TO BE BETWEEN 10 AND 100 MG/D, ALTHOUGH THIS IS ALMOST CERTAINLY AN UNDERESTIMATE DUE TO THE LACK OF KNOWLEDGE OF THEIR CONCENTRATIONS IN
MANY FOODS. SEVERAL FLAVONOIDS HAVE BEEN SHOWN TO HAVE ANTIOXIDANT ACTIVITY AND TO AFFECT CELL SIGNALING. FROM OBSERVATIONAL EPIDEMIOLOGIC STUDIES AND FROM LIMITED CLINICAL HUMAN AND ANIMAL STUDIES, FLAVONOIDS HAVE BEEN POSTULATED TO PLAY A ROLE IN THE PREVENTION OF SEVERAL CHRONIC DISEASES, INCLUDING NEURODEGENERATIVE DISEASE, DIABETES, AND OSTEOPOROSIS. THE ULTIMATE IMPORTANCE AND USEFULNESS OF THEIR COMPOUNDS AGAINST HUMAN DISEASE HAVE YET TO BE DEMONSTRATED.

VITAMIN A

VITAMIN A, IN THE STRICTEST SENSE, REFERS TO RETINOL. HOWEVER, THE OXIDIZED METABOLITES, RETINALDEHYDE AND RETINOIC ACID, ARE ALSO BIOLOGICALLY ACTIVE COMPOUNDS. THE TERM RETINOIDS INCLUDES ALL MOLECULES (INCLUDING SYNTHETIC MOLECULES) THAT ARE CHEMICALLY RELATED TO RETINOL. RETINALDEHYDE (11-CIS) IS THE ESSENTIAL FORM OF VITAMIN A THAT IS REQUIRED FOR NORMAL VISION, WHEREAS RETINOIC ACID IS NECESSARY FOR NORMAL MORPHOGENESIS, GROWTH, AND CELL DIFFERENTIATION. RETINOIC ACID DOES NOT FUNCTION IN VISION AND, IN CONTRAST TO RETINOL, IS NOT INVOLVED IN REPRODUCTION. VITAMIN A ALSO PLAYS A ROLE IN IRON UTILIZATION, HUMORAL IMMUNITY, T CELL-MEDIATED IMMUNITY, NATURAL KILLER CELL ACTIVITY, AND PHAGOCYTOSIS. VITAMIN A IS COMMERCIALY AVAILABLE IN ESTERIFIED FORMS (E.G., ACETATE, PALMITATE) SINCE IT IS MORE STABLE AS AN ESTER. THERE ARE MORE THAN 600 CAROTENOIDS IN NATURE, AND APPROXIMATELY 50 OF THESE CAN BE METABOLIZED TO VITAMIN A. *-CAROTENE IS THE MOST PREVALENT CAROTENOID IN THE FOOD SUPPLY THAT HAS PROVITAMIN A ACTIVITY. IN HUMANS, SIGNIFICANT FRACTIONS OF CAROTENOIDS ARE ABSORBED INTACT AND ARE STORED IN LIVER AND FAT. IT IS NOW ESTIMATED THAT 12 *G OR GREATER OF DIETARY #-CAROTENE IS EQUIVALENT TO 1 *G OF RETINOL, WHEREAS 24 *G OR GREATER OF OTHER DIETARY PROVITAMIN A CAROTENOIDS (E.G., CRYPTOXANTHIN, #-CAROTENE) IS EQUIVALENT TO 1 *G OF RETINOL.

METABOLISM THE LIVER CONTAINS APPROXIMATELY 90% OF THE VITAMIN A RESERVES AND SECRETES VITAMIN A IN THE FORM OF RETINOL, WHICH IS BOUND TO RETINOL-BINDING PROTEIN. ONCE THIS HAS OCCURRED, THE RETINOL-BINDING PROTEIN COMPLEX INTERACTS WITH A SECOND PROTEIN, TRANSTHYRETIN. THIS TRIMOLECULAR COMPLEX FUNCTIONS TO PREVENT VITAMIN A FROM BEING FILTERED BY THE KIDNEY GLOMERULUS, TO PROTECT THE BODY AGAINST THE TOXICITY OF RETINOL AND TO ALLOW RETINOL TO BE TAKEN UP BY SPECIFIC CELL-SURFACE RECEPTORS THAT RECOGNIZE RETINOL-BINDING PROTEIN. A CERTAIN AMOUNT OF VITAMIN A ENTERS PERIPHERAL CELLS EVEN IF IT IS NOT BOUND TO RETINOL-BINDING PROTEIN. AFTER RETINOL IS INTERNALIZED BY THE CELL, IT BECOMES BOUND TO A SERIES OF CELLULAR RETINOL-BINDING PROTEINS, WHICH FUNCTION AS SEQUESTERING AND TRANSPORTING AGENTS AS WELL AS CO-LIGANDS FOR
ENZYMATIC REACTIONS. CERTAIN CELLS ALSO CONTAIN RETINOIC ACID-BINDING PROTEINS, WHICH HAVE SEQUESTERING FUNCTIONS BUT ALSO SHUTTLE RETINOIC ACID TO THE NUCLEUS AND ENABLE ITS METABOLISM. RETINOIC ACID IS A LIGAND FOR CERTAIN NUCLEAR RECEPTORS THAT ACT AS TRANSCRIPTION FACTORS. TWO FAMILIES OF RECEPTORS (RAR AND RXR RECEPTORS) ARE ACTIVE IN RETINOID-MEDIATED GENE TRANSCRIPTION. RETINOID RECEPTORS REGULATE TRANSCRIPTION BY BINDING AS DIMERIC COMPLEXES TO SPECIFIC DNA SITES, THE RETINOIC ACID RESPONSE ELEMENTS, IN TARGET GENES (CHAP. 332). THE RECEPTORS CAN EITHER STIMULATE OR REPRESS GENE EXPRESSION IN RESPONSE TO THEIR LIGANDS. RAR BINDS ALL-TRANS RETINOIC ACID AND 9-CIS RETINOIC ACID, WHEREAS RXR BINDS ONLY 9-CIS RETINOIC ACID.

PAGE NO. 90

447 CHAPTER 71 VITAMIN AND TRACE MINERAL DEFICIENCY AND EXCESS

THE RETINOID RECEPTORS PLAY AN IMPORTANT ROLE IN CONTROLLING CELL PROLIFERATION AND DIFFERENTIATION. RETINOIC ACID IS USEFUL IN THE TREATMENT OF PROMYEOCYTIC LEUKEMIA (CHAP. 104) AND IS ALSO USED IN THE TREATMENT OF CYSTIC ACNE BECAUSE IT INHIBITS KERATINIZATION, DECREASES SEBUM SECRETION, AND POSSIBLY ALTERS THE INFLAMMATORY REACTION (CHAP. 53). RXRS DIMERIZE WITH OTHER NUCLEAR RECEPTORS TO FUNCTION AS COREGULATORS OF GENES RESPONSIVE TO RETINOIDS, THYROID HORMONE, AND CALCITRIOL. RXR AGONISTS INDUCE INSULIN SENSITIVITY EXPERIMENTALLY, PERHAPS BECAUSE RXR IS A COFACTOR FOR THE PEROXISOME-PROLIFERATOR-ACTIVATED RECEPTORS (PPARS), WHICH ARE TARGETS FOR THE THIAZOLIDINEDIONE DRUGS SUCH AS ROSIGLITAZONE AND TROGLITAZONE (CHAP. 338).

DIETARY SOURCES THE RETINOL ACTIVITY EQUIVALENT (RAE) IS USED TO EXPRESS THE VITAMIN A VALUE OF FOOD. ONE RAE IS DEFINED AS 1 *G OF RETINOL (0.003491 MMOL), 12 *G OF -*CAROTENE, AND 24 *G OTHER PROVITAMIN A CAROTENOIDS. IN OLDER LITERATURE, VITAMIN A WAS OFTEN EXPRESSED IN INTERNATIONAL UNITS (IU), WITH 1 RAE BEING EQUAL TO 3.33 IU OF RETINOL AND 20 1U OF -*CAROTENE, BUT THESE UNITS ARE NO LONGER IN CURRENT SCIENTIFIC USE.

LIVER, FISH, AND EGGS ARE EXCELLENT FOOD SOURCES FOR PREFORMED VITAMIN A; VEGETABLE SOURCES OF PROVITAMIN A CAROTENOIDS INCLUDE DARK GREEN AND DEEPLY COLORED FRUITS AND VEGETABLES. MODERATE COOKING OF VEGETABLES ENHANCES CAROTENOID RELEASE FOR UPTAKE IN THE GUT. CAROTENOID ABSORPTION IS ALSO AIDED BY SOME FAT IN A MEAL. INFANTS ARE PARTICULARLY SUSCEPTIBLE TO VITAMIN A DEFICIENCY BECAUSE NEITHER BREAST NOR COW’S MILK SUPPLIES ENOUGH VITAMIN A TO PREVENT DEFICIENCY. IN DEVELOPING COUNTRIES, CHRONIC DIETARY DEFICIT IS THE MAIN CAUSE OF VITAMIN A DEFICIENCY AND IS EXACERBATED BY INFECTION. IN EARLY CHILDHOOD, LOW VITAMIN A STATUS RESULTS FROM INADEQUATE INTAKES OF ANIMAL FOOD SOURCES AND EDIBLE OILS, BOTH OF WHICH ARE EXPENSIVE, COUPLED WITH SEASONAL UN-
AVAILABILITY OF VEGETABLES AND FRUITS, AND LACK OF MARKETED FORTIFIED FOOD PRODUCTS. CONCURRENT ZINC DEFICIENCY CAN INTERFERE WITH THE MOBILIZATION OF VITAMIN A FROM LIVER STORES. ALCOHOL INTERFERES WITH THE CONVERSION OF RETINOL TO RETINALDEHYDE IN THE EYE BY COMPETING FOR ALCOHOL (RETINOL) DEHYDROGENASE. DRUGS THAT INTERFERE WITH THE ABSORPTION OF VITAMIN A INCLUDE MINERAL OIL, NEOMYCIN, AND CHOLESTYRAMINE.

DEFICIENCY VITAMIN A DEFICIENCY IS ENDEMIC WHERE DIETS ARE CHRONICALLY POOR, ESPECIALLY IN SOUTHERN ASIA, SUB-SAHARAN AFRICA, SOME AREAS OF LATIN AMERICA, AND THE WESTERN PACIFIC, INCLUDING PARTS OF CHINA. VITAMIN A STATUS IS USUALLY ASSESSED BY MEASURING SERUM RETINOL [NORMAL RANGE, 1.05-3.50 *MOL/L (30-100 *G/DL)] OR BLOOD SPOT RETINOL OR BY TESTS OF DARK ADAPTATION. STABLE ISOTOPIC OR INVASIVE LIVER BIOPSY METHODS EXIST TO ESTIMATE TOTAL BODY STORES OF VITAMIN A. BASED ON DEFICIENT SERUM RETINOL [<0.70 *MOL/L (20 *G/DL)], THERE ARE MORE THAN 125 MILLION PRESCHOOL-AGE CHILDREN WITH VITAMIN A DEFICIENCY, AMONG WHOM ~4 MILLION HAVE AN OCULAR MANIFESTATION OF DEFICIENCY TERMED XEROPHTHALMIA. THIS CONDITION INCLUDES MILD STAGES OF NIGHT BLINDNESS AND CONJUNCTIVAL XEROSIS (DRYNESS) WITH BITOT’S SPOTS (WHITE PATCHES OF KERATINIZED EPITHELIMUM APPEARING ON THE SCLERA) AS WELL AS RARE, POTENTIALLY BLINDING CORNEAL ULCERATION AND NECROSIS. KERATOMALACIA (SOFTENING OF THE CORNEA) LEADS TO CORNEAL SCARRING THAT BLINDS AT LEAST A QUARTER OF A MILLION CHILDREN EACH YEAR AND IS ASSOCIATED WITH A FATALITY RATE OF 4-25%. HOWEVER, VITAMIN A DEFICIENCY AT ANY STAGE POSES AN INCREASED RISK OF MORTALITY FROM DIARRHEA, DYSENTERY, MEASLES, MALARIA, AND RESPIRATORY DISEASE. VITAMIN A DEFICIENCY CAN COMPROMISE BARRIER AND INNATE AND ACQUIRED IMMUNE DEFENSES TO INFECTION. VITAMIN A SUPPLEMENTATION CAN MARKEDLY REDUCE RISK OF CHILD MORTALITY (23-34%, ON AVERAGE) WHERE DEFICIENCY IS WIDELY PREVALENT. ABOUT 10% OF PREGNANT WOMEN IN UNDERNOURISHED SETTINGS ALSO DEVELOP NIGHT BLINDNESS, ASSESSED BY HISTORY, DURING THE LATTER HALF OF PREGNANCY AND THIS MODERATE VITAMIN A DEFICIENCY IS ASSOCIATED WITH AN INCREASED RISK OF MATERNAL INFECTION AND MORTALITY.

VITAMIN A DEFICIENCY

ANY STAGE OF XEROPHTHALMIA SHOULD BE TREATED WITH 60 MG OF VITAMIN A IN OILY SOLUTION, USUALLY CONTAINED IN A SOFT-GEL CAPSULE. THE SAME DOSE IS REPEATED 1 AND 14 DAYS LATER. DOSES SHOULD BE REDUCED BY HALF FOR PATIENTS 6-11 MONTHS OF AGE. MOTHERS WITH NIGHT BLINDNESS OR BITOT’S SPOTS SHOULD BE GIVEN VITAMIN A ORALLY, EITHER 3 MG DAILY OR 7.5 MG TWICE A WEEK FOR 3 MONTHS. THESE REGIMENS ARE EFFICACIOUS, AND THEY ARE LESS EXPENSIVE AND MORE WIDELY AVAILABLE THAN INJECTABLE WATER-MISCIBLE VITAMIN A. A COMMON APPROACH TO PREVENTION IS TO SUPPLEMENT YOUNG CHILDREN LIVING IN HIGH-RISK AREAS WITH 60 MG EVERY 4-6 MONTHS, WITH A HALF-DOSE GIVEN TO INFANTS 6-11 MONTHS OF AGE. UNCOMPLICATED VITAMIN A DEFICIENCY RARELY OCCURS IN INDUSTRIALIZED COUNTRIES. ONE HIGH-RISK GROUP, EXTREMELY LOW-BIRTH-WEIGHT INFANTS (<1000
IS LIKELY TO BE VITAMIN A-DEFICIENT AND SHOULD BE SUPPLEMENTED WITH 1500 *G (OR RAE) OF VITAMIN A, THREE TIMES A WEEK FOR 4 WEEKS. SEVERE MEASLES IN ANY SOCIETY CAN LEAD TO SECONDARY VITAMIN A DEFICIENCY. CHILDREN HOSPITALIZED WITH MEASLES SHOULD RECEIVE TWO 60-MG DOSES OF VITAMIN A ON TWO CONSECUTIVE DAYS. VITAMIN A DEFICIENCY MOST OFTEN OCCURS IN PATIENTS WITH MALABSORPTIVE DISEASES (E.G., CELIAC SPRUE, SHORT-BOWEL SYNDROME), WHO HAVE ABNORMAL DARK ADAPTATION OR SYMPTOMS OF NIGHT BLINDNESS WITHOUT OTHER OCULAR CHANGES. TYPICALLY, SUCH PATIENTS ARE TREATED FOR 1 MONTH WITH 15 MG/D OF A WATER-MISCIBLE PREPARATION OF VITAMIN A. THIS IS FOLLOWED BY A LOWER MAINTENANCE DOSE WITH THE EXACT AMOUNT DETERMINED BY MONITORING SERUM RETINOL.

THERE ARE NO SPECIFIC DEFICIENCY SIGNS OR SYMPTOMS THAT RESULT FROM CAROTENOID DEFICIENCY. IT WAS POSTULATED THAT *-CAROTENE WOULD BE AN EFFECTIVE CHEMOPREVENTIVE AGENT FOR CANCER BECAUSE NUMEROUS EPIDEMIOLOGIC STUDIES HAD SHOWN THAT DIETS HIGH IN *-CAROTENE WERE ASSOCIATED WITH LOWER INCIDENCES OF CANCERS OF THE RESPIRATORY AND DIGESTIVE SYSTEMS. HOWEVER, INTERVENTION STUDIES IN SMOKERS FOUND THAT TREATMENT WITH HIGH DOSES OF *-CAROTENE ACTUALLY RESULTED IN MORE LUNG CANCERS THAN DID TREATMENT WITH PLACÉBO. NON-PROVITAMIN A CAROTENOIDS, SUCH AS LUTEIN AND ZEAXANTHIN, HAVE BEEN SUGGESTED TO PROTECT AGAINST MACULAR DEGENERATION. THE NON-PROVITAMIN A CAROTENOID LYCOPENE HAS BEEN PROPOSED TO PROTECT AGAINST PROSTATE CANCER. HOWEVER, THE EFFECTIVENESS OF THESE AGENTS HAS NOT BEEN PROVEN BY INTERVENTION STUDIES, AND THE MECHANISMS UNDERLYING THESE PURPORTED BIOLOGIC ACTIONS ARE UNKNOWN.

TOXICITY ACUTE TOXICITY OF VITAMIN A WAS FIRST NOTED IN ARCTIC EXPLORERS WHO ATE POLAR BEAR LIVER AND HAS ALSO BEEN SEEN AFTER ADMINISTRATION OF 150 MG IN ADULTS OR 100 MG IN CHILDREN. ACUTE TOXICITY IS MANIFESTED BY INCREASED INTRACRANIAL PRESSURE, VERTIGO, DIPLOPIA, BULGING FONTANELS IN CHILDREN, SEIZURES, AND EXFOLIATIVE DERMATITIS; IT MAY RESULT IN DEATH.

IN CHILDREN BEING TREATED FOR VITAMIN A DEFICIENCY ACCORDING TO THE PROTOCOLS OUTLINED ABOVE, TRANSIENT BULGING OF FONTANELS OCCURS IN 2% OF INFANTS, AND TRANSIENT NAUSEA, VOMITING, AND HEADACHE OCCUR IN 5% OF PRESCHOOLERS. CHRONIC VITAMIN A INTOXICATION IS LARGELY A CONCERN IN INDUSTRIALIZED COUNTRIES AND HAS BEEN SEEN IN NORMAL ADULTS WHO INGEST 15 MG/D AND CHILDREN WHO INGEST 6 MG/D OF VITAMIN A OVER A PERIOD OF SEVERAL MONTHS. MANIFESTATIONS INCLUDE DRY SKIN, CHEILOSIS, GLOSSITIS, VOMITING, ALOPECIA, BONE DEMINERALIZATION AND PAIN, HYPERCALCEMIA, LYMPH NODE ENLARGEMENT, HYPERLIPIDEMIA, AMENORRHEA, AND FEATURES OF PSEUDOTUMOR CEREBRI WITH INCREASED INTRACRANIAL PRESSURE AND PAPILLEDEMA. LIVER FIBROSIS WITH PORTAL HYPERTENSION AND BONE DEMINERALIZATION MAY RESULT FROM CHRONIC VITAMIN A INTOXICATION. WHEN VITAMIN A IS PROVIDED IN EXCESS TO PREGNANT WOMEN, CONGENITAL MALFORMATIONS HAVE INCLUDED SPONTANEOUS ABORTIONS, CRANIOFACIAL ABNORMALITIES, AND VALVULAR HEART DISEASE. IN PREGNANCY, THE DAILY DOSE OF VITAMIN A SHOULD NOT EXCEED 3 MG. COMMERCIALLY AVAILABLE RETINOID DERIVATIVES ARE ALSO TOXIC, INCLUDING 13-CIS-RETINOIC ACID, WHICH HAS BEEN ASSOCIATED WITH BIRTH DEFECTS. AS A RESULT, CONTRACEPTION SHOULD BE
CONTINUED FOR AT LEAST 1 YEAR, AND POSSIBLY LONGER, IN WOMEN WHO HAVE TAKEN 13-CIS RETINOIC ACID. HIGH DOSES OF CAROTENOIDS DO NOT RESULT IN TOXIC SYMPTOMS BUT SHOULD BE AVOIDED IN SMOKERS DUE TO AN INCREASED RISK OF LUNG CANCER. CAROTENEMIA, WHICH IS CHARACTERIZED BY A YELLOWING OF THE SKIN (CREASES OF THE PALMS AND SOLES) BUT NOT THE SCLERAE, MAY BE PRESENT AFTER INGESTION OF >30 MG OF *-CAROTENE DAILY. HYPOTHYROID PATIENTS ARE PARTICULARLY SUSCEPTIBLE TO THE DEVELOPMENT OF CAROTENEMIA DUE TO IMPAIRED BREAKDOWN OF CAROTENE TO VITAMIN A. REDUCTION OF CAROTENES FROM THE DIET RESULTS IN THE DISAPPEARANCE OF SKIN YELLOWING AND CAROTENEMIA OVER A PERIOD OF 30-60 DAYS.

PAGE NO. 91

448 PART 5: NUTRITION

VITAMIN D

SEE CHAP. 346, FIG. 71-1, AND TABLE 71-1.

VITAMIN E


ABSORPTION AND METABOLISM AFTER ABSORPTION, VITAMIN E IS TAKEN UP FROM CHYLOMICRONs BY THE LIVER, AND A HEPATIC *-TOCOPHEROL TRANSPORT PROTEIN MEDIATES INTRACELLULAR VITAMIN E TRANSPORT AND INCORPORATION INTO VERY LOW-DENSITY LIPOPROTEIN (VLDL). THE TRANSPORT PROTEIN HAS PARTICULAR AFFINITY FOR THE RRR ISOMERIC FORM OF *-TOCOPHEROL; THUS THIS NATURAL ISOMER HAS THE MOST BIOLOGIC ACTIVITY.

REQUIREMENT VITAMIN E IS WIDELY DISTRIBUTED IN THE FOOD SUPPLY AND IS PARTICULARLY HIGH IN SUNFLOWER OIL, SAFFLOWER OIL, AND WHEAT GERM OIL; *-TOCOTRIENOLS ARE NOTABLY PRESENT IN SOYBEAN AND CORN OILS. VITAMIN E IS ALSO FOUND IN MEATS, NUTS, AND CEREAL GRAINS, AND SMALL AMOUNTS ARE PRESENT IN FRUITS AND VEGETABLES. VITAMIN E PILLS CONTAINING DOSES OF 50-1000 MG ARE INGESTED BY A LARGE FRACTION OF THE U.S. POPULATION. THE RDA FOR VITAMIN E IS 15 MG/D (34.9 *MOL OR 22.5 IU) FOR ALL ADULTS. DIETS HIGH IN POLYUNSATURATED FATS MAY NECESSITATE A SLIGHTLY
HIGHER REQUIREMENT FOR VITAMIN E.

DIETARY DEFICIENCY OF VITAMIN E DOES NOT EXIST. VITAMIN E DEFICIENCY IS SEEN IN ONLY SEVERE AND PROLONGED MALABSORPTIVE DISEASES, SUCH AS CELIAC DISEASE, OR AFTER SMALL-INTESTINAL RESECTION. CHILDREN WITH CYSTIC FIBROSIS OR PROLONGED CHOLESTASIS MAY DEVELOP VITAMIN E DEFICIENCY CHARACTERIZED BY AREFLEXIA AND HEMOLYTIC ANEMIA. CHILDREN WITH ABETALIPOPROTEINEMIA CANNOT ABSORB OR TRANSPORT VITAMIN E AND BECOME DEFICIENT QUITE RAPIDLY. A FAMILIAL FORM OF ISOLATED VITAMIN E DEFICIENCY ALSO EXISTS; IT IS DUE TO A DEFECT IN THE A TOCOPHEROL TRANSPORT PROTEIN. VITAMIN E DEFICIENCY CAUSES AXONAL DEGENERATION OF THE LARGE MYELINATED AXONS AND RESULTS IN POSTERIOR COLUMN AND SPINOCEREBELLAR SYMPTOMS. PERIPHERAL NEUROPATHY IS INITIALLY CHARACTERIZED BY AREFLEXIA, WITH PROGRESSION TO AN ATAXIC GAIT, AND BY DECREASED VIBRATION AND POSITION SENSATIONS. OPHTHALMOPLEGIA, SKELETAL MYOPATHY, AND PIGMENTED RETINOPATHY MAY ALSO BE FEATURES OF VITAMIN E DEFICIENCY. EITHER VITAMIN E OR SELENIUM DEFICIENCY IN THE HOST HAS BEEN SHOWN TO INCREASE CERTAIN VIRAL MUTATIONS AND, THEREFORE, VIRULENCE. THE LABORATORY DIAGNOSIS OF VITAMIN E DEFICIENCY IS MADE ON THE BASIS OF LOW BLOOD LEVELS OF * TOCOPHEROL (<5 *G/ML, OR <0.8 MG OF * TOCOPHEROL PER GRAM OF TOTAL LIPIDS).

VITAMIN E DEFICIENCY

SYMPTOMATIC VITAMIN E DEFICIENCY SHOULD BE TREATED WITH 800-1200 MG OF * TOCOPHEROL PER DAY. PATIENTS WITH ABETALIPOPROTEINEMIA MAY NEED AS MUCH AS 5000-7000 MG/D. CHILDREN WITH SYMPTOMATIC VITAMIN E DEFICIENCY SHOULD BE TREATED WITH 400 MG/D ORALLY OF WATER-MISCIBLE ESTERS; ALTERNATIVELY, 2 MG/KG PERD MAY BE ADMINISTERED INTRAMUSCULARLY. VITAMIN E IN HIGH DOSES MAY PROTECT AGAINST OXYGEN-INDUCED RETROLENTAL FIBROPLASIA AND BRONCHOPULMONARY DYSPLASIA, A SWELL AS INTRAVENTRICULAR HEMORRHAGE OF PREMATURE. VITAMIN E HAS BEEN SUGGESTED TO INCREASE SEXUAL PERFORMANCE, TO TREAT INTERMITTENT CLAUDICATION, AND TO SLOW THE AGING PROCESS, BUT EVIDENCE FOR THESE PROPERTIES IS LACKING. WHEN GIVEN IN COMBINATION WITH OTHER ANTIOXIDANTS, VITAMIN E MAY HELP TO PREVENT MACULAR DEGENERATION. HIGH DOSES (60-800 MG/D) OF VITAMIN E HAVE BEEN SHOWN IN CONTROLLED TRIALS TO IMPROVE PARAMETERS OF IMMUNE FUNCTION AND TO REDUCE COLDS IN NURSING HOME RESIDENTS, BUT INTERVENTION STUDIES USING VITAMIN E TO PREVENT CARDIOVASCULAR DISEASE OR CANCER HAVE NOT SHOWN EFFICACY AND, AT DOSES >400 MG/D, MAY EVEN INCREASE ALL-CAUSE MORTALITY.

TOXICITY ALL FORMS OF VITAMIN E ARE ABSORBED AND COULD CONTRIBUTE
TOXICITY. HIGH DOSES OF VITAMIN E (>800 MG/D) MAY REDUCE PLATELET AGGREGATION AND INTERFERE WITH VITAMIN K METABOLISM AND ARE THEREFORE CONTRAINDICATED IN PATIENTS TAKING WARFARIN. NAUSEA, FLATULENCE, AND DIARRHEA HAVE BEEN REPORTED AT DOSES >1 G/D.

VITAMIN K

THERE ARE TWO NATURAL FORMS OF VITAMIN K: VITAMIN K1, ALSO KNOWN AS PHYLLOQUINONE, FROM VEGETABLE AND ANIMAL SOURCES, AND VITAMIN K2, OR MENAQUINONE, WHICH IS SYNTHESIZED BY BACTERIAL FLORA AND FOUND IN HEPATIC TISSUE. PHYLLOQUINONE CAN BE CONVERTED TO MENAQUINONE IN SOME ORGANS.

VITAMIN K IS REQUIRED FOR THE POSTTRANSLATIONAL CARBOXYLATION OF GLUTAMIC ACID, WHICH IS NECESSARY FOR CALCIUM BINDING TO CARBOXYLATED PROTEINS SUCH AS PROTHROMBIN (FACTOR II); FACTORS VII, IX, AND X; PROTEIN C; PROTEIN S; AND PROTEINS FOUND IN BONE (OSTEOCALCIN) AND VASCULAR SMOOTH MUSCLE (E.G., MATRIX GLA PROTEIN). HOWEVER, THE IMPORTANCE OF VITAMIN K FOR BONE MINERALIZATION AND PREVENTION OF VASCULAR CALCIFICATION IS NOT KNOWN. WARFARIN-TYPE DRUGS INHIBIT CARBOXYLATION BY PREVENTING THE CONVERSION OF VITAMIN K TO ITS ACTIVE HYDROQUINONE FORM.

DIETARY SOURCES VITAMIN K IS FOUND IN GREEN LEAFY VEGETABLES SUCH AS KALE AND SPINACH, AND APPRECIABLE AMOUNTS ARE ALSO PRESENT IN MARGARINE AND LIVER. VITAMIN K IS PRESENT IN VEGETABLE OILS AND IS PARTICULARLY RICH IN OLIVE, CANOLA, AND SOYBEAN OILS. THE AVERAGE DAILY INTAKE BY AMERICANS IS ESTIMATED TO BE APPROXIMATELY 100 MG/D.

DEFICIENCY THE SYMPTOMS OF VITAMIN K DEFICIENCY ARE DUE TO HEMORRHAGE, AND NEWBORNS ARE PARTICULARLY SUSCEPTIBLE BECAUSE OF LOW FAT STORES, LOW BREAST MILK LEVELS OF VITAMIN K, STERILITY OF THE INFANTILE INTESTINAL TRACT, LIVER IMMATURITY, AND POOR PLACENTAL TRANSPORT.

INTRACRANIAL BLEEDING, AS WELL AS GASTROINTESTINAL AND SKIN BLEEDING, CAN OCCUR IN VITAMIN K-DEFICIENT INFANTS 1-7 DAYS AFTER BIRTH. THUS, VITAMIN K (1 MG IM) IS GIVEN PROPHYLACTICALLY AT THE TIME OF DELIVERY. VITAMIN K DEFICIENCY IN ADULTS MAY BE SEEN IN PATIENTS WITH CHRONIC SMALL-INTESTINAL DISEASE (E.G., CELIAC DISEASE, CROHN’S DISEASE), IN THOSE WITH OBSTRUCTED BILIARY TRACTS, OR AFTER SMALL-BOWEL RESECTION. BROAD-SPECTRUM ANTIBIOTIC TREATMENT CAN PRECIPITATE VITAMIN K DEFICIENCY BY REDUCING GUT BACTERIA, WHICH SYNTHESIZE MENAQUINONES, AND BY INHIBITING THE METABOLISM OF VITAMIN K. IN PATIENTS WITH WARFARIN THERAPY, THE ANTIOBESITY DRUG ORLISTAT CAN LEAD TO INR CHANGES DUE TO VITAMIN K MALABSORPTION. THE DIAGNOSIS OF VITAMIN K DEFICIENCY IS USUALLY MADE ON THE BASIS OF AN ELEVATED PROTHROMBIN TIME OR REDUCED CLOTTING FACTORS, ALTHOUGH VITAMIN K MAY ALSO BE MEASURED DIRECTLY BY HPLC. VITAMIN K DEFICIENCY IS TREATED USING A PARENTERAL DOSE OF 10 MG. FOR PATIENTS WITH CHRONIC MALABSORPTION, 1-2 MG/D OF VITAMIN K SHOULD BE GIVEN ORALLY, OR 1-2 MG/WEEK CAN BE TAKEN PARENTERALLY. PATIENTS WITH LIVER DISEASE MAY HAVE AN ELEVATED PROTHROMBIN TIME BECAUSE OF LIVER CELL DESTRUCTION AS WELL AS VITAMIN K
DEFICIENCY. IF AN ELEVATED PROTHROMBIN TIME DOES NOT IMPROVE ON VITAMIN K THERAPY, IT CAN BE DEDUCED THAT IT IS NOT THE RESULT OF VITAMIN K DEFICIENCY.

TOXICITY TOXICITY FROM DIETARY PHYLLOQUINONES AND MENAQUINONES HAS NOT BEEN DESCRIBED. HIGH DOSES OF VITAMIN K CAN IMPAIR THE ACTIONS OF ORAL ANTICOAGULANTS.

MINERALS

TABLE 71-2.

CALCIUM

SEE CHAP. 346.

ZINC

ZINC IS AN INTEGRAL COMPONENT OF MANY METALLOENZYMES IN THE BODY; IT IS INVOLVED IN THE SYNTHESIS AND STABILIZATION OF PROTEINS, DNA, AND RNA AND PLAYS A STRUCTURAL ROLE IN RIBOSOMES AND MEMBRANES. ZINC IS NECESSARY FOR THE BINDING OF STEROID HORMONE RECEPTORS AND SEVERAL OTHER TRANSCRIPTION FACTORS TO DNA. ZINC IS ABSOLUTELY REQUIRED FOR NORMAL SPERMATOGENESIS, FETAL GROWTH, AND EMBRYONIC DEVELOPMENT.

ABSORPTION THE ABSORPTION OF ZINC FROM THE DIET IS INHIBITED BY DIETARY PHYTATE, FIBER, OXALATE, IRON, AND COPPER, AS WELL AS BY CERTAIN DRUGS INCLUDING PENICILLAMINE, SODIUM VALPROATE, AND ETHAMBUTOL. MEAT, SHELLFISH, NUTS, AND LEGUMES ARE GOOD SOURCES OF BIOAVAILABLE ZINC, WHEREAS ZINC IN GRAINS AND LEGUMES IS LESS AVAILABLE FOR ABSORPTION.

DEFICIENCY MILD ZINC DEFICIENCY HAS BEEN DESCRIBED IN MANY DISEASES, INCLUDING DIABETES MELLITUS, HIV/AIDS, CIRRHOSIS, ALCOHOLISM, INFLAMMATORY BOWEL DISEASE, MALABSORPTION SYNDROMES, AND SICKLE CELL DISEASE. IN THESE DISEASES, MILD CHRONIC ZINC DEFICIENCY CAN CAUSE STunted GROWTH IN CHILDREN, DECREASED TASTE SENSATION (HYPOGEUSIA), AND IMPAIRED IMMUNE FUNCTION. SEVERE CHRONIC ZINC DEFICIENCY HAS BEEN DESCRIBED AS A CAUSE OF HYPOGONADISM AND DWARFISM IN SEVERAL MIDDLE EASTERN COUNTRIES. IN THESE CHILDREN, HYPOPigMENTED HAIR IS ALSO PART OF THE SYNDROME. ACRODERMATITIS ENTEROPATHICA IS A RARE AUTOSOMAL RECESSIVE DISORDER CHARACTERIZED BY ABNORMALITIES IN ZINC ABSORPTION. CLINICAL MANIFESTATIONS INCLUDE DIARRHEA, ALOPECIA, MUSCLE WASTING, DEPRESSION, IRRITABILITY, AND A RASH INVOLVING THE EXTREMITIES, FACE, AND PERINEUM. THE
RASH IS CHARACTERIZED BY VESICULAR AND PUSTULAR CRUSTING WITH SCALING AND ERYTHEMA. OCCASIONAL PATIENTS WITH WILSON’S DISEASE HAVE DEVELOPED ZINC DEFICIENCY AS A CONSEQUENCE OF PENICILLAMINE THERAPY (CHAP. 354).

THE DIAGNOSIS OF ZINC DEFICIENCY IS USUALLY MADE BY A SERUM ZINC LEVEL OF <12 *MOL/L (<70 *G/DL). PREGNANCY AND BIRTH CONTROL PILLS MAY CAUSE A SLIGHT DEPRESSION IN SERUM ZINC LEVELS, AND HYPOALBUMINEMIA FROM ANY CAUSE CAN RESULT IN HYPOZINCEMIA. IN ACUTE STRESS SITUATIONS, ZINC MAY BE REDISTRIBUTED FROM SERUM INTO TISSUES. ZINC DEFICIENCY MAY BE TREATED WITH 60 MG ELEMENTAL ZINC, ORALLY TWICE A DAY. ZINC GLUCONATE LOZENGES (13 MG ELEMENTAL ZINC EVERY 2 H WHILE AWAKE) HAVE BEEN REPORTED TO REDUCE THE DURATION AND SYMPTOMS OF THE COMMON COLD IN ADULTS, BUT STUDIES ARE CONFLICTING.

ZINC DEFICIENCY IS PREVALENT IN MANY DEVELOPING COUNTRIES AND USUALLY COEXISTS WITH OTHER MICRONUTRIENT DEFICIENCIES (ESPECIALLY IRON). ZINC (20 MG/D) MAY BE AN EFFECTIVE ADJUNCTIVE THERAPEUTIC STRATEGY FOR DIARRHEAL DISEASE IN CHILDREN.

TOXICITY ACUTE ZINC TOXICITY AFTER ORAL INGESTION CAUSES NAUSEA, VOMITING, AND FEVER. ZINC FUMES FROM WELDING MAY ALSO BE TOXIC AND CAUSE FEVER, RESPIRATORY DISTRESS, EXCESSIVE SALIVATION, SWEATING, AND HEADACHE. CHRONIC LARGE DOSES OF ZINC MAY DEPRESS IMMUNE FUNCTION AND CAUSE HYPOCHROMIC ANEMIA AS A RESULT OF COPPER DEFICIENCY.

COPPER

COPPER IS AN INTEGRAL PART OF NUMEROUS ENZYME SYSTEMS INCLUDING AMINE OXIDASES, FERROXIDASE (CERULOPLASMIN), CYTOCHROME-C OXIDASE, SUPEROXIDE DISMUTASE, AND DOPAMINE HYDROXYLASE. COPPER IS ALSO A COMPONENT OF FERROPROTEIN, A TRANSPORT PROTEIN INVOLVED IN THE BASOLATERAL TRANSFER OF IRON DURING ABSORPTION FROM THE ENTEROCYTE. AS SUCH, COPPER PLAYS A ROLE IN IRON METABOLISM, MELANIN SYNTHESIS, ENERGY PRODUCTION, NEUROTRANSMITTER SYNTHESIS, AND CNS FUNCTION; THE SYNTHESIS AND CROSS-LINKING OF ELASTIN AND COLLAGEN; AND THE SCAVENGING OF SUPEROXIDE RADICALS. DIETARY SOURCES OF COPPER INCLUDE SHELLFISH, LIVER, NUTS, LEGUMES, BRAN, AND ORGAN MEATS.

DEFICIENCY DIETARY COPPER DEFICIENCY IS RELATIVELY RARE, ALTHOUGH IT HAS BEEN DESCRIBED IN PREMATURE INFANTS WHO ARE FED MILK DIETS AND IN INFANTS WITH MALABSORPTION (TABLE 71-2). COPPER-DEFICIENCY ANEMIA HAS BEEN REPORTED IN PATIENTS WITH MALABSORPTIVE DISEASES AND NEPHROTIC SYNDROME AND IN PATIENTS TREATED FOR WILSON’S DISEASE WITH CHRONIC HIGH DOSES OF ORAL ZINC, WHICH CAN INTERFERE WITH COPPER ABSORPTION. MENKES KINKY HAIR SYNDROME IS AN X-LINKED METABOLIC DISTURBANCE OF COPPER METABOLISM CHARACTERIZED BY MENTAL RETARDATION, HYPOCUPREMIA, AND DECREASED CIRCULATING CERULOPLASMIN (CHAP. 357). IT IS CAUSED BY MUTA-
TIONS IN THE COPPER-TRANSPORTING ATP7A GENE. CHILDREN WITH THIS DISEASE OFTEN DIE WITHIN 5 YEARS BECAUSE OF DISSECTING ANEURYSMS OR CARDIAC RUP-
TURE. ACERULOPLASMINEMIA IS A RARE AUTOSOMAL RECESSIVE DISEASE CHARAC-
TERIZED BY TISSUE IRON OVERLOAD, MENTAL DETERIORATION, MICROCYTIC ANEMIA,
AND LOW SERUM IRON AND COPPER CONCENTRATIONS. THE DIAGNOSIS OF COPPER DEFICIENCY IS USUALLY MADE ON THE BASIS OF LOW SERUM LEVELS OF COPPER (<65 *G/DL) AND LOW CERULOPLASMIN LEVELS (<20 MG/DL). SERUM LEVELS OF COPPER MAY BE ELEVATED IN PREGNANCY OR STRESS CONDITIONS SINCE CERULOPLASMIN IS AN ACUTE-PHASE REACTANT AND 90% OF CIRCULATING COPPER IS BOUND TO CERULOPLASMIN.

TOXICITY COPPER TOXICITY IS USUALLY ACCIDENTAL (TABLE 71-2). IN SEVERE CASES, KIDNEY FAILURE, LIVER FAILURE, AND COMA MAY ENSUE. IN WILSON’S DISEASE, MUTATIONS IN THE COPPER-TRANSPORTING ATP7B GENE LEAD TO AC-
CUMULATION OF COPPER IN THE LIVER AND BRAIN, WITH LOW BLOOD LEVELS DUE TO DECREASED CERULOPLASMIN (CHAP. 354).

SELENIUM

SELENIUM, IN THE FORM OF SELENOCYSTEINE, IS A COMPONENT OF THE ENZYME GLUTATHIONE PEROXIDASE, WHICH SERVES TO PROTECT PROTEINS, CELL MEMBRANES, LIPIDS, AND NUCLEIC ACIDS FROM OXI-
DANT MOLECULES. AS SUCH, SELENIUM IS BEING ACTIVELY STUDIED AS A CHEMOPREVENTIVE AGENT AGAINST CERTAIN CANCERS, SUCH AS PROSTATE. SELE-
NOCYSTEINE IS ALSO FOUND IN THE DEIODINASE ENZYMES, WHICH MEDIATE THE DEIODINATION OF THYROXINE TO TRIIODOTHYRONINE (CHAP. 335). RICH DI-
ETARY SOURCES OF SELENIUM INCLUDE SEAFOOD, MUSCLE MEAT, AND CEREALS, ALTHOUGH THE SELENIUM CONTENT OF CEREAL IS DETERMINED BY THE SOIL CON-
CENTRATION. COUNTRIES WITH LOW SOIL CONCENTRATIONS INCLUDE PARTS OF SCANDINAVIA, CHINA, AND NEW ZEALAND. KESHAN DISEASE IS AN ENDEMIC CARDIOMYOPATHY FOUND IN CHILDREN AND YOUNG WOMEN RESIDING IN RE-
GIONS OF CHINA WHERE DIETARY INTAKE OF SELENIUM IS LOW (<20 *G/D). CONCOMITANT DEFICIENCIES OF IODINE AND SELENIUM MAY WORSEN THE CLIN-
ICAL MANIFESTATIONS OFCRETINISM. CHRONIC INGESTION OF HIGH AMOUNTS OF SELENIUM LEADS TO SELENOSIS CHARACTERIZED BY HAIR AND NAIL BRITTleness AND LOSS, GARLIC BREATH ODOR, SKIN RASH, MYOPATHY, IRRITABILITY, AND OTHER ABNORMALITIES OF THE NERVOUS SYSTEM.

CHROMIUM

CHROMIUM POTENTIATES THE ACTION OF INSULIN IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE, PRESUMABLY BY INCREASING INSULIN RECEPTOR-
MEDIATED SIGNALING, ALTHOUGH ITS USEFULNESS IN TREATING TYPE II DIABETES IS UNCER-
TAIN. IN ADDITION, IMPROVEMENT IN BLOOD LIPID PROFILES HAS BEEN REPORTED IN SOME PATIENTS. THE USEFULNESS OF CHROMIUM SUPPLEMENTS IN MUSCLE BUILDING IS NOT SUBSTANTIATED. RICH FOOD SOURCES OF CHROMIUM INCLUDE YEAST, MEAT, AND GRAIN PRODUCTS. CHROMIUM IN THE TRIVALENT STATE IS FOUND IN SUPPLEMENTS AND IS LARGELY NONTOXIC; HOWEVER, CHROMIUM-6 IS A
PRODUCT OF STAINLESS STEEL WELDING AND IS A KNOWN PULMONARY CARCINOGEN, AS WELL AS A CAUSE OF LIVER, KIDNEY, AND CNS DAMAGE.

MAGNESIUM

SEE CHAP. 346.

FLOURIDE, MANGANESE, AND ULTRATRACE ELEMENTS

AN ESSENTIAL FUNCTION FOR FLUORIDE IN HUMANS HAS NOT BEEN DESCRIBED, ALTHOUGH IT IS USEFUL FOR THE MAINTENANCE OF STRUCTURE IN TEETH AND BONE. ADULT FLUOROSIS RESULTS IN MOTTLED AND PITTED DEFECTS IN TOOTH ENAMEL AS WELL AS BRITTLE BONE (SKELETAL FLUOROSIS). MANGANESE AND MOLYBDENUM DEFICIENCIES HAVE BEEN REPORTED IN PATIENTS WITH RARE GENETIC ABNORMALITIES AND IN A FEW PATIENTS RECEIVING PROLONGED TOTAL PARENTERAL NUTRITION. SEVERAL MANGANESE-SPECIFIC ENZYMES HAVE BEEN IDENTIFIED (E.G., MANGANESE SUPEROXIDE DISMUTASE). DEFICIENCIES OF MANGANESE HAVE BEEN REPORTED TO RESULT IN BONE DE-MINERALIZATION, POOR GROWTH, ATAXIA, DISTURBANCES IN CARBOHYDRATE AND LIPID METABOLISM, AND CONVULSIONS. ULTRATRACE ELEMENTS ARE DEFINED AS THOSE NEEDED IN AMOUNTS <1 MG/D. ESSENTIALITY HAS NOT BEEN ESTABLISHED FOR MOST ULTRATRACE ELEMENTS, AL-

450 PART 5: NUTRITION

THOUGH SELENIUM, CHROMIUM, AND IODINE ARE CLEARLY ESSENTIAL (CHAP. 335). MOLYBDENUM IS NECESSARY FOR THE ACTIVITY OF SULFITE AND XANTHINE OXIDASE, AND MOLYBDENUM DEFICIENCY MAY RESULT IN SKELETAL AND BRAIN LESIONS.

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MILLER ER ET AL: META-ANALYSIS: HIGH-DOSAGE VITAMIN E SUPPLEMENTATION MAY INCREASE ALL-CAUSE MORTALITY. ANN INTERN M ED 142:37, 2005
MORRIS MC ET AL: DIETARY FOLATE AND VITAMIN B12 INTAKE AND COGNITIVE 72 MALNUTRITION AND NUTRITIONAL ASSESSMENT
MALNUTRITION CAN ARISE FROM PRIMARY OR SECONDARY CAUSES, WITH THE FORMER RESULTING FROM INADEQUATE OR POOR-QUALITY FOOD INTAKE AND THE LATTER FROM DISEASES THAT ALTER FOOD INTAKE OR NUTRIENT REQUIREMENTS, METABOLISM, OR ABSORPTION. PRIMARY MALNUTRITION OCCURS MAINLY IN DEVELOPING COUNTRIES AND UNDER CONDITIONS OF WAR OR FAMINE. SECONDARY MALNUTRITION, THE MAIN FORM ENCOUNTERED IN INDUSTRIALIZED COUNTRIES, WAS LARGELY UNRECOGNIZED UNTIL THE EARLY 1970S, WHEN IT BECAME APPRECIATED THAT PERSONS WITH ADEQUATE FOOD SUPPLIES CAN BECOME MALNOURISHED AS A RESULT OF ACUTE OR CHRONIC DISEASES THAT ALTER NUTRIENT INTAKE OR METABOLISM. VARIOUS STUDIES HAVE SHOWN THAT PROTEIN-ENERGY MALNUTRITION (PEM) AFFECTS ONE-THIRD TO ONE-HALF OF PATIENTS ON GENERAL MEDICAL AND SURGICAL WARDS IN TEACHING HOSPITALS. THE CONSISTENT FINDING THAT NUTRITIONAL STATUS INFLUENCES PATIENT PROGNOSIS UNDERSCORES THE IMPORTANCE OF PREVENTING, DETECTING, AND TREATING MALNUTRITION.

PROTEIN-ENERGY MALNUTRITION

THE TWO MAJOR TYPES OF PEM ARE MARASMUS AND KWASIORKOR. THESE CONDITIONS ARE COMPARED IN TABLE 72-1. MARASMUS AND KWASIORKOR CAN OCCUR SINGLY OR IN COMBINATION, AS MARASMICKWASIORKOR. KWASIORKOR CAN OCCUR RAPIDLY, WHEREAS MARASMUS IS THE END RESULT OF A GRADUAL WASTING PROCESS THAT PASSES THROUGH STAGES OF UNDERWEIGHT, THEN MILD, MODERATE, AND SEVERE CACHEXIA.

MARASMUS

THE END STAGE OF CACHEXIA, MARASMUS IS A STATE IN WHICH VIRTUALLY ALL AVAILABLE BODY FAT STORES HAVE BEEN EXHAUSTED DUE TO STARVATION. CONDITIONS THAT PRODUCE MARASMUS IN DEVELOPED COUNTRIES TEND TO BE CHRONIC AND INDOLENT, SUCH AS CANCER, CHRONIC PULMONARY DISEASE, AND ANOREXIA NERVOSA. MARASMUS IS EASY TO DETECT BECAUSE OF THE PATIENT’S STARVED APPEARANCE. THE DIAGNOSIS IS BASED ON SEVERE FAT AND MUSCLE WASTAGE RESULTING FROM PROLONGED CALORIE DEFICIENCY. DIMINISHED SKIN-FOLD THICKNESS REFLECTS THE LOSS OF FAT RESERVES; REDUCED ARM MUSCLE CIRCUMFERENCE WITH TEMPORAL AND INTEROSSEOUS MUSCLE WASTING REFLECTS THE CATABOLISM OF PROTEIN THROUGHOUT THE BODY, INCLUDING VITAL ORGANS SUCH AS THE HEART, LIVER, AND KIDNEYS.
THE LABORATORY FINDINGS IN MARASMUS ARE RELATIVELY UNREMARKABLE. THE CREATININE-HEIGHT INDEX (THE 24-H URINARY CREATININE EXCRETION COMPARED WITH NORMAL VALUES BASED ON HEIGHT) IS LOW, REFLECTING THE LOSS OF MUSCLE MASS. OCCASIONALLY, THE SERUM ALBUMIN LEVEL IS REDUCED, BUT IT STAYS ABOVE 2.8 G/DL IN UNCOMPLICATED CASES. DESPITE A MORBID APPEARANCE, IMMUNOCOMPETENCE, WOUND HEALING, AND THE ABILITY TO HANDLE SHORT-TERM STRESS ARE REASONABLY WELL PRESERVED IN MOST PATIENTS WITH MARASMUS. MARASMUS IS A CHRONIC, FAIRLY WELL-ADAPTED FORM OF STARVATION RATHER THAN AN ACUTE ILLNESS; IT SHOULD BE TREATED CAUTIOUSLY, IN AN ATTEMPT TO REVERSE THE DOWNWARD TREND GRADUALLY. ALTHOUGH NUTRITIONAL SUPPORT IS NECESSARY, OVERLY AGGRESSIVE REPLETION CAN RESULT IN SEVERE, EVEN LIFE-THREATENING METABOLIC IMBALANCES SUCH AS HYPOPHOSPHATEMIA AND CARDIORESPIRATORY FAILURE. WHEN POSSIBLE, ORAL OR ENTERAL NUTRITIONAL SUPPORT IS PREFERRED; TREATMENT STARTED SLOWLY ALLOWS READAPTATION OF METABOLIC AND INTESTINAL FUNCTIONS (CHAP. 73).

KWASHIORKOR

IN CONTRAST TO MARASMUS, KWASHIORKOR IN DEVELOPED COUNTRIES OCCURS MAINLY IN CONNECTION WITH ACUTE, LIFE-THREATENING ILLNESSES SUCH AS TRAUMA AND SEPSIS, AND CHRONIC ILLNESSES THAT INVOLVE ACUTE-PHASE IN-

TABLE 72-1 COMPARISON OF MARASMUS AND KWASHIORKOR

CLINICAL SETTING
TIME COURSE TO DEVELOP
CLINICAL FEATURES

LABORATORY FINDINGS

CLINICAL COURSE

MORTALITY
DIAGNOSTIC CRITERIA

MARASMUS

* ENERGY INTAKE
MONTHS OR YEARS
STARVED APPEARANCE
WEIGHT <80% STANDARD FOR HEIGHT
TRICEPS SKINFOLD <3 MM
MID-ARM MUSCLE CIRCUMFERENCE <15 CM
CREATININE-HEIGHT INDEX <60% STANDARD

REASONABLY PRESERVED RESPONSIVENESS TO SHORT-TERM STRESS

LOW UNLESS RELATED TO UNDERLYING DISEASE
TRICEPS SKINFOLD <3 MM
MID-ARM MUSCLE CIRCUMFERENCE <15 CM

KWASHIORKOR

* PROTEIN INTAKE DURING STRESS STATE
WEEKS
WELL-NOURISHED APPEARANCE
EASY HAIR PLUCKABILITY

EDEMA

SERUM ALBUMIN <2.8 G/DL
TOTAL IRON-BINDING CAPACITY <200 *G/DL
LYMPHOCYTES < 1500/*L
ANERGY
INFECTIONS
POOR WOUND HEALING, DECUBITUS ULCERS, SKIN BREAKDOWN
HIGH

SERUM ALBUMIN <2.8 G/DL
AT LEAST ONE OF THE FOLLOWING:
POOR WOUND HEALING,
DECUBITUS ULCERS, OR SKIN BREAKDOWN
EASY HAIR PLUCKABILITY
EDEMA
The findings used to diagnose kwashiorkor must be unexplained by other causes. Tested by firmly pulling a lock of hair from the top (not the sides or back), grasping with the thumb and forefinger. An average of three or more hairs removed easily and painlessly is considered abnormal hair pluckability.

Chapter 72 Malnutrition and Nutritional Assessment

Flammatory responses. The physiologic stress produced by these illnesses increases protein and energy requirements at a time when intake is often limited. A classic scenario for kwashiorkor is the acutely stressed patient who receives only 5% dextrose solutions for periods as brief as 2 weeks. Although the etiologic mechanisms are not clear, the protein-sparing response normally seen in starvation is blocked by the stressed state and by carbohydrate infusion. In its early stages, the physical findings of kwashiorkor are few and subtle. Fat reserves and muscle mass are initially unaffected, giving the deceptive appearance of adequate nutrition. Signs that support the diagnosis of kwashiorkor include easy hair pluckability, edema, skin breakdown, and poor wound healing. The major sine qua non is severe reduction of levels of serum proteins such as albumin (<2.8 G/DL) and transferrin (<150 MG/DL) or iron-binding capacity (<200 *G/DL). Cellular immune function is depressed, reflected by lymphopenia (<1500 lymphocytes/*L in adults and older children) and lack of response to skin test antigens (anergy).

The prognosis of adult patients with full-blown kwashiorkor is not good, even with aggressive nutritional support. Surgical wounds often dehisce (fail to heal), pressure sores develop, gastroparesis and diarrhea can occur with enteral feeding, the risk of gastrointestinal bleeding from stress ulcers is increased, host defenses are compromised, and death from overwhelming infection may occur despite antibiotic therapy. Unlike treatment in marasmus, aggressive nutritional support is indicated to restore better metabolic balance rapidly (chap. 73). Although kwashiorkor in children is less foreboding, perhaps because a lesser degree of stress is required to precipitate the disorder, it is still a serious condition.

Marasmic Kwashiorkor
MARASMIC KWASHIORKOR, THE COMBINED FORM OF PEM, DEVELOPS WHEN THE CACHETIC OR MARASMIC PATIENT EXPERIENCES ACUTE STRESS SUCH AS SURGERY, TRAUMA, OR SEPSIS, SUPERIMPOSING KWASHIORKOR ONTO CHRONIC STARVATION. AN EXTREMELY SERIOUS, LIFE-THREATENING SITUATION CAN OCCUR BECAUSE OF THE HIGH RISK OF INFECTION AND OTHER COMPLICATIONS. IT IS IMPORTANT TO DETERMINE THE MAJOR COMPONENT OF PEM SO THAT THE APPROPRIATE NUTRITIONAL PLAN CAN BE DEVELOPED. IF KWASHIORKOR PREDOMINATES, THE NEED FOR VIGOROUS NUTRITIONAL THERAPY IS URGENT; IF MARASMUS PREDOMINATES, FEEDING SHOULD BE MORE CAUTIOUS.

PHYSIOLOGIC CHARACTERISTICS OF HYPO-and HYPER-METABOLIC STATES

THE METABOLIC CHARACTERISTICS AND NUTRITIONAL NEEDS OF HYPERMETABOLIC PATIENTS WHO ARE STRESSED FROM INJURY, INFECTION, OR CHRONIC INFLAMMATORY ILLNESS DIFFER FROM THOSE OF HYPOMETABOLIC PATIENTS WHO ARE UNSTRESSED BUT CHRONICALLY STARVED. IN BOTH CASES, NUTRITIONAL SUPPORT IS IMPORTANT, BUT MISJUDGMENTS IN SELECTING THE APPROPRIATE APPROACH MAY HAVE DISASTROUS CONSEQUENCES. THE HYPOMETABOLIC PATIENT IS TYPIFIED BY THE RELATIVELY UNSTRESSED BUT MILDLY CATABOLIC AND CHRONICALLY STARVED INDIVIDUAL WHO, WITH TIME, WILL DEVELOP MARASMUS. THE HYPERMETABOLIC PATIENT STRESSED FROM INJURY OR INFECTION IS CATABOLIC (EXPERIENCING RAPID BREAKDOWN OF BODY MASS) AND IS AT HIGH RISK FOR DEVELOPING KWASHIORKOR, IF NUTRITIONAL NEEDS ARE NOT MET AND/OR THE ILLNESS DOES NOT RESOLVE QUICKLY. AS SUMMARIZED IN TABLE 72-2, THE TWO STATES ARE DISTINGUISHED BY DIFFERING PERTURBATIONS OF METABOLIC RATE, RATES OF PROTEIN BREAKDOWN (PROTEOLYSIS), AND RATES OF GLUCONEOGENESIS. THESE DIFFERENCES ARE MEDIATED BY PROINFLAMMATORY CYTOKINES AND COUNTERREGULATORY HORMONES—TUMOR NECROSIS FACTOR, INTERLEUKINS 1 AND 6, C-REA CTIVE PROTEIN, CATECHOLAMINES (EPINEPHRINE AND NOREPINEPHRINE), GLUCAGON, AND CORTISOL—THAT ARE RELATIVELY REDUCED IN HYPOMETABOLIC PATIENTS AND INCREASED IN HYPERMETABOLIC PATIENTS. ALTHOUGH INSULIN LEVELS ARE ALSO ELEVATED IN STRESSED PATIENTS, INSULIN RESISTANCE IN THE TARGET TISSUES PREVENTS INSULIN-MEDIATED ANABOLIC ACTIONS.

METABOLIC RATE

IN STARVATION AND SEMISTARVATION, THE RESTING METABOLIC RATE FALLS BETWEEN 10% AND 30% AS AN ADAPTIVE RESPONSE TO ENERGY RESTRICTION, SLOWING THE RATE OF WEIGHT LOSS. BY CONTRAST, RESTING METABOLIC RATE RISES

| TABLE 72-2 PHYSIOLOGIC CHARACTERISTICS OF HYPOMETABOLIC AND |
HYPERMETABOLIC STATES

PHYSIOLOGIC CHARACTERISTICS

CYTOKINES, CATECHOLAMINES, GLUCAGON, CORTISOL, INSULIN
METABOLIC RATE, O2 CONSUMPTION
PROTEOLYSIS, GLUCONEOGENESIS
UREAGENESIS, UREA EXCRETION
FAT CATABOLISM, FATTY ACID UTILIZATION
ADAPTATION TO STARVATION

HYPOMETABOLIC, NONSTRESSED PATIENT (CACHECTIC, MARASMIC)

NORMAL

HYPERMETABOLIC, STRESSED PATIENT (KWASHIORKOR RISK)

ABNORMAL

### A THESE CHANGES CHARACTERIZE THE STRESSED, KWASHIORKOR-RISK PATIENT SEEN IN DEVELOPED COUNTRIES; THEY DIFFER IN SOME RESPECTS FROM THE CHARACTERISTICS OF PRIMARY KWASHIORKOR SEEN IN DEVELOPING COUNTRIES.

IN THE PRESENCE OF PHYSIOLOGIC STRESS IN PROPORTION TO THE DEGREE OF THE INSULT. IT MAY INCREASE BY ABOUT 10% AFTER ELECTIVE SURGERY, 20-30% AFTER BONE FRACTURES, 30-60% WITH SEVERE INFECTIONS SUCH AS PERITONITIS OR
GRAM-NEGATIVE SEPTICEMIA, AND AS MUCH AS 110% AFTER MAJOR BURNS. IF THE METABOLIC RATE (ENERGY REQUIREMENT) IS NOT MATCHED BY ENERGY INTAKE, WEIGHT LOSS RESULTS SLOWLY IN HYPOMETABOLISM AND QUICKLY IN HYPERMETABOLISM. LOSSES OF UP TO 10% OF BODY WEIGHT ARE UNLIKELY TO BE DETRIMENTAL; HOWEVER, LOSSES GREATER THAN THIS IN ACUTELY ILL HYPERMETABOLIC PATIENTS MAY BE ASSOCIATED WITH RAPID DETERIORATION IN BODY FUNCTION.

PROTIEN CATABOLISM

THE RATE OF ENDOGENOUS PROTEIN BREAKDOWN (CATABOLISM) TO SUPPLY ENERGY NEEDS NORMALLY FALLS DURING UNCOMPLICATED ENERGY DEPRIVATION. AFTER ABOUT 10 DAYS OF TOTAL STARVATION, THE UNSTRESSED INDIVIDUAL LOSES ABOUT 12-18 G/D PROTEIN (EQUIVALENT TO APPROXIMATELY 2 OZ OF MUSCLE TISSUE OR 2-3 G OF NITROGEN). BY CONTRAST, IN INJURY AND SEPSIS, PROTEIN BREAKDOWN ACCELERATES IN PROPORTION TO THE DEGREE OF STRESS, TO 30-60 G/D AFTER ELECTIVE SURGERY, 60-90 G/D WITH INFECTION, 100-130 G/D WITH SEVERE SEPSIS OR SKELETAL TRAUMA, AND >175 G/D WITH MAJOR BURNS OR HEAD INJURIES. THESE LOSSES ARE REFLECTED BY PROPORTIONAL INCREASES IN THE EXCRETION OF UREA NITROGEN, THE MAJOR BYPRODUCT OF PROTEIN BREAKDOWN.

GLUCONEOGENESIS

THE MAJOR AIM OF PROTEIN CATABOLISM DURING A STATE OF STARVATION IS TO PROVIDE THE GLUCOGENIC AMINO ACIDS (ESPECIALLY ALANINE AND GLUTAMINE) THAT SERVE AS SUBSTRATES FOR ENDOGENOUS GLUCOSE PRODUCTION (GLUCONEOGENESIS) IN THE LIVER. IN THE HYPOMETABOLIC/STARVED STATE, PROTEIN BREAKDOWN FOR GLUCONEOGENESIS IS MINIMIZED, ESPECIALLY AS KETONES DERIVED FROM FATTY ACIDS BECOME THE SUBSTRATE PREFERRED BY CERTAIN TISSUES. IN THE HYPERMETABOLIC/STRESS STATE, GLUCONEOGENESIS INCREASES DRAMATICALLY AND IN PROPORTION TO THE DEGREE OF THE INSULT, TO INCREASE THE SUPPLY OF GLUCOSE (THE MAJOR FUEL OF REPARATION). GLUCOSE IS THE ONLY FUEL THAT CAN BE UTILIZED BY HYPOXIC TISSUES (ANAEROBIC GLYCOLYSIS), WHITE BLOOD CELLS, AND NEWLY GENERATED FIBROBLASTS. INFUSIONS OF GLUCOSE PARTIALLY OFFSET A NEGATIVE ENERGY BALANCE BUT DO NOT SIGNIFICANTLY SUPPRESS THE HIGH RATES OF GLUCONEOGENESIS IN THE CATABOLIC PATIENT. HENCE, ADEQUATE SUPPLIES OF PROTEIN ARE NEEDED TO REPLACE THE AMINO ACIDS UTILIZED FOR THIS METABOLIC RESPONSE. IN SUMMARY, THE HYPOMETABOLIC PATIENT IS ADAPTED TO STARVATION
AND CONSERVES BODY MASS BY REDUCING THE METABOLIC RATE AND USING FAT AS THE PRIMARY FUEL (RATHER THAN GLUCOSE AND ITS PRECURSOR AMINO ACIDS). THE HYPERMETABOLIC PATIENT ALSO USES FAT AS A FUEL BUT RAPIDLY BREAKS DOWN BODY PROTEIN TO PRODUCE GLUCOSE, CAUSING LOSS OF MUSCLE AND ORGAN TISSUE AND ENDANGERING VITAL BODY FUNCTIONS.

MICRONUTRIENT MALNUTRITION

THE SAME ILLNESSES AND REDUCTIONS IN NUTRIENT INTAKE THAT LEAD TO PEM OFTEN PRODUCE DEFICIENCIES OF VITAMINS AND MINERALS AS WELL (CHAP. 71).

452 PART 5: NUTRITION

DEFICIENCIES OF NUTRIENTS THAT ARE STORED IN SMALL AMOUNTS (SUCH AS THE WATER-SOLUBLE VITAMINS) ARE LOST THROUGH EXTERNAL SECRETIONS, SUCH AS ZINC IN DIARRHEA FLUID OR BURN EXUDATE, AND ARE PROBABLY MORE COMMON THAN GENERALLY RECOGNIZED. DEFICIENCIES OF VITAMIN C, FOLIC ACID, AND ZINC ARE REASONABLY COMMON IN SICK PATIENTS. SIGNS OF SCURVY SUCH AS CORKSCREW HAIRS ON THE LOWER EXTREMITIES ARE FREQUENTLY FOUND IN CHRONICALLY ILL AND/OR ALCOHOLIC PATIENTS. THE DIAGNOSIS CAN BE CONFIRMED WITH PLASMA VITAMIN C LEVELS. FOLIC ACID INTAKES AND BLOOD LEVELS ARE OFTEN LESS THAN OPTIMAL, EVEN AMONG HEALTHY PERSONS; WHEN ILLNESS, ALCOHOLISM, POVERTY, OR POOR DENTITION IS PRESENT, DEFICIENCIES ARE COMMON. LOW BLOOD ZINC LEVELS ARE PREVALENT IN PATIENTS WITH MALABSORPTION SYNDROMES SUCH AS INFLAMMATORY BOWEL DISEASE. PATIENTS WITH ZINC DEFICIENCY OFTEN EXHIBIT POOR WOUND HEALING, PRESSURE ULCER FORMATION, AND IMPAIRED IMMUNITY. THIAMINE DEFICIENCY IS A COMMON COMPLICATION OF ALCOHOLISM, BUT ITS MANIFESTATIONS ARE OFTEN PREVENTED BY THERAPEUTIC DOSES OF THIAMINE IN PATIENTS TREATED FOR ALCOHOL ABUSE. PATIENTS WITH LOW PLASMA VITAMIN C LEVELS USUALLY RESPOND TO THE DOSES FOUND IN MULTIVITAMIN PREPARATIONS, BUT PATIENTS WITH DEFICIENCIES SHOULD BE SUPPLEMENTED WITH 250-500 MG/D. FOLIC ACID IS ABSENT FROM SOME ORAL MULTIVITAMIN PREPARATIONS; PATIENTS WITH DEFICIENCIES SHOULD BE SUPPLEMENTED WITH ABOUT 1 MG/D. PATIENTS WITH ZINC DEFICIENCIES RESULTING FROM LARGE EXTERNAL LOSSES SOMETIMES REQUIRE ORAL DAILY SUPPLEMENTATION WITH 220 MG OF ZINC SULFATE ONE TO THREE TIMES DAILY. FOR THESE REASONS, LABORATORY ASSESSMENTS OF THE MICRONUTRIENT STATUS OF PATIENTS AT HIGH RISK ARE DESIRABLE. HYPOPHOSPHATEMIA DEVELOPS IN HOSPITALIZED PATIENTS WITH REMARKABLE FREQUENCY AND GENERALLY RESULTS FROM RAPID INTRACELLULAR SHIFTS OF PHOSPHATE IN CACHETIC OR ALCOHOLIC PATIENTS RECEIVING INTRAVENOUS GLU-
COSE (CHAP. 46). THE ADVERSE CLINICAL SEQUELAE ARE NUMEROUS; SOME, SUCH AS ACUTE CARDIOPULMONARY FAILURE, CAN BE LIFE-THREATENING.

NUTRITIONAL ASSESSMENT

BECAUSE INTERACTIONS BETWEEN ILLNESS AND NUTRITION ARE COMPLEX, MANY PHYSICAL AND LABORATORY FINDINGS REFLECT BOTH UNDERLYING DISEASE AND NUTRITIONAL STATUS. THEREFORE, THE NUTRITIONAL EVALUATION OF A PATIENT REQUIRES AN INTEGRATION OF THE HISTORY, PHYSICAL EXAMINATION, ANTHROPOMETRIES, AND LABORATORY STUDIES. THIS APPROACH HELPS BOTH TO DETECT NUTRITIONAL PROBLEMS AND TO AVOID CONCLUDING THAT ISOLATED FINDINGS INDICATE NUTRITIONAL PROBLEMS WHEN THEY DO NOT. FOR EXAMPLE, HYPOALBUMINEMIA CAUSED BY AN UNDERLYING ILLNESS DOES NOT NECESSARILY INDICATE MALNUTRITION.

NUTRITIONAL HISTORY

A NUTRITIONAL HISTORY IS DIRECTED TOWARD IDENTIFYING UNDERLYING MECHANISMS THAT PUT PATIENTS AT RISK FOR NUTRITIONAL DEPLETION OR EXCESS. THESE MECHANISMS INCLUDE INADEQUATE INTAKE, IMPAIRED ABSORPTION, DECREASED UTILIZATION, INCREASED LOSSES, AND INCREASED REQUIREMENTS OF NUTRIENTS. INDIVIDUALS WITH THE CHARACTERISTICS LISTED IN TABLE 72-3 ARE AT PARTICULAR RISK FOR NUTRITIONAL DEFICIENCIES.

PHYSICAL EXAMINATION

PHYSICAL FINDINGS THAT SUGGEST VITAMIN, MINERAL, AND PROTEIN-ENERGY DEFICIENCIES AND EXCESSES ARE OUTLINED IN TABLE 72-4. MOST OF THE PHYS-

TABLE 72-3 THE HIGH-RISK PATIENT

UNDERWEIGHT (BODY MASS INDEX <185) AND/OR RECENT LOSS OF *10% OF USUAL BODY WEIGHT
POOR INTAKE: ANOREXIA, FOOD AVOIDANCE (EG, PSYCHIATRIC CONDITION), OR NPO
STATUS FOR MORE THAN ABOUT 5 DAYS
PROTRACTED NUTRIENT LOSSES: MALABSORPTION, ENTERIC FISTULAE, DRAINING ABSCESSES
OR WOUNDS, RENAL DIALYSIS
HYPERMETABOLIC STATES: SEPSIS, PROTRACTED FEVER, EXTENSIVE TRAUMA OR BURNS
ALCOHOL ABUSE OR USE OF DRUGS WITH ANTINUTRIENT OR CATABOLIC PROPERTIES:
STERoids, ANTIMETABOLITES (EG, METHOTREXATE), IMMUNOSUPPRESSANTS,
ANTITUMOR AGENTS
IMPOVERISHMENT, ISOLATION, ADVANCED AGE

ICAL FINDINGS ARE NOT SPECIFIC FOR INDIVIDUAL NUTRIENT DEFICIENCIES, AND THEY MUST BE INTEGRATED WITH THE HISTORIC, ANTHROPOMETRIC, AND LABORATORY FINDINGS. FOR EXAMPLE, THE FINDING OF FOLLICULAR HYPERKERATOSIS
ON THE BACK OF THE ARMS IS A FAIRLY COMMON, NORMAL FINDING. ON THE OTHER HAND, IF IT IS WIDESPREAD IN A PERSON WHO CONSUMES LITTLE FRUIT AND VEGETABLES AND SMOKES REGULARLY (INCREASING ASCORBIC ACID REQUIREMENTS), VITAMIN C DEFICIENCY IS LIKELY. SIMILARLY, EASILY PLUCKABLE HAIR MAY BE A CONSEQUENCE OF CHEMOTHERAPY, BUT IN A HOSPITALIZED PATIENT WHO HAS POORLY HEALING SURGICAL WOUNDS AND HYPOALBUMINEMIA, IT SUGGESTS KWASHIORKOR.

ANTHROPOMETRICS

ANTHROPOMETRIC MEASUREMENTS PROVIDE INFORMATION ON BODY MUSCLE MASS AND FAT RESERVES. THE MOST PRACTICAL AND COMMONLY USED MEASUREMENTS ARE BODY WEIGHT, HEIGHT, TRICEPS SKINFOLD (TSF), AND MID-ARM MUSCLE CIRCUMFERENCE (MAMC). BODY WEIGHT IS ONE OF THE MOST USEFUL NUTRITIONAL PARAMETERS TO FOLLOW IN PATIENTS WHO ARE ACUTELY OR CHRONICALLY ILL. UNINTENTIONAL WEIGHT LOSS DURING ILLNESS OF TEN REFLECTS LOSS OF LEAN BODY MASS (MUSCLE AND ORGAN TISSUE), ESPECIALLY IF IT IS RAPID AND NOT CAUSED BY DIURESIS. THIS CAN BE AN OMINOUS SIGN SINCE IT INDICATES USE OF VITAL BODY PROTEIN STORES AS A METABOLIC FUEL. THE REFERENCE STANDARD FOR NORMAL BODY WEIGHT, BODY MASS INDEX (BMI, OR WEIGHT IN KILOGRAMS DIVIDED BY HEIGHT, IN METERS, Squared), IS DISCUSSED IN CHAP 75. BM IS <18.5 ARE CONSIDERED UNDERWEIGHT, 18.5-24.9 ARE NORMAL, 25-29.9 ARE OVERWEIGHT, AND *30 ARE OBESE.

MEASUREMENT OF SKINFOLD THICKNESS IS USEFUL FOR ESTIMATING BODY FAT STORES, BECAUSE ABOUT 50% OF BODY FAT IS NORMALLY LOCATED IN THE SUBCUTANEOUS REGION. SKINFOLD THICKNESSES CAN ALSO PERMIT DISCRIMINATION OF FAT MASS FROM MUSCLE MASS. THE TSF IS A CONVENIENT SITE THAT IS GENERALLY REPRESENTATIVE OF THE BODY'S OVERALL FAT LEVEL. A THICKNESS OF <3 MM SUGGESTS VIRTUALLY COMPLETE EXHAUSTION OF FAT STORES. THE MAMC, OFTEN USED TO ESTIMATE SKELETAL MUSCLE MASS, IS CALCULATED AS FOLLOWS:

MAMC (CM) = UPPER ARM CIRCUMFERENCE (CM) - [0.314 X TSF (MM)]

LABORATORY STUDIES

A NUMBER OF LABORATORY TESTS USED ROUTINELY IN CLINICAL MEDICINE CAN YIELD VALUABLE INFORMATION ABOUT A PATIENT'S NUTRITIONAL STATUS IF A SLIGHTLY DIFFERENT APPROACH TO THEIR INTERPRETATION IS USED. FOR EXAMPLE, ABNORMALLY LOW SERUM ALBUMIN LEVELS, TOTAL IRON-BINDING CAPACITY, AND ANERGY MAY HAVE A DISTINCT EXPLANATION, BUT COLLECTIVELY THEY MAY REPRESENT KWASHIORKOR. IN THE CLINICAL SETTING OF A HYPERMETABOLIC, ACUTELY ILL PATIENT WHO IS EDEMATOUS AND HAS EASILY PLUCKABLE HAIR AND INADEQUATE PROTEIN INTAKE, THE DIAGNOSIS OF KWASHIORKOR IS CLEAR-CUT. COMMONLY USED LABORATORY TESTS FOR ASSESSING NUTRITIONAL STATUS ARE OUTLINED IN TABLE 72-5. THE TABLE ALSO PROVIDES TIPS TO HELP AVOID ASSIGNING NUTRITIONAL SIGNIFICANCE TO TESTS THAT MAY BE ABNORMAL FOR NON-
NUTRITIONAL REASONS.

ASSESSMENT OF CIRCULATING (VISCERAL) PROTEINS  THE SERUM PROTEINS MOST USED TO ASSESS NUTRITIONAL STATUS INCLUDE ALBUMIN, TOTAL IRON-BINDING CAPACITY (OR TRANSFERRIN), THYROXINE-BINDING PREALBUMIN (OR TRANS-THYRETIN), AND RETINOL-BINDING PROTEIN.  BECAUSE THEY HAVE DIFFERING SYNTHESIS RATES AND HALF-LIVES-THE HALF-LIFE OF SERUM ALBUMIN IS ABOUT 21 DAYS WHEREAS THOSE OF PREALBUMIN AND RETINOL-BINDING PROTEIN ARE ABOUT 2 DAYS AND 12 H, RESPECTIVELY-SOME OF THESE PROTEINS REFLECT CHANGES IN NUTRITIONAL STATUS MORE QUICKLY THAN OTHERS.  HOWEVER, RAPID FLUCTUATIONS CAN ALSO MAKE SHORTER-HALF-LIFE PROTEINS LESS RELIABLE. LEVELS OF CIRCULATING PROTEINS ARE INFLUENCED BY THEIR RATES OF SYNTHESIS AND CATABOLISM, “THIRD SPACING” (LOSS INTO INTERSTITIAL SPACES), AND, IN SOME CASES, EXTERNAL LOSS.  ALTHOUGH AN ADEQUATE INTAKE OF CALORIES AND PROTEIN IS NECESSARY TO ACHIEVE OPTIMAL CIRCULATING PROTEIN LEVELS, SERUM PROTEIN LEVELS GENERALLY DO NOT REFLECT PROTEIN INTAKE. FOR EXAMPLE, A DROP IN THE SERUM LEVEL OF ALBUMIN OR TRANSFERRIN OFTEN ACCOMPANIES SIGNIFICANT PHYSIOLOGIC STRESS (E.G., FROM INFECTION

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PAGE NO. 96

453 CHAPTER 72 MALNUTRITION AND NUTRITIONAL ASSESSMENT

TABLE 72-4 PHYSICAL FINDINGS OF NUTRITIONAL DEFICIENCIES

CLINICAL FINDINGS

HAIR, NAILS

CORKSCREW HAIRS AND UNEMERGED COILED HAIRS EASILY PLUCKABLE HAIR FLAG SIGN (TRANSVERSE DEPIGMENTATION OF HAIR) SPARSE HAIR TRANSVERSE RIDGING OF NAILS

SKIN

CELOPHANE APPEARANCE CRACKING (FLAKY PAINT OR CRAZY PAVEMENT DERMATOSIS) FOLLICULAR HYPERKERATOSIS PETECHIAE (ESPECIALLY PERIFOLLICULAR) PURPURA PIGMENTATION, SCALING OF SUN-EXPOSED AREAS POOR WOUND HEALING, DECUBITUS ULCERS SCALING YELLOW PIGMENTATION SPARING SCLERAE (BENIGN)
EYES
NIGHT BLINDNESS
PAPILLEDEMA

PERIORAL
ANGULAR STOMATITIS
CHEILOSIS (DRY, CRACKING, ULCERATED LIPS)

ORAL
ATROPHIC LINGUAL PAPILLAE (SLICK TONGUE)
GLOSSITIS (SCARLET, RAW TONGUE)
HYPOGEUSESTHESIA, HYPSMIA
SWOLLEN, RETRACTED, BLEEDING GUMS (IF TEETH PRESENT)

BONES, JOINTS
BEADING OF RIBS, EPIPHYSEAL SWELLING, BOWLEGS
TENDERNESS, SUBPERIOSTEAL HEMORRHAGE IN CHILDREN

NEUROLOGIC
CONFABULATION, DISORIENTATION
DROWSINESS, LETHARGY, VOMITING
DEMENTIA
HEADACHE
OPHTHALMOPEGIA
PERIPHERAL NEUROPATHY (EG, WEAKNESS,
PARESTHESIAS, ATAXIA, FOOT DROP, AND
DECREASED TENDON REFLEXES, FINE TACTILE SENSE, VIBRATORY SENSE, AND POSITION SENSE)
TETANY

OTHER
EDEMA
HEART FAILURE
HEPATOMEGALY
PAROTID ENLARGEMENT
SUDDEN HEART FAILURE, DEATH

POSSIBLE DEFICIENCY###A
VITAMIN C
PROTEIN
PROTEIN

PROTEIN, BIOTIN, ZINC
PROTEIN
PROTEIN

VITAMINS A, C
VITAMIN C
VITAMINS C, K
NIACIN
PROTEIN, VITAMIN C, ZINC
VITAMIN A, ESSENTIAL FATTY ACIDS, BIOTIN
ZINC (HYPERPIGMENTED)

VITAMIN A

RIBOFLAVIN, PYRIDOXINE, NIACIN
RIBOFLAVIN, PYRIDOXINE, NIACIN

RIBOFLAVIN, NIACIN, FOLATE, VITAMIN B12, PROTEIN, IRON
RIBOFLAVIN, NIACIN, PYRIDOXINE, FOLATE, VITAMIN B12
ZINC
VITAMIN C

VITAMIN D
VITAMIN C

THIAMINE (KORSAKOFF PSYCHOSIS)

NIACIN, VITAMIN B12, FOLATE

THIAMINE, PHOSPHORUS
THIAMINE, PYRIDOXINE, VITAMIN B12

CALCIUM MAGNESIUM

PROTEIN, THIAMINE

THIAMINE (“WET” BERIBERI), PHOSPHORUS
PROTEIN
PROTEIN (CONSIDER ALSO BULIMIA)
VITAMIN C

POSSIBLE
EXCESS

VITAMIN A

VITAMIN A
CAROTENE

VITAMIN A
VITAMIN A
PYRIDOXINE

VITAMIN A

###AIN THIS TABTE, “PROTEIN DEFICIENCY” IS USED TO SIGNIFY KWASHIORKOR.

OR INJURY) AND IS NOT NECESSARILY AN INDICATION OF MALNUTRITION OR POOR INTAKE. A LOW SERUM ALBUMIN LEVEL IN A BURNED PATIENT WITH BOTH HYPERMETABOLISM AND INCREASED DERMAL LOSSES OF PROTEIN MAY NOT INDICATE MALNUTRITION. ON THE OTHER HAND, ADEQUATE NUTRITIONAL SUPPORT OF THE PATIENT’S CALORIE AND PROTEIN NEEDS IS CRITICAL FOR RETURNING CIRCULATING PROTEINS TO NORMAL LEVELS AS STRESS RESOLVES. THUS LOW VALUES BY THEMSELVES DO NOT DEFINE MALNUTRITION, BUT THEY OFTEN POINT TO INCREASED RISK OF MALNUTRITION BECAUSE OF THE HYPERMETABOLIC STRESS STATE. AS LONG AS SIGNIFICANT PHYSIOLOGIC STRESS PERSISTS, SERUM PROTEIN LEVELS REMAIN LOW, EVEN WITH AGGRESSIVE NUTRITIONAL SUPPORT. HOWEVER, IF THE LEVELS DO NOT RISE AFTER THE UNDERLYING ILLNESS IMPROVES, THE PATIENT’S PROTEIN AND CALORIE NEEDS SHOULD BE REASSESSED TO ENSURE THAT INTAKE IS SUFFICIENT.

**ASSESSMENT OF VITAMIN AND MINERAL STATUS**  THE USE OF LABORATORY TESTS TO CONFIRM SUSPECTED MICRONUTRIENT DEFICIENCIES IS DESIRABLE BECAUSE THE PHYSICAL FINDINGS FOR THESE ARE OFTEN EQUIVOCAL OR NONSPECIFIC. LOW BLOOD MICRONUTRIENT LEVELS CAN PREDATE MORE SERIOUS CLINICAL MANIFESTATIONS AND MAY ALSO INDICATE DRUG-NUTRIENT INTERACTIONS.

**ESTIMATING ENERGY AND PROTEIN REQUIREMENTS**

A PATIENT’S BASAL ENERGY EXPENDITURES (BEE, MEASURED IN KILOCALORIES PER DAY) CAN BE ESTIMATED FROM HEIGHT, WEIGHT, AGE, AND GENDER USING THE HARRIS-BENEDICT EQUATIONS:

MEN: $\text{BEE} = 66.47 + 13.75W + 5.00H - 6.76A$

WOMEN: $\text{BEE} = 655.10 + 9.56W + 1.85H - 4.68A$

WHERE W IS WEIGHT IN KG; H IS HEIGHT IN CM, AND A IS AGE IN YEARS. AFTER SOLVING THESE EQUATIONS, TOTAL ENERGY REQUIREMENTS ARE ESTIMATED BY MULTIPLYING THE BEE BY A FACTOR THAT ACCOUNTS
FOR THE STRESS OF ILLNESS. MULTIPLYING BY 1.1-1.4 YIELDS A RANGE 10-40% ABOVE BASAL THAT ESTIMATES THE 24-H ENERGY EXPENDITURE OF THE MAJORITY OF PATIENTS. THE LOWER VALUE (1.1) IS USED FOR PATIENTS WITHOUT EVIDENCE OF SIGNIFICANT PHYSIOLOGIC STRESS; THE HIGHER VALUE (1.4) IS APPROPRIATE FOR PATIENTS WITH MARKED STRESS SUCH AS SEPSIS OR TRAUMA. THE RESULT IS USED AS A 24-H ENERGY GOAL FOR FEEDING.

WHEN IT IS IMPORTANT TO HAVE A MORE ACCURATE ASSESSMENT OF ENERGY EXPENDITURE, IT CAN BE MEASURED AT THE BEDSIDE USING INDIRECT CALORIMETRY. THIS TECHNIQUE IS USEFUL IN PATIENTS WHO ARE BELIEVED TO BE HYPERMETABOLIC FROM SEPSIS OR TRAUMA AND WHOSE BODY WEIGHTS CANNOT BE OBTAINED ACCURATELY. INDIRECT CALORIMETRY CAN ALSO BE USEFUL IN PATIENTS HAVING DIFFICULTY WEANING FROM A VENTILATOR, AS THEIR ENERGY NEEDS SHOULD NOT BE EXCEEDED TO AVOID EXCESSIVE CO##2 PRODUCTION. PATIENTS AT THE EXTREMES OF WEIGHT (E.G., OBESE PERSONS) AND/OR AGE ARE GOOD CANDIDATES AS WELL, BECAUSE THE HARRIS-BENEDICT EQUATIONS WERE DEVELOPED FROM MEASUREMENTS IN ADULTS WITH ROUGHLY NORMAL BODY WEIGHTS.

BECAUSE UREA IS A MAJOR BYPRODUCT OF PROTEIN CATABOLISM, THE AMOUNT OF UREA NITROGEN EXCRETED EACH DAY CAN BE USED TO ESTIMATE THE RATE OF PROTEIN CATABOLISM AND TO DETERMINE IF PROTEIN INTAKE IS ADEQUATE TO OFFSET IT. TOTAL PROTEIN LOSS AND PROTEIN BALANCE CAN BE CALCULATED FROM THE URINARY UREA NITROGEN (UUN) AS FOLLOWS:

\[
\text{PROTEIN CATABOLIC RATE (G/D)} = \left[ 24\text{-H UUN (G)} + 4 \right] \times 6.25 \text{ (G PROTEIN/G NITROGEN)}
\]

THE VALUE OF 4 G ADDED TO THE UUN REPRESENTS A LIBERAL ESTIMATE OF THE UNMEASURED NITROGEN LOST IN THE URINE (E.G., CREATININE AND URIC ACID), SWEAT, HAIR, SKIN, AND FECES. WHEN PROTEIN INTAKE IS LOW (E.G., LESS

454 PART 5: NUTRITION

TABLE 72-5 LABORATORY TESTS FOR NUTRITIONAL ASSESSMENT

TEST (NORMAL VALUES)

SERUM ALBUMIN (35-55 G/DL)
SERUM PREALBUMIN, ALSO CALLED TRANSTHYRETIN (20-40 MG/DL; LOWER IN PREPUBERTAL CHILDREN)
SERUM TOTAL IRON BINDING CAPACITY (TIBC) 240-450 *G/DL

PROTHROMBIN TIME
12.0-15.5 SEC

SERUM CREATININE
0.6-1.6 MG/DL

24-H URINARY CREATININE
500-1200 MG/D (STANDARDIZED FOR HEIGHT AND SEX)

24-H URINARY UREA NITROGEN (UUN) <5 G/D (DEPENDS ON LEVEL OF PROTEIN INTAKE)

BLOOD UREA NITROGEN (BUN)
8-23 MG/DL

NUTRITIONAL USE

2.8-35: COMPROMISED PROTEIN STATUS
<2.8: POSSIBLE KWASHIORKOR
INCREASING VALUE REFLECTS POSITIVE PROTEIN BALANCE

10-15 MG/DL: MILD PROTEIN DEPLETION
5-10 MG/DL: MODERATE PROTEIN DEPLETION
<5 MG/DL: SEVERE PROTEIN DEPLETION
INCREASING VALUE REFLECTS POSITIVE PROTEIN BALANCE
<200: COMPROMISED PROTEIN STATUS, POSSIBLE KWASHIORKOR
INCREASING VALUE REFLECTS POSITIVE PROTEIN BALANCE
MORE LABILE THAN ALBUMIN

PROLONGATION: VITAMIN K DEFICIENCY

<0.6: MUSCLE WASTING DUE TO PROLONGED ENERGY DEFICIT
REFLECTS MUSCLE MASS

LOW VALUE: MUSCLE WASTING DUE TO PROLONGED ENERGY DEFICIT

DETERMINE LEVEL OF CATABOLISM (AS LONG AS PROTEIN INTAKE IS *10 G BELOW CALCULATED PROTEIN LOSS OR <20 G TOTAL, BUT AT LEAST 100 G CARBOHYDRATE IS PROVIDED)
5-10 G/D = MILD CATABOLISM OR NORMAL FED STATE
10-15 G/D = MODERATE CATABOLISM
>15 G/D = SEVERE CATABOLISM
ESTIMATE PROTEIN BALANCE
PROTEIN BALANCE = PROTEIN INTAKE - PROTEIN LOSS
WHERE PROTEIN LOSS (PROTEIN CATABOLIC RATE) = [24-H
UUN (G) + 4] X 6.25
ADJUSTMENTS REQUIRED IN BURN PATIENTS AND OTHERS WITH
LARGE NONURINARY NITROGEN LOSSES AND IN PATIENTS WITH
FLUCTUATING BUN LEVELS (EG, RENAL FAILURE)
<8: POSSIBLY INADEQUATE PROTEIN INTAKE
12-23: POSSIBLY ADEQUATE PROTEIN INTAKE
>23: POSSIBLY EXCESSIVE PROTEIN INTAKE
IF SERUM CREATININE IS NORMAL, USE BUN
IF SERUM CREATININE IS ELEVATED, USE BUN/CREATININE
RATIO (NORMAL RANGE IS ESSENTIALLY THE SAME AS FOR
BUN)

CAUSES OF NORMAL VALUE
DESPITE MALNUTRITION

DEHYDRATION
INFUSION OF ALBUMIN, FRESH
FROZEN PLASMA, OR
WHOLE BLOOD

CHRONIC RENAL FAILURE

IRON DEFICIENCY

>24-H COLLECTION
DECREASING SERUM
CREATININE

OTHER CAUSES OF
ABNORMAL VALUE

LOW
COMMON:
INFECTION AND OTHER STRESS, ESPE-
CIALLY WITH POOR PROTEIN INTAKE
BURNS, TRAUMA
CONGESTIVE HEART FAILURE
FLUID OVERLOAD
SEVERE LIVER DISEASE
UNCOMMON:
NEPHROTIC SYNDROME
ZINC DEFICIENCY
BACTERIAL STASIS/OVERGROWTH OF
SMALL INTESTINE
SIMILAR TO SERUM ALBUMIN

LOW
SIMILAR TO SERUM ALBUMIN
HIGH
IRON DEFICIENCY
PROLONGED
ANTICOAGULANT THERAPY (WARFARIN)
SEVERE LIVER DISEASE
HIGH
DESPITE MUSCLE WASTING:
RENAL FAILURE
SEVERE DEHYDRATION
LOW
INCOMPLETE URINE COLLECTION
INCREASING SERUM CREATININE
NEUROMUSCULAR WASTING

LOW
SEVERE LIVER DISEASE
ANABOLIC STATE
SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE
HIGH
DESPITE POOR PROTEIN INTAKE:
RENAL FAILURE (USE BUN/CREATININE RATIO)
CONGESTIVE HEART FAILURE
GASTROINTESTINAL HEMORRHAGE

THAN ABOUT 20 G/D), THE EQUATION INDICATES BOTH THE PATIENT’S PROTEIN RE-
QUIREMENT AND THE SEVERITY OF THE CATABOLIC STATE (TABLE 72-5). MORE
SUB-
STANTIAL PROTEIN INTAKES CAN RAISE THE UUN BECAUSE SOME OF THE
INGESTED
(OR INFUSED) PROTEIN IS CATABOLIZED AND CONVERTED TO UUN. THUS AT
LOWER
PROTEIN INTAKES THE EQUATION IS USEFUL FOR ESTIMATING REQUIREMENTS, AND
AT
HIGHER PROTEIN INTAKES IT IS USEFUL FOR ASSESSING PROTEIN BALANCE.

PROTEIN BALANCE (G/D) = PROTEIN INTAKE - PROTEIN CATABOLIC RATE

FURTHER READINGS

AMERICAN SOCIETY FOR PARENTERAL AND ENTERAL NUTRITION:

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PHILADELPHIA, MOSBY ELSEVIER, 2006
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BALTIMORE, LIPPINCOTT WILLIAMS & WILKINS, 2005
THE ABILITY TO PROVIDE SPECIALIZED NUTRITIONAL SUPPORT (SNS) REPRESENTS A MAJOR ADVANCE IN MEDICAL THERAPY. NUTRITIONAL SUPPORT, VIA EITHER ENTERAL OR PARENTERAL ROUTES, IS USED IN TWO MAIN SETTINGS: (1) TO PROVIDE ADEQUATE NUTRITIONAL INTAKE DURING THE RECUPERATIVE PHASE OF ILLNESS OR INJURY, WHEN THE PATIENT’S ABILITY TO INGEST OR ABSORB NUTRIENTS IS IMPAIRED, AND (2) TO SUPPORT THE PATIENT DURING THE SYSTEMIC RESPONSE TO INFLAMMATION, INJURY, OR INFECTION DURING AN EXTENDED CRITICAL ILLNESS. SNS IS ALSO USED IN PATIENTS WITH PERMANENT LOSS OF INTESTINAL LENGTH OR FUNCTION. IN ADDITION, AN INCREASING NUMBER OF ELDERLY PATIENTS LIVING IN NURSING HOMES AND CHRONIC CARE FACILITIES RECEIVE ENTERAL FEEDING, USUALLY AS A CONSEQUENCE OF INADEQUATE NUTRITIONAL INTAKE. ENTERAL REFERS TO FEEDING VIA A TUBE PLACED INTO THE GUT TO DELIVER LIQUID FORMULAS CONTAINING ALL ESSENTIAL NUTRIENTS. PARENTERAL REFERS TO THE INFUSION OF COMPLETE NUTRIENT SOLUTIONS INTO THE BLOODSTREAM VIA A PERIPHERAL VEIN OR, MORE COMMONLY, BY CENTRAL VENOUS ACCESS TO MEET NUTRITIONAL NEEDS. ENTERAL FEEDING IS GENERALLY THE PREFERRED ROUTE BECAUSE OF BENEFITS DERIVED FROM MAINTAINING THE DIGESTIVE, ABSORPTIVE, AND IMMUNOLOGIC BARRIER FUNCTIONS OF THE GASTROINTESTINAL TRACT. SMALL-BORE PLIABLE TUBES HAVE LARGELY REPLACED LARGE-BORE RUBBER TUBES, MAKING PLACEMENT EASIER AND MORE ACCEPTABLE TO PATIENTS. INFUSION PUMPS HAVE ALSO IMPROVED THE DELIVERY OF NUTRIENT SOLUTIONS. FOR SHORT-TERM USE, ENTERAL TUBES CAN BE PLACED VIA THE NOSE INTO THE STOMACH, DUODENUM, OR JEJUNUM. FOR LONG-TERM USE, THESE SITES CAN BE ACCESSSED THROUGH THE ABDOMINAL WALL USING ENDOSCOPIC, RADIOLOGIC, OR SURGICAL PROCEDURES. INTESTINAL TOLERANCE OF TUBE FEEDING MAY BE LIMITED DURING ACUTE ILLNESS BY GASTRIC RETENTION OR DIARRHEA. PARENTERAL FEEDING HAS GREATER RISK OF INFECTION, REFLECTING THE NEED FOR VENOUS ACCESS, AND A GREATER PROPENSITY FOR INDUCING HYPERGLYCEMIA. HOWEVER, THESE RISKS CAN GENERALLY BE MANAGED SUCCESSFULLY BY SNS TEAMS. FOR THE POSTOPERATIVE PATIENT WITH PREEXISTING MALNUTRITION, OR IN TRAUMA PATIENTS WHO WERE PREVIOUSLY WELL NOURISHED, SNS IS STRIKINGLY COST-EFFECTIVE. IN THE MOST CRITICALLY ILL PATIENT IN THE INTENSIVE CARE UNIT, SNS CAN DRAMATICALLY ENHANCE SURVIVAL. ALTHOUGH ENTERAL NUTRITION (EN)
CAN BE PROVIDED BY MOST HEALTH CARE TEAMS CARING FOR HOSPITALIZED PATIENTS, SAFE AND EFFECTIVE PARENTERAL NUTRITION (PN) USUALLY REQUIRES SPECIALIZED TEAMS.

**APPROACH TO THE PATIENT:**

**REQUIREMENTS FOR SPECIALIZED NUTRITIONAL SUPPORT**

**INDICATIONS FOR SPECIALIZED NUTRITIONAL SUPPORT**  
ALTHOUGH AT LEAST 15-20% OF PATIENTS IN ACUTE CARE HOSPITALS HAVE EVIDENCE OF SIGNIFICANT MALNUTRITION, ONLY A SMALL FRACTION WILL BENEFIT FROM SNS. FOR OTHERS, WASTING IS AN INEVITABLE COMPONENT OF A TERMINAL DISEASE AND THE COURSE OF THE DISEASE WILL NOT BE ALTERED BY SNS. THE DECISION TO USE SNS SHOULD BE BASED ON THE LIKELIHOOD THAT PREVENTING PROTEIN-CALORIE MALNUTRITION (PCM) WILL INCREASE THE LIKELIHOOD OF RECOVERY, REDUCE INFECTION RATES, IMPROVE HEALING, OR OTHERWISE SHORTEN THE HOSPITAL STAY. IN THE CASE OF THE ELDERLY OR CHRONICALLY ILL PATIENT FOR WHOM FULL RECOVERY IS NOT ANTICIPATED, THE DECISION TO FEED IS USUALLY BASED ON WHETHER SNS WILL EXTEND THE DURATION AND QUALITY OF LIFE. THE DECISION-MAKING PROCESS USED TO DECIDE WHEN TO USE SNS IS DEPICTED IN FIG. 73-1.

THE FIRST STEP IN DECIDING TO ADMINISTER SNS IS TO CONSIDER THE NUTRITIONAL IMPLICATIONS OF THE DISEASE PROCESS. IS THE CONDITION OR ITS TREATMENT LIKELY TO IMPAIR FOOD INTAKE AND ABSORPTION FOR A PROLONGED PERIOD OF TIME? FOR EXAMPLE, A WELL-NOURISHED INDIVIDUAL CAN TOLERATE APPROXIMATELY 7 DAYS OF STARVATION WHILE EXPERIENCING A SYSTEMIC RESPONSE TO INFLAMMATION (SRI). THE SECOND STEP IS TO DETERMINE IF THE PATIENT IS ALREADY SIGNIFICANTLY MALNOURISHED TO THE DEGREE THAT CRITICAL FUNCTIONS SUCH AS WOUND HEALING, IMMUNE FUNCTION, OR VENTILATORY FUNCTION ARE IMPAIRED (CHAP. 72). AN UNINTENTIONAL WEIGHT LOSS OF >10% DURING THE PREVIOUS 6 MONTHS OR A WEIGHT/HEIGHT <90% OF STANDARD, WHEN ASSOCIATED WITH PHYSIOLOGIC IMPAIRMENT, REPRESENTS SIGNIFICANT PCM. WEIGHT LOSS >20% OF USUAL OR <80% OF STANDARD REFLECTS SEVERE PCM. THE PRESENCE OR ABSENCE OF SRI SHOULD BE NOTED, SINCE INFLAMMATION, INJURY, AND INFECTION INCREASE THE RATE OF LEAN TISSUE LOSS. SRI ALSO HAS PATHOPHYSIOLOGIC EFFECTS THAT INFLUENCE NUTRITIONAL RESPONSES SUCH AS FLUID RETENTION AND HYPERGLYCEMIA, AS WELL AS IMPAIRMENT OF ANABOLIC RESPONSES TO NUTRITIONAL SUPPORT.

ONCE IT IS DETERMINED THAT A PATIENT IS ALREADY OR AT RISK OF BECOMING MALNOURISHED, THE NEXT STEP IS TO DECIDE WHETHER SNS WILL IMPACT POSITIVELY ON THE PATIENT’S RESPONSE TO DISEASE. IN THE END STAGES OF MANY CHRONIC ILLNESSES WITH ACCOMPANYING PCM, PARTICULARLY THOSE DUE TO CANCER OR TERMINAL NEUROLOGIC DISORDERS, NUTRITION MAY NOT REVERSE THE PCM OR IMPROVE QUALITY OF LIFE. WHILE THE PROVISION OF FOOD AND WATER IS PART OF BASIC MEDICAL CARE, NUTRITION DELIVERED BY TUBE OR CATHETER, EITHER ENTERALLY OR PARENTERALLY, IS ASSOCIATED WITH RISK AND DISCOMFORT. Thus, SNS SHOULD BE RECOMMENDED ONLY WHEN POTENTIAL BENEFITS EXCEED RISKS, AND SHOULD BE UNDERTAKEN WITH THE CONSENT OF THE PATIENT. LIKE OTHER LIFE SUPPORT MEASURES, ENTERAL OR PARENTERAL THERAPY IS DIFFICULT TO WITHDRAW ONCE STARTED. INITIATING NUTRITION SUPPORT MAY BE APPROPRIATE BEFORE A FINAL PROGNOSIS CAN BE DETERMINED, BUT THIS SHOULD NOT PRECLUDE ITS SUBSEQUENT WITHDRAWAL. IF PREVENTING OR TREATING PCM WITH SNS IS APPROPRIATE, NUTRITIONAL REQUIREMENTS AND THE METHOD
OF DELIVERY SHOULD BE DETERMINED. THE OPTIMAL ROUTE DEPENDS ON THE DEGREE OF GUT FUNCTION AND SOMEWHEAT ON THE AVAILABLE TECHNICAL RESOURCES.

THE TIMING OF NUTRITIONAL SUPPORT IS BASED ON EVALUATION OF THE PREEXISTING NUTRITIONAL STATUS, THE PRESENCE AND EXTENT OF SRI, AND THE ANTICIPATED CLINICAL COURSE. SRI IS IDENTIFIED BY THE STANDARD CLINICAL SIGNS OF LEUKOCYTOSIS, TACHYCARDIA, TACHYPNEA, AND/OR TEMPERATURE ELEVATION OR DEPRESSION. ALTHOUGH THE DEGREE OF HYPOALBUMINEMIA PROVIDES AN ESTIMATE OF SRI SEVERITY, NORMAL SERUM ALBUMIN LEVELS WILL NOT BE RESTORED BY ADEQUATE NUTRITIONAL SUPPORT UNTIL THE SRI REMITS, EVEN THOUGH NUTRITIONAL BENEFITS CAN BE ACHIEVED BY ADEQUATE FEEDING.

THE SRI CAN BE GRADED AS SEVERE, MODERATE, OR MILD. EXAMPLES OF SEVERE SRI INCLUDE SEPSIS OR OTHER INFLAMMATORY CONDITIONS LIKE PANCREATITIS REQUIRING ICU CARE, MULTIPLE TRAUMA WITH AN INJURY SEVERITY SCORE > 20-25 OR APACHE II > 25, CLOSED HEAD INJURY WITH A GLASGOW COMA SCALE < 8, OR MAJOR THIRD-DEGREE BURNS OF >40% OF BODY SURFACE AREA. MODERATE SRI INCLUDES LESS SEVERE INFECTIONS, INJURIES, OR INFLAMMATORY CONDITIONS LIKE PNEUMONIA, MAJOR SURGERY, ACUTE HEPATIC OR RENAL INSUFFICIENCY, AND EXACERBATIONS OF ULCERATIVE COLITIS OR REGIONAL ENTERITIS REQUIRING HOSPITALIZATION. PCM SHOULD ALSO BE DEFINED AS SEVERE, MODERATE, OR MINIMAL AS ASSESSED BY WEIGHT/HEIGHT, PERCENT RECENT WEIGHT LOSS, AND BODY MASS INDEX. THE BODY MASS INDEX IN RELATION TO NUTRITIONAL STATUS IS LISTED IN TABLE 73-1. A PATIENT WITH A SEVERE SRI REQUIRES EARLY FEEDING WITHIN THE FIRST SEVERAL DAYS OF CARE BECAUSE THE CONDITION IS LIKELY TO PRODUCE INADEQUATE SPONTANEOUS INTAKE OVER THE NEXT 7 DAYS. A MODERATE SRI, AS COMMONLY SEEN DURING A POSTOPERATIVE PERIOD WITHOUT ORAL INTAKE THAT EXCEEDS 5 DAYS, BENEFITS FROM ADEQUATE FEEDING BY DAY 5-7 IF THE PATIENT WAS INITIALLY WELL NOURISHED. IF SEVERELY MALNOURISHED, CANDIDATES FOR ELECTIVE MAJOR SURGERY BENEFIT FROM PREOPERATIVE NUTRITIONAL REPLETION FOR 5-7 DAYS. HOWEVER, THIS IS NOT OFTEN POSSIBLE. THUS, EARLY POSTOPERATIVE FEEDING IS INDICATED. PATIENTS WITH A MODERATE SRI AND MODERATE PCM ALSO BENEFIT FROM EARLIER FEEDING WITHIN THE FIRST SEVERAL DAYS.

EFFECTIC OF SNS IN DIFFERENT DISEASE STATES EFFICACY STUDIES HAVE SHOWN THAT MALNOURISHED PATIENTS UNDERGOING MAJOR THORACOABDOMINAL SURGERY BENEFIT FROM SNS. CRITICAL ILLNESS REQUIRING ICU CARE INCLUDING MAJOR BURNS, MAJOR TRAUMA, SEVERE SEPSIS, CLOSED

FIGURE 73-1 DECISION-MAKING FOR THE IMPLEMENTATION OF SPECIALIZED NUTRITION SUPPORT (SNS). CVC, CENTRAL VENOUS CATHETER; PICC, PERIPHERALLY INSERTED CENTRAL CATHETER. (ADAPTED FROM PREVIOUS CHAPTER BY LYN HOWARD, MD.)

PAGE NO. 99

456 PART 5: NUTRITION
HEAD INJURY, AND SEVERE PANCREATITIS [POSITIVE CT SCAN AND ACUTE PHYSIOLOGY AND CHRONIC HEALTH EVALUATION II (APACHE II) > 10] ALL BENEFIT BY EARLY SNS, AS INDICATED BY REDUCED MORTALITY AND MORBIDITY. IN CRITICAL ILLNESS, INITIATION OF SNS WITHIN 24 H OF INJURY OR ICU ADMISSION IS ASSOCIATED WITH A ~50% REDUCTION IN MORTALITY. PATIENTS WITH NITROGEN ACCUMULATION DISORDERS OF RENAL AND HEPATIC FAILURE HAVE A LIKELIHOOD OF PCM OF >50% AND AT LEAST A MODERATE SRI. IMPROVEMENTS IN MORBIDITY, INCLUDING INFECTION RATES, ENCEPHALOPATHY, LIVER OR RENAL FUNCTION, AND LENGTH OF HOSPITAL STAY HAVE BEEN FOUND WITH SNS. INFLAMMATORY BOWEL DISEASE INCLUDING CROHN’S DISEASE PARTICULARLY, AND, TO A LESSER DEGREE, ULCERATIVE COLITIS-OFTEN PRODUCE PCM. IN THE OUTPATIENT SETTING, SNS IN CROHN’S DISEASE CAN IMPROVE NUTRITIONAL STATUS, QUALITY OF LIFE, AND THE LIKELIHOOD OF REMISSION. WITH PULMONARY DISEASE IN THE CRITICALLY ILL, SNS IMPROVES VENTILATORY STATUS, AND IN ACUTE LUNG INJURY THE USE OF OMEGA 3 FATS AS A COMPONENT OF SNS IMPROVES GAS EXCHANGE AND RESPIRATORY DYNAMICS AND REDUCES THE NEED FOR MECHANICAL VENTILATION. LOW BODY WEIGHT IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE IS ASSOCIATED WITH DIMINISHED PULMONARY STATUS AND EXERCISE CAPACITY AND HIGHER MORTALITY RATES. HOWEVER, THERE IS LITTLE CONVINCING EVIDENCE THAT SNS AS CALORIC SUPPLEMENTATION IMPROVES NUTRITION OR PULMONARY FUNCTION. PCM IS ALSO COMMON IN THE COURSE OF CANCER AND HIV DISEASE, ALTHOUGH LESS SO IN THE LATTER WITH THE ADVENT OF HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY. WHEN PCM DEVELOPS AS A CONSEQUENCE OF SRI IN THESE CONDITIONS, THERE IS LIMITED LIKELIHOOD OF SUBSTANTIAL EFFICACY OR BENEFIT FROM SNS. HOWEVER, WHEN PCM DEVELOPS AS A CONSEQUENCE OF GASTROINTESTINAL DYSFUNCTION, SNS CAN BE EFFECTIVE. ALTHOUGH NO RANDOMIZED TRIALS HAVE BEEN PERFORMED FOR SNS PROVIDED FOR HYPEREMESIS GRAVIDARUM, THERE IS CONSIDERABLE CLINICAL EVIDENCE THAT IT IMPROVES PREGNANCY OUTCOMES.

**RISKS AND BENEFITS OF SPECIALIZED NUTRITION SUPPORT** THE RISKS ARE DETERMINED PRIMARILY BY PATIENT FACTORS SUCH AS STATE OF ALERTNESS, SWALLOWING COMPETENCE, THE ROUTE OF DELIVERY, UNDERLYING CONDITIONS, AND THE EXPERIENCE OF THE SUPERVISING CLINICAL TEAM. THE SAFEST AND LEAST COSTLY APPROACH IS TO AVOID SNS BY CLOSE ATTENTION TO ORAL FOOD INTAKE, BY ADDING AN ORAL LIQUID SUPPLEMENT, OR IN CERTAIN CHRONIC
CONDITIONS BY USING MEDICATIONS TO STIMULATE APPETITE. NUTRIENT INTAKE MONITORING BY FREQUENT CALORIE COUNTS OR ORAL FORMULA SELECTION IS BEST PERFORMED BY A NUTRITIONIST. ENTERAL TUBE FEEDING IS OFTEN REQUIRED IN PATIENTS WITH ANOREXIA, IMPAIRED SWALLOWING, OR BOWEL DISEASE. THE BOWEL AND ITS ASSOCIATED DIGESTIVE ORGANS DERIVE 70% OF THEIR REQUIRED NUTRIENTS DIRECTLY FROM FOOD IN THE LUMEN. ARGinine, GLUTAMINE, SHORT-CHAIN FATTY ACIDS, LONG-CHAIN OMEGA 3 FATTY ACIDS, AND NUCLEOTIDES AVAILABLE IN SOME SPECIALTY ENTERAL FORMULAS ARE PARTICULARLY IMPORTANT FOR MAINTAINING IMMUNITY. ENTERAL FEEDING ALSO SUPPORTS GUT FUNCTION BY STIMULATING SPLANCHNIC BLOOD FLOW, NEURONAL ACTIVITY, IGA ANTIBODY RELEASE, AND SECRETION OF GASTROINTESTINAL HORMONES THAT STIMULATE GUT TROPHIC ACTIVITY. THESE FACTORS

TABLE 73-1 BODY MASS INDEX (BMI) AND NUTRITIONAL STATUS

BMI

>30 KG/M²
>25-30 KG/M²
20-25 KG/M²
<185 KG/M²
<16 KG/M²
<13 KG/M²
<11 KG/M²

NUTRITIONAL STATUS

OBESE
OVERWEIGHT
NORMAL
MODERATE MALNUTRITION
SEVERE MALNUTRITION
LETHAL IN MALES
LETHAL IN FEMALES

SUPPORT THE GUT AS AN IMMUNOLOGIC BARRIER AGAINST ENTERIC PATHOGENS. FOR THESE REASONS, SOME LUMINAL NUTRITION SHOULD BE PROVIDED, EVEN WHEN PN IS REQUIRED TO PROVIDE MOST OF THE NUTRITIONAL SUPPORT. THE COMBINATION OF SOME ENTERAL FEEDING EITHER BY MOUTH OR BY ENTERAL TUBE WITH PARENTERAL FEEDING OFTEN SHORTENS THE TRANSITION TO FULL ENTERAL FEEDING, WHICH CAN GENERALLY BE USED WHEN >50% OF REQUIREMENTS CAN BE MET ENTERALLY. SUBSTANTIAL NUTRITIONAL BENEFIT CAN BE ACHIEVED BY PROVIDING ~50% OF ENERGY NEEDS FOR PERIODS OF UP TO 10 DAYS, IF PROTEIN AND OTHER ESSENTIAL NUTRIENT REQUIREMENTS ARE MET. FOR LONGER PERIODS OF TIME, IT MAY BE PREFERABLE TO PROVIDE 75-80% OF ENERGY NEEDS, RATHER THAN FULL FEEDING, IF THIS IMPROVES GASTROINTESTINAL TOLERANCE, GLYCEMIC CONTROL, AND AVOIDANCE OF EXCESS FLUID ADMINISTRATION.

IN THE PAST, BOWEL REST THROUGH PN WAS THE CORNERSTONE OF TREATMENT FOR MANY SEVERE GASTROINTESTINAL DISORDERS. HOWEVER, THE VALUE OF PROVIDING EVEN MINIMAL AMOUNTS OF EN IS NOW WIDELY ACCEPTED. THE DEVELOPMENT OF PROTOCOLS TO FACILITATE MORE WIDESPREAD USE OF EN INCLUDE INITIATION WITHIN 24 H OF ICU ADMISSION; AGGRESSIVE USE OF THE HEAD-UPRIGHT POSITION; POSTPYLORIC AND NASOJEJUNAL FEEDING TUBES; PROKINETIC AGENTS; MORE RAPID INCREASES IN FEEDING RATES; TOLERANCE OF HIGHER GASTRIC RESIDUALS; AND NURSE-ADMINISTERED ALGORITHMS. PN ALONE IS GENERALLY NECESSARY ONLY FOR SEVERE GUT DYSFUNCTION DUE TO PROLONGED ILEUS, OBSTRUCTION, OR SEVERE HEMORRHAGIC PANCREATITIS. IN THE CRITICALLY ILL, FEEDING ADEQUATELY BY PN BEGINNING WITHIN THE FIRST 24 H OF CARE IMPROVES MORTALITY AND IS MORE EFFECTIVE THAN DELAYED EN. EARLY FEEDING OF THE CRITICALLY ILL IN THE ICU IS ASSOCIATED WITH A 50% REDUCTION IN MORTALITY, BUT THERE IS ALSO A 50% INCREASE IN INFECTION RISK. MUCH OF THE INCREASE IN MORBIDITY RELATED TO PN AND EN IS DUE TO HYPERGLYCEMIA, WHICH CAN BE SIGNIFICANTLY REDUCED BY INSULIN THERAPY. THE LEVEL OF GLYCEMIA NECESSARY TO ACCOMPLISH THIS GOAL, WHETHER <110 MG/DL OR ONLY <150 MG/DL, IS NOT YET DEFINED. ALTHOUGH PN WAS INITIALLY RELATIVELY EXPENSIVE, ITS COMPONENTS ARE OFTEN LESS EXPENSIVE THAN SPECIALTY ENTERAL FORMULAS. PERCUTANEOUS PLACEMENT OF A CENTRAL VENOUS CATHETER INTO THE SUBCLAVIAN OR INTERNAL JUGULAR VEIN WITH ADVANCEMENT INTO THE SUPERIOR VENA CAVA CAN BE ACCOMPLISHED AT THE BEDSIDE BY TRAINED PERSONNEL USING STERILE TECHNIQUES. PERIPHERALLY INSERTED CENTRAL CATHETERS CAN ALSO BE PLACED WITHIN THE LUMEN IN THE CENTRAL VEIN, BUT THIS TECHNIQUE IS USUALLY MORE APPROPRIATE FOR NON-ICU PATIENTS. THE SUBCLAVIAN OR INTERNAL JUGULAR LINES CAN BE CHANGED OVER A WIRE, BUT THIS CARRIES A GREATER RISK OF PNEUMOTHORAX OR SERIOUS VASCULAR DAMAGE. THE PERIPHERALLY INSERTED CATHETERS ARE SUBJECT TO POSITION-RELATED FLOW, AND THE CATHETER CANNOT BE CHANGED OVER A WIRE. INSERTING A NASOGASTRIC TUBE IS A BEDSIDE PROCEDURE, BUT MANY CRITICALLY ILL PATIENTS HAVE IMPAIRED GASTRIC EMPTYING THAT INCREASES THE RISK OF ASPIRATION PNEUMONIA. THIS RISK CAN BE REDUCED BY FEEDING DIRECTLY INTO THE JEJUNUM BEYOND THE LIGAMENT OF TREITZ. THIS USUALLY REQUIRES FLUOROSCOPIC GUIDANCE OR ENDOSCOPIC PLACEMENT. IN PATIENTS WHO HAVE PLANNED LAPAROTOMIES OR OTHER CONDITIONS LIABLE TO REQUIRE A PROLONGED NEED FOR SNS, IT IS ADVANTAGEOUS TO PLACE A JEJUNAL FEEDING TUBE AT THE TIME OF SURGERY. ALTHOUGH MOST SNS IS DELIVERED IN HOSPITALS, SOME PATIENTS REQUIRE IT ON A LONG-TERM BASIS. IF THEY HAVE A SAFE ENVIRONMENT AND A
WILLINGNESS TO LEARN THE SELF-CARE TECHNIQUES, SNS CAN BE ADMINIST- 
TERED AT HOME. THE CLINICAL OUTCOMES OF PATIENTS WITH SEVERE INTESTI- 
NAL DISORDERS TREATED WITH HOME PN OR EN ARE SUMMARIZED IN 
TABLE 73-2. PN INFUSED AT HOME IS USUALLY CYCLED OVERNIGHT TO GIVE 
greater daytime freedom. OTHER IMPORTANT CONSIDERATIONS IN DETER-
MINING THE APPROPRIATENESS OF HOME PN OR EN ARE THAT THE PA-
tIENT'S PROGNOSIS IS LONGER THAN SEVERAL MONTHS AND THAT THE THERAPY 
BENEFITS QUALITY OF LIFE.

DISEASE-SPECIFIC NUTRITIONAL SUPPORT SNS IS BASICALLY A SUPPORT 
THERAPY AND IS PRIMARY THERAPY ONLY FOR THE TREATMENT OR PREVEN-
TION OF MALNUTRITION. CERTAIN CONDITIONS REQUIRE MODIFICATION OF 
NUTRITIONAL SUPPORT BECAUSE OF ORGAN OR SYSTEM IMPAIRMENT. FOR 
INSTANCE, IN NITROGEN ACCUMULATION DISORDERS, PROTEIN INTAKE MAY 
NEED TO BE REDUCED. HOWEVER, IN RENAL DISEASE, EXCEPT FOR BRIEF PERI-
ODS OF SEVERAL DAYS, PROTEIN INTAKES SHOULD APPROACH REQUIREMENT 
LEVELS OF AT LEAST 0.8 G/KG OR HIGHER UP TO 1.2 G/KG AS LONG AS THE 

TABLE 73-2 SUMMARY OF OUTCOMES FOR PATIENTS ON HOME PARENTERAL 
AND ENTERAL NUTRITION (HPEN)

DIAGNOSIS

HOME PARENTERAL NUTRITION

CROHN'S DISEASE
ISCHEMIC TOWEL DISEASE
MOTILITY DISORDER
CONGENITAL BOWEL 
DEFECT
HYPEREMESIS
GRAVIDARUM
CHRONIC PANCREATITIS
RADIATION ENTERITIS
CHRONIC ADHESIVE 
OBSTRUCTIONS
CYSTIC FIBROSIS
CANCER
AIDS

HOME ENTERAL NUTRITION

NEUROLOGIC DISORDERS 
OF SWALLOWING
CANCER

NUMBER 
IN GROUP

562
331
299
172
112
<table>
<thead>
<tr>
<th>AGE IN YEARS</th>
<th>% SURVIVAL ON THERAPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>96</td>
</tr>
<tr>
<td>49</td>
<td>87</td>
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<tr>
<td>45</td>
<td>87</td>
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</tr>
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<td>42</td>
<td>90</td>
</tr>
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<td>58</td>
<td>87</td>
</tr>
<tr>
<td>53</td>
<td>83</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>44</td>
<td>20</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>65</td>
<td>55</td>
</tr>
<tr>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Therapy Status</td>
<td>% at 1 Year</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>FULL ORAL NUTRITION</strong></td>
<td></td>
</tr>
<tr>
<td>70 27 31 42</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>82 28 47</td>
<td></td>
</tr>
<tr>
<td>38 26 13</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>CONTINUED ON HPEN RX</strong></td>
<td></td>
</tr>
<tr>
<td>25 48 44 47</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10 49 34</td>
<td></td>
</tr>
<tr>
<td>13 8 6</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>DIED</strong></td>
<td></td>
</tr>
<tr>
<td>2 19 21 9</td>
<td></td>
</tr>
</tbody>
</table>
REHABILITATION###C
STATUS, % IN 1ST YEAR

C
60
53
49
63

83
60
42
23

24
29
8

5
21

P
38
41
39
27

16
38
49
68

66
57
63

24
COMPLICATIONS PER PATIENT-YEAR

HPEN

0.9
1.4
1.3
2.1
1.5
1.2
0.8
1.7
0.8
1.1
1.6
0.3
0.4

NONHPEN

1.1
1.1
1.1
1.0
458 PART 5: NUTRITION

BLOOD UREA NITROGEN DOES NOT EXCEED 100 MG/DL. IF THIS IS NOT POSSIBLE, THEN DIALYSIS OR HEMOFILTRATION SHOULD BE CONSIDERED TO ALLOW BETTER FEEDING. IN HEPATIC FAILURE, INTAKES OF 1.2-1.4 G/KG UP TO THE OPTIMAL 1.5 G/KG SHOULD BE ATTEMPTED, AS LONG AS ENCEPHALOPATHY DUE TO PROTEIN INTOLERANCE IS NOT ENCOUNTERED. IN THE PRESENCE OF PROTEIN INTOLERANCE, FORMULAS CONTAINING 33-50% BRANCHED-CHAIN AMINO ACIDS ARE AVAILABLE AT THE 1.2-1.4-G/KG LEVEL. CARDIAC PATIENTS, AND MANY SEVERELY STRESSED PATIENTS, OFTEN BENEFIT FROM FLUID AND SODIUM RESTRICTION TO LEVELS OF 1000 ML OF TOTAL PARENTERAL NUTRITION (TPN) FORMULA AND 5-20 MEQ OF SODIUM PER DAY. IN PATIENTS WITH SEVERE CHRONIC PCM CHARACTERIZED BY SEVERE WEIGHT LOSS AND TISSUE WASTING, TPN MUST BE INSTITUTED GRADUALLY BECAUSE OF THE PROFOUND ANTINATRIURESIS, ANTIDIURESIS, AND INTRACELLULAR ACCUMULATION OF POTASSIUM, MAGNESIUM, AND PHOSPHORUS. THIS IS USUALLY ACCOMPLISHED BY LIMITING FLUID INTAKES INITIALLY TO ABOUT 1000 ML
CONTAINING MODEST CARBOHYDRATE CONTENT OF 10-20% DEXTROSE, LOW SODIUM, AND AMPLE POTASSIUM, MAGNESIUM, AND PHOSPHORUS, WITH CAREFUL ASSESSMENT OF FLUID AND ELECTROLYTE STATUS. PROTEIN NEED NOT BE RESTRICTED.

THE DESIGN OF INDIVIDUAL REGIMENS

FLUID REQUIREMENTS

THE NORMAL DAILY REQUIREMENT FOR FLUID IS 30 ML/KG OF BODY WEIGHT FROM ALL SOURCES (IV INFUSIONS, PER TUBE, OR ORAL INTAKE), PLUS ANY REPLACEMENT OF ABNORMAL LOSSES SUCH AS AN OSMOTIC DIURESIS, NASOGASTRIC DRAINAGE, WOUND OUTPUT, OR DIARRHEAL/OSTOMY LOSSES. ELECTROLYTE AND MINERAL LOSSES CAN BE ESTIMATED OR MEASURED AND ALSO NEED TO BE REPLACED (TABLE 73-3). FLUID RESTRICTION MIGHT BE NECESSARY IN PATIENTS WITH FLUID OVERLOAD, AND FLUID INPUTS CAN BE LIMITED TO 1200 ML/D IF URINE IS THE ONLY SIGNIFICANT FLUID OUTPUT. WHEN SEVERE FLUID OVERLOAD OCCURS, THE OPTIMAL PN SOLUTION FOR CENTRAL VENOUS ADMINISTRATION IS A CONCENTRATED 1-L SOLUTION OF 7% CRYSTALLINE AMINO ACIDS (70 G) AND 21% DEXTROSE (210 G), WHICH PROVIDES AN AMOUNT OF NITROGEN AND GLUCOSE THAT IS EFFECTIVE AT PROTEIN-SPARING. PATIENTS REQUIRING PN OR EN IN THE ACUTE CARE SETTING GENERALLY HAVE SOME ELEMENT OF ASSOCIATED HORMONAL ADAPTATIONS (E.G., INCREASED SECRETION OF ANTIDIURETIC HORMONE, ALDOSTERONE, INSULIN, GLUCAGON, OR CORTISOL) THAT CAUSE FLUID RETENTION AND HYPERGLYCEMIA. WEIGHT GAIN IN THE CRITICALLY ILL, WHETHER RECEIVING SNS OR NOT, IS INEVITABLY THE CONSEQUENCE OF FLUID RETENTION, SINCE LEAN TISSUE ACCRETION IS MINIMAL IN THE ACUTE PHASE OF ILLNESS. BECAUSE EXCESS FLUID REMOVAL CAN BE DIFFICULT, LIMITING FLUID INTAKE TO ALLOW FOR BALANCED INTAKE AND OUTPUT IS MORE EFFECTIVE.

ENERGY REQUIREMENTS

TOTAL ENERGY EXPENDITURE COMPRISSES RESTING ENERGY EXPENDITURE (TWO-THIRDS) PLUS ACTIVITY ENERGY EXPENDITURE (ONE-THIRD) (CHAP. 72). RESTING ENERGY EXPENDITURE INCLUDES THE CALORIES NECESSARY FOR BASAL METABOLISM AT BED REST. ACTIVITY ENERGY EXPENDITURE REPRESENTS ONE-FOURTH TO ONE-THIRD OF THE TOTAL, AND THE THERMAL EFFECT OF FEEDING IS ABOUT 10% OF THE TOTAL ENERGY EXPENDITURE. FOR NORMALLY NOURISHED HEALTHY INDIVIDUALS, THE TOTAL ENERGY EXPENDITURE IS ABOUT 30-35 KCAL/KG. ALTHOUGH CRITICAL ILLNESS INCREASES RESTING ENERGY EXPENDITURE, ONLY IN INITIALLY

TABLE 73-3 ENTERIC FLUID VOLUMES AND THEIR ELECTROLYTE CONTENT###A

ORAL INTAKE
ENTERIC SECRETIONS
SALIVA
GASTRIC JUICE
BILE
PANCREATIC
SMALL INTESTINE

**L/D**

2-3
1-2
1.5-2
0.5-1.5
0.5-1
1-2

**NA**

15
50-70
120-150
100-140
80-140

**K**

30
5-15
5-15
10
10-20

**CL**

15
90-120
80-120
70-100
80-120

**HCO###3**

50
0
30-50
60-110
20-40

**H**

- 
70-100
- 
- 
-
WELL-NOURISHED INDIVIDUALS WITH THE HIGHEST SYSTEMIC INFLAMMATORY RESPONSE, SUCH AS THAT FROM SEVERE MULTIPLE TRAUMA, BURNS, CLOSED HEAD INJURY, OR SEPSIS, DO TOTAL ENERGY EXPENDITURES REACH 40-45 KCAL/KG. THE CHRONICALLY ILL PATIENT WITH LEAN TISSUE LOSS HAS REDUCED BASAL ENERGY EXPENDITURE, AND INACTIVITY WHICH RESULTS IN A TOTAL ENERGY EXPENDITURE OF ABOUT 20-25 KCAL/KG. ABOUT 95% OF SUCH PATIENTS NEED <30 KCAL/KG TO ACHIEVE ENERGY BALANCE. BECAUSE PROVIDING ABOUT 50% OF MEASURED ENERGY EXPENDITURE AS SNS IS AT LEAST EQUALLY EFFICACIOUS FOR THE FIRST 10 DAYS OF CRITICAL ILLNESS, ACTUAL MEASUREMENT OF ENERGY EXPENDITURE IS NOT GENERALLY NECESSARY IN THE EARLY PERIOD OF SNS. HOWEVER, IN PATIENTS WHO REMAIN CRITICALLY ILL BEYOND SEVERAL WEEKS, IN THE SEVERELY MALNOURISHED FOR WHOM ESTIMATES OF ENERGY EXPENDITURE ARE UNRELIABLE, OR IN THOSE WHO ARE DIFFICULT TO WEAN FROM VENTILATORS, IT IS REASONABLE TO ACTUALLY MEASURE ENERGY EXPENDITURE AND TO AIM FOR ENERGY BALANCE WITH SNS. INSULIN RESISTANCE IS ASSOCIATED WITH INCREASED GLUCONEOGENESIS AND REDUCED GLUCOSE UTILIZATION, PREDISPOSING A PATIENT TO HYPERGLYCEMIA. THIS IS AGGRAVATED IN PATIENTS RECEIVING EXOGENOUS CARBOHYDRATE FROM SNS. NORMALIZATION OF BLOOD GLUCOSE LEVELS BY INSULIN INFUSION IN CRITICALLY ILL PATIENTS RECEIVING SNS REDUCES MORBIDITY AND MORTALITY. IN MILD OR MODERATELY MALNOURISHED PATIENTS, A REASONABLE GOAL IS TO PROVIDE METABOLIC SUPPORT TO IMPROVE PROTEIN SYNTHESIS AND MAINTAIN METABOLIC HOMEOSTASIS. HYPOCALORIC NUTRITION PROVIDING ONLY ABOUT 1000 KCAL/D AND 70 G PROTEIN FOR UP TO 10 DAYS REQUIRES LESS FLUID AND REDUCES THE LIKELIHOOD OF POOR GLYCEMIC CONTROL. ENERGY CONTENT CAN BE ADVANCED TO 20-25 KCAL/KG WITH 1.5 G PROTEIN/KG AS CONDITIONS PERMIT AND DEFINITELY DURING THE SECOND WEEK OF SNS. PATIENTS WITH MULTIPLE TRAUMA, CLOSED HEAD INJURY, AND SEVERE BURNS OFTEN HAVE MUCH HIGHER ENERGY EXPENDITURES, BUT THERE IS LITTLE EVIDENCE THAT PROVIDING MORE THAN 30 KCAL/KG HAS ADDITIONAL BENEFIT, AND IT RISKS HYPERGLYCEMIA. GENERALLY, BECAUSE GLUCOSE IS AN ESSENTIAL TISSUE FUEL, GLUCOSE AND AMINO ACIDS ARE PROVIDED PARENTERALLY UNTIL THE LEVEL OF RESTING ENERGY EXPENDITURE IS REACHED. AT THIS POINT, ADDING FAT BECOMES BENEFICIAL, SINCE MORE PARENTERAL GLUCOSE STIMULATES DE NOVO LIPOGENESIS BY THE LIVER—AN ENERGY-INEFFICIENT PROCESS. POLYUNSATURATED LONG-CHAIN TRIGLYCERIDES ARE THE CHIEF INGREDIENT IN MOST PARENTERAL FAT EMULSIONS AND THE MAJORITY OF THE FAT IN ENTERAL FEEDING FORMULAS. THESE VEGETABLE OIL-BASED EMULSIONS PROVIDE ESSENTIAL FATTY ACIDS. ENTERAL FEEDING FORMULAS HAVE FAT CONTENT THAT RANGES FROM 3% OF CALORIES UP TO AS MUCH AS 50% OF CALORIES, WHILE PARENTERAL FAT COMES IN SEPARATE CONTAINERS AS 10,20,
AND 30% EMULSIONS THAT CAN BE INFUSED SEPARATELY OR MIXED BY THE
PHARMACY UNDER CONTROLLED CONDITIONS AS ALL-IN-ONE OR TOTAL NUTRIENT
ADMIXTURE WITH GLUCOSE, AMINO ACIDS, LIPID, ELECTROLYTES, VITAMINS, AND
MINERALS. ALTHOUGH PARENTERAL FAT IS REQUIRED AT ONLY ABOUT 3% OF
ENERGY REQUIREMENTS TO MEET ESSENTIAL FATTY ACID REQUIREMENTS, WHEN PRO-
VIDED AS AN ALL-IN-ONE MIXTURE OF CARBOHYDRATE, FAT, AND PROTEIN, 2-3% 
FAT IN THE TPN MIXTURES, REPRESENTING ABOUT 20-30% OF CALORIES AS FAT, IS PROVIDED TO ENSURE EMULSION STABILITY. IF GIVEN SEPARATELY, 
PARENTERAL FAT SHOULD NOT BE PROVIDED AT RATES EXCEEDING 0.11 G/KG
BODY WEIGHT 
PER H OR ABOUT 100 G OVER 12 H-EQUIVALENT TO 1 L OF 10% PARENTERAL FAT 
AND 500 ML OF 20% PARENTERAL FAT. MEDIUM-CHAIN TRIGLYCERIDES, WHICH CONTAIN SATURATED FATTY ACIDS WITH 
CHAIN LENGTHS OF 6, 8,10, OR 12 CARBONS, ARE PROVIDED IN A NUMBER OF EN-
TERAL FEEDING FORMULAS BECAUSE THEY ARE ABSORBED PREFERENTIALLY. FISH OIL 
CONTAINS POLYUNSATURATED FATTY ACIDS OF THE OMEGA 3 FAMILY, WHICH HAVE 
BEEN SHOWN TO IMPROVE IMMUNE FUNCTION AND 
REDUCE THE INFLAMMATORY RESPONSE. PARENTERAL 
EMULSIONS CONTAINING MEDIUM-CHAIN TRIGLYCER-
IDES, OLIVE OIL, AND FISH OIL ARE AVAILABLE IN EUROPE 
AND JAPAN BUT NOT YET IN THE UNITED STATES. CARBOHYDRATES ARE PROVIDED AS HYDROUS GLU-
COSE PROVIDING 3.4 KCAL/G IN PN FORMULAS. IN EN-
TERAL FORMULAS, GLUCOSE IS THE CARBOHYDRATE 
SOURCE IN SO-CALLED MONOMICER DIETS. THESE DIETS 
PROVIDE PROTEIN AS AMINO ACIDS AND FAT IN MINI-
MAL AMOUNTS (3%) TO MEET ESSENTIAL FATTY ACID 
REQUIREMENTS. MONOMERIC FORMULAS ARE DE-
SIGNED TO OPTIMIZE ABSORPTION IN THE SERIOUSLY 
COMPROMISED GUT. THESE FORMULAS, LIKE THE IM-

459 CHAPTER 73 ENTERAL AND PARENTERAL NUTRITION 
THERAPY 

MUNEE-ENHANCING DIETS, ARE QUITE EXPENSIVE. IN POLYMERIC DIETS, THE CAR-
BOHYDRATE SOURCE IS USUALLY AN OSMOTICALLY LESS ACTIVE 
POLYSACCHARIDE, 
PROTEIN IS USUALLY SOY OR CASEIN PROTEIN, AND FAT IS PRESENT IN AMOUNTS 
FROM 25 TO 50%. SUCH FORMULAS ARE USUALLY WELL TOLERATED BY PATIENTS 
WITH 
NORMAL INTESTINAL LENGTH, AND SOME ARE ACCEPTABLE FOR ORAL 
CONSUMPTION.

PROTIEN OR AMINO ACID REQUIREMENTS
ALTHOUGH THE RECOMMENDED DIETARY ALLOWANCE FOR PROTEIN IS 0.8 G/KG PER D, MAXIMAL RATES OF REPLETION OCCUR WITH 1.5 G/KG IN THE MALNOURISHED. IN THE SEVERELY CATABOLIC PATIENT, THIS HIGHER LEVEL MINIMIZES PROTEIN LOSS. IN PATIENTS REQUIRING SNS IN THE ACUTE CARE SETTING, AT LEAST 1 G/KG IS RECOMMENDED, WITH GREATER AMOUNTS UP TO 1.5 G/KG AS VOLUME, RENAL, AND HEPATIC TOLERANCES ALLOW. THE STANDARD PARENTERAL AND ENTERAL FORMULAS CONTAIN PROTEIN OF HIGH BIOLOGIC VALUE AND MEET THE REQUIREMENTS FOR THE EIGHT ESSENTIAL AMINO ACIDS. IN PROTEIN-INTOLERANT CONDITIONS SUCH AS RENAL AND HEPATIC FAILURE, MODIFIED AMINO ACID FORMULAS SHOULD BE CONSIDERED. IN HEPATIC FAILURE, HIGHER BRANCHED-CHAIN AMINO ACID-ENRICHED FORMULAS APPEAR TO IMPROVE OUTCOMES. CONDITIONALLY ESSENTIAL AMINO ACIDS LIKE ARGinine AND GLUTAMINE MAY ALSO HAVE SOME BENEFIT IN SUPPLEMENTAL AMOUNTS.

PROTEIN (NITROGEN) BALANCE PROVIDES A MEASURE OF FEEDING EFFICACY OF PN OR EN. IT IS CALCULATED AS PROTEIN INTAKE/6.25 BECAUSE PROTEINS ARE ON AVERAGE 16% NITROGEN (N), MINUS THE 24-H URINE UREA N (UUN) PLUS 4 G N, WHICH REFLECTS OTHER N LOSSES. IN THE CRITICALLY ILL, A MILD NEGATIVE BALANCE OF 2-4 G N/D IS USUALLY ACHIEVABLE WITH A SIMILARLY MILD POSITIVE BALANCE IN THE RECUPERATING PATIENT. EACH G N REPRESENTS APPROXIMATELY 30 G LEAN TISSUE.

MINERAL AND VITAMIN REQUIREMENTS

PARENTERAL ELECTROLYTE, VITAMIN, AND TRACE MINERAL REQUIREMENTS ARE SUMMARIZED IN TABLES 73-4, 73-5, AND 73-6. ELECTROLYTE MODIFICATIONS ARE NECESSARY WITH SUBSTANTIAL GASTROINTESTINAL LOSSES FROM NASOGASTRIC DRAINAGE OR INTESTINAL LOSSES FROM FISTULAS, DIARRHEA OR OSTOMY OUTPUTS. SUCH LOSSES ALSO IMPLY EXTRA CALCIUM, MAGNESIUM, AND ZINC LOSSES. EXCESSIVE URINE OR POTASSIUM LOSSES WITH AMPHOTERICIN, OR MAGNESIUM LOSSES WITH CISPLATIN OR IN RENAL FAILURE, NECESSITATE ADJUSTMENTS IN SODIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND ACID-BASE BALANCE. VITAMIN AND TRACE ELEMENT REQUIREMENTS ARE MET BY THE DAILY PROVISION OF A COMPLETE PARENTERAL VITAMIN SUPPLEMENT AND TRACE ELEMENTS FOR PN, AND WITH THE PROVISION OF ADEQUATE AMOUNTS OF ENTERAL FEEDING FORMULAS THAT CONTAIN THESE MICRONUTRIENTS.

PARENTERAL NUTRITION INFUSION TECHNIQUE AND PATIENT MONITORING

PARENTERAL FEEDING THROUGH A PERIPHERAL VEIN IS LIMITED BY OSMOLALITY AND VOLUME CONSTRAINTS. SOLUTIONS THAT CONTAIN MORE THAN 3% AMINO ACIDS AND 5% GLUCOSE (290 KCAL/L) ARE POORLY TOLERATED PERIPHERALLY. PARENTERAL FAT (20%) CAN BE GIVEN TO INCREASE THE CALORIES DELIVERED. THE TOTAL VOLUME REQUIRED TO PROVIDE A MARGINAL PROTEIN INTAKE OF 60 G AND 1680 TOTAL KCAL IS 2.5 L. HOWEVER, THE RISK OF SIGNIFICANT MOR-

TABLE 73-4 USUAL DAILY ELECTROLYTE ADDITIONS TO PARENTERAL NUTRITION
ELECTROLYTE

SODIUM

POTASSIUM

CHLORIDE

ACETATE

CALCIUM

MAGNESIUM

PHOSPHORUS

PARENTERAL EQUIVALENT OF RDA

10 MEQ
10 MEQ
30 MMOL

USUAL INTAKE

1-2 MEQ/KG + REPLACEMENT, BUT CAN BE AS LOW AS 5-40 MEQ/D
40-100 MEQ/D + REPLACEMENT OF UNUSUAL LOSSES
AS NEEDED FOR ACID-BASE BALANCE, BUT USUALLY 2:1 TO 1:1 WITH ACETATE
AS NEEDED FOR ACID-BASE BALANCE
10-20 MEQ/D
8-16 MEQ/D
20-40 MMOL

TABLE 73-5 PARENTERAL MULTIVITAMIN REQUIREMENTS FOR ADULTS

VITAMIN

VITAMIN A
THIAMIN (B###1)
RIBOFLAVIN (B###2)
NIACIN (B###3)
FOLIC ACID
PANTOTHENIC ACID
PYRIDOXINE (B###6)
CYANOCOBALAMIN (B###12)
BIOTIN
ASCORBIC ACID (C)
VITAMIN D
VITAMIN E
VITAMIN K###A

RECENTLY REVISED VALUE

3300 IU
### A PRODUCT IS AVAILABLE THAT DOES NOT CONTAIN VITAMIN K. VITAMIN K SUPPLEMENTATION IS RECOMMENDED AT 2-4 MG/WEEK IN PATIENTS NOT RECEIVING ORAL ANTICOAGULATION THERAPY IF USING THIS PRODUCT.

BIDITY AND MORTALITY FROM INCOMPATIBILITIES OF CALCIUM AND PHOSPHATE SALTS IS GREATEST IN THESE LOW-OSMOLALITY, LOW-GLUCOSE REGIMENS. PARENTERAL FEEDING VIA A PERIPHERAL VEIN IS GENERALLY INTENDED AS A SUPPLEMENT TO ORAL FEEDING AND IS NOT OPTIMAL FOR THE CRITICALLY ILL. PARENTERAL NUTRITION MAY BENEFIT FROM SMALL AMOUNTS OF HEPARIN AT 1000 U/L AND CO-INFUSION WITH PARENTERAL FAT TO REDUCE OSMOLALITY, BUT VOLUME CONSTRAINTS STILL LIMIT THE VALUE OF THIS THERAPY. PERIPHERALLY INSERTED CENTRAL CATHETERS (PICCS) CAN BE USED FOR THE SHORT TERM TO PROVIDE CONCENTRATED GLUCOSE PARENTERAL SOLUTIONS OF 20-25% DEXTROSE AND 4-7% AMINO ACIDS, WHILE AVOIDING SOME OF THE COMPLICATIONS OF CATHETER PLACEMENT VIA A LARGE CENTRAL VEIN.

WITH PICC LINES, HOWEVER, FLOW CAN BE POSITION-RELATED, AND THE LINES CANNOT BE EXCHANGED OVER A WIRE FOR INFECTION MONITORING. FOR THESE REASONS, IN THE CRITICALLY ILL, CENTRALLY PLACED CATHETERS ARE PREFERRED. THE SUBCLAVIAN APPROACH IS BEST TOLERATED BY THE PATIENT AND IS THE EASIEST TO DRESS. THE JUGULAR APPROACH IS LESS LIKELY TO LEAD TO A PNEUMOTHORAX. THE FEMORAL APPROACH IS DISCOURAGED BECAUSE OF THE GREATER RISK OF CATHETER INFECTION. FOR LONG-TERM FEEDING IN THE HOME, TUNNELED CATHETERS AND IMPLANTED PORTS REDUCE INFECTION RISK AND ARE MORE ACCEPTABLE TO PATIENTS. HOWEVER, TUNNELED CATHETERS REQUIRE PLACEMENT IN THE OPERATING ROOM.

CATHETERS ARE MADE OF SILASTIC, POLYURETHANE, OR POLYVINYL CHLORIDE. SILASTIC CATHETERS ARE LESS THROMBOGENIC AND ARE BEST FOR TUNNELED CATHETERS. POLYURETHANE IS BEST FOR TEMPORARY CATHETERS. DRESSING CHANGES WITH DRY GAUZE AT REGULAR INTERVALS SHOULD BE PERFORMED BY NURSES SKILLED IN CATHETER CARE TO AVOID INFECTION. CHLORHEXIDINE SOLUTION IS MORE EFFECTIVE THAN ALCOHOL OR IODINE COMPOUNDS. APPROPRIATE MONITORING FOR PATIENTS RECEIVING PN IS SUMMARIZED IN TABLE 73-7.

TABLE 73-6 PARENTERAL TRACE METAL SUPPLEMENTATION FOR ADULTS### A TRACE MINERAL
ZINC
COPPER
MANGANESE
CHROMIUM
SELENIUM
MOLYBDENUM
IODINE

**INTAKE**

2.5-4 MG/D, AN ADDITIONAL 10-15 MG/D PER LOF STOOL OR ILEOSTOMY OUTPUT
0.5-1.5 MG/D, POSSIBILITY OF RETENTION IN BILIARY TRACT OBSTRUCTION
0.1-0.3 MG/D, POSSIBILITY OF RETENTION IN BILIARY TRACT OBSTRUCTION
10-15 *G/D
20-100 *G/D, NECESSARY FOR LONG-TERM PN, OPTIONAL FOR SHORT-TERM TPN
20-120 *G/D, NECESSARY FOR LONG-TERM PN, OPTIONAL FOR SHORT-TERM PN
75-150 *G/D, NECESSARY FOR LONG-TERM PN, OPTIONAL FOR SHORT-TERM PN

###ACOMMERCIAL PRODUCTS ARE AVAILABLE THAT HAVE THE FIRST FOUR, FIRST FIVE, AND ALL SEVEN OF THESE METALS IN RECOMMENDED AMOUNTS.

*NOTE:* PN, PARENTERAL NUTRITION; TPN, TOTAL PARENTERAL NUTRITION.

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**460 PART 5: NUTRITION**

**TABLE 73-7 MONITORING THE PATIENT ON PARENTERAL NUTRITION**

**CLINICAL DATA MONITORED DAILY**

GENERAL SENSE OF WELL-BEING
STRENGTH AS EVIDENCED IN GETTING OUT OF BED, WALKING, RESISTANCE EXERCISE AS APPROPRIATE
VITAL SIGNS INCLUDING TEMPERATURE, BLOOD PRESSURE, PULSE, AND RESPIRATORY RATE
FLUID BALANCE: WEIGHT AT LEAST SEVERAL TIMES WEEKLY, FLUID INTAKE (PARENTERAL AND ENTERAL) VS FLUID OUTPUT (URINE, STOOL, GASTRIC DRAINAGE, WOUND, OSTOMY)
PARENTERAL NUTRITION DELIVER/EQUIPMENT: TUBING, PUMP, FILTER, CATHETER, DRESSING
NUTRIENT SOLUTION COMPOSITION

LABORATORY DAILY

FINGER-STICK GLUCOSE
BLOOD GLUCOSE, NA, K, CL, HCO₃, BUN

SERUM CREATININE, ALBUMIN, PO₄, CA,
MG, HB/HCT, WBC
INR
MICRONUTRIENT TESTS

THREE TIMES DAILY UNTIL STABLE
DAILY UNTIL STABLE AND FULLY ADVANCED,
THEN TWICE WEEKLY
BASELINE, THEN TWICE WEEKLY
BASELINE, THEN WEEKLY
AS INDICATED

NOTE: HB HEMOGLOBIN; HCT, HEMATOCRIT; INR, INTERNATIONAL NORMALIZED RATIO,
WBC,
WHITE BLOOD CELL COUNT.
SOURCE: ADAPTED FROM CHAPTER BY LYN HOWARD, MD, IN HPIM, 16E.

COMPLICATIONS

MECHANICAL  THE INSERTION OF A CENTRAL VENOUS CATHETER SHOULD BE PERFORMED BY TRAINED AND EXPERIENCED PERSONNEL USING ASEPTIC TECHNIQUES TO LIMIT THE MAJOR COMMON COMPLICATIONS OF PNEUMOTHORAX AND INADVERTENT ARTERIAL PUNCTURE OR INJURY.  CATHETER POSITION SHOULD BE RADIOGRAPHICALLY CONFIRMED TO BE IN THE SUPERIOR VENA CAVA DISTAL TO THE JUNCTION WITH THE JUGULAR OR SUBCLAVIAN VEIN AND NOT DIRECTLY AGAINST THE VESSEL WALL.  THROMBOSIS RELATED TO THE CATHETER MAY OCCUR AT THE SITE OF ENTRY INTO THE VEIN AND EXTEND TO ENCASE THE CATHETER.  CATHETER INFECTION PREDISPOSES TO THROMBOSIS, AS DOES THE SYSTEMIC INFLAMMATORY RESPONSE.  THE ADDITION OF 6000 U OF HEPARIN IN THE DAILY PARENTERAL FORMULA IN HOSPITALIZED PATIENTS WITH TEMPORARY CATHETERS REDUCES THE RISK OF FIBRIN SHEATH FORMATION AND CATHETER INFECTION.  TEMPORARY CATHETERS THAT DEVELOP A THROMBUS SHOULD BE REMOVED AND, BASED ON CLINICAL FINDINGS, TREATED WITH ANTICOAGULANTS.  THROMBOLYTIC THERAPY CAN BE CONSIDERED FOR PATIENTS WITH PERMANENT CATHETERS DEPENDING ON THE EASE OF REPLACEMENT AND PRESENCE OF ALTERNATE, REASONABLY ACCEPTABLE VENOUS ACCESS SITES.  LOW-DOSE WARFARIN THERAPY OF 1 MG/D REDUCES THE RISK OF THROMBOSIS IN PERMANENT CATHETERS USED FOR HOME PN, BUT FULL ANTICOAGULATION MAY BE REQUIRED IN PATIENTS WHO HAVE
RECURRENT THROMBOSIS RELATED TO PERMANENT CATHETERS. A RECENT U.S. FOOD AND DRUG ADMINISTRATION MANDATE TO REFORMULATE PARENTERAL MULTIVITAMINS TO INCLUDE VITAMIN K AT A DOSE OF 150 *G DAILY MAY AFFECT THE EFFICACY OF LOW-DOSE WARFARIN THERAPY. THERE IS A “NO VITAMIN K” VERSION AVAILABLE FOR PATIENTS RECEIVING THIS THERAPY. CATHETERS CAN BECOME MECHANICALLY OCCLUDED AND MAY ALSO BECOME OCCLUDED BY FIBRIN AT THE TIP, OR BY FAT, MINERALS, OR DRUGS INTRALUMINALLY. THESE OCCLUSIONS CAN BE MANAGED WITH LOW-DOSE ALTEPLASE FOR FIBRIN, WITH INDIWELLING 70% ALCOHOL FOR FAT, WITH 0.1 N HYDROCHLORIC ACID FOR MINERAL PRECIPITATES, AND WITH EITHER 0.1 N HYDROCHLORIC ACID OR 0.1 N SODIUM HYDROXIDE FOR DRUGS, DEPENDING ON THEIR PH.

METABOLIC THE MOST COMMON PROBLEMS RELATED TO PN ARE FLUID OVERLOAD AND HYPERGLYCEMIA (TABLE 73-8). HYPERTONIC DEXTROSE STIMULATES A MUCH HIGHER INSULIN LEVEL THAN MEAL FEEDING. BECAUSE INSULIN IS A POTENT ANTINATRIURETIC AND ANTIDIURETIC HORMONE, HYPERINSULINEMIA LEADS TO SODIUM AND FLUID RETENTION. IN THE ABSENCE OF GASTROINTESTINAL LOSSES OR RENAL DYSFUNCTION, NET FLUID RETENTION IS LIKELY WHEN TOTAL FLUID INTAKE EXCEEDS 2000 ML/D. CLOSE MONITORING OF BODY WEIGHT, AS WELL AS FLUID INTAKE AND OUTPUT, IS NECESSARY TO PREVENT THIS COMPLICATION. IN THE ABSENCE OF SIGNIFICANT RENAL IMPAIRMENT, THE SODIUM CONTENT OF THE URINE IS LIKELY TO BE <10 MEQ/L. PROVIDING SODIUM IN LIMITED AMOUNTS OF 40 MEQ/D AND THE USE OF BOTH GLUCOSE AND FAT IN THE PN MIXTURE TO LOWER TOTAL GLUCOSE AND SODIUM WILL HELP REDUCE FLUID RETENTION. THE ELEVATED INSULIN ALSO INCREASES THE INTRACELLULAR TRANSPORT OF POTASSIUM, MAGNESIUM, AND PHOSPHORUS, WHICH CAN PRECIPITATE A DANGEROUS REFEEDING SYNDROME IF THE TOTAL GLUCOSE CONTENT OF THE PN SOLUTION IS ADVANCED TOO QUICKLY IN SEVERELY MALNOURISHED PATIENTS. IT IS GENERALLY BEST TO START PN WITH <200 G GLUCOSE/D TO ASSESS GLUCOSE TOLERANCE. REGULAR INSULIN CAN BE ADDED TO THE PN FORMULA TO ESTABLISH GLYCEMIC CONTROL, AND THE INSULIN DOSES CAN BE INCREASED PROPORTIONATELY AS THE GLUCOSE IS ADVANCED. AS A GENERAL RULE, PATIENTS WITH INSULIN-DEPENDENT DIABETES REQUIRE ABOUT TWICE THEIR USUAL HOME INSULIN DOSES WHEN THEY ARE RECEIVING TPN AT 20-25 KCAL/KG, LARGELY AS A CONSEQUENCE OF PARENTERAL GLUCOSE ADMINISTRATION AND SOME LOSS OF INSULIN TO THE TPN CONTAINER. AS A ROUGH ESTIMATE, THE AMOUNT OF INSULIN CAN BE PROVIDED IN A SIMILAR PROPORTION TO THE AMOUNT OF CALORIES PROVIDED AS TPN RELATIVE TO FULL FEEDING, AND THE INSULIN CAN BE PLACED IN THE TPN FORMULA. SUBCUTANEOUS REGULAR INSULIN CAN BE PROVIDED TO IMPROVE GLUCOSE CONTROL AS ASSESSED BY MEASUREMENTS OF BLOOD GLUCOSE EVERY 6 H. ABOUT TWO-

TABLE 73-8 SELECTED METABOLIC DISTURBANCES AND THEIR CORRECTION

DISTURBANCE

HYPONATREMIA
HYPERNATREMIA

HYPOKALEMIA

HYPERKALEMIA

HYPOCALCEMIA

HYPERCALCEMIA

HYPOMAGNESEMIA

HYPOPHOSPHATEMIA

HYPERPHOSPHATEMIA

AZOTEMIA

CAUSE

INCREASED TOTAL BODY WATER OR DECREASED TOTAL BODY SODIUM OCCURS COMMONLY WITH EXCESSIVE ISOTONIC OR HYPERTONIC FLUID FOLLOWED BY DIURETIC ADMINISTRATION WITH FREE WATER CLEARANCE; CAN ALSO OCCUR WITH DEHYDRATION AND NORMAL TOTAL BODY SODIUM INADEQUATE INTAKE RELATIVE TO NEED EXCESSIVE DIURESIS, TUBULAR DYSFUNCTION MAGNESIUM DEFICIENCY METABOLIC ALKALOSIS HYPERINSULINEMIA

EXCESSIVE PROVISION METABOLIC ACIDOSIS

RENEAL DETERIORATION

RECIPOCAL RESPONSE TO PHOSPHORUS REPLETION CRITICAL ILLNESS EFFECT SEVERE MALABSORPTION EXCESSIVE ADMINISTRATION OR PATHOLOGIC (CANCER, HYPERPARATHYROIDISM) INCREASED REQUIREMENTS DUE TO DIURETIC USE ALCOHOLISM, MALABSORPTION, MALNUTRITION
CRITICAL ILLNESS
INADEQUATE INTAKE RELATIVE TO NEEDS
RELATED TO MALNUTRITION, ALCOHOL USE
INCREASED CALCIUM INTAKE
EXCESSIVE ADMINISTRATION OR WORSENING
RENAL FUNCTION
EXCESSIVE AMINO ACID INFUSION OR
WORSENING RENAL FUNCTION

CORRECTIVE ACTION WITH PN

DECREASE FREE WATER OR INCREASE
SODIUM
INCREASE FREE WATER TO PRODUCE NET
POSITIVE FLUID BALANCE MAINTAIN-
ING SODIUM AND CHLORIDE
BALANCE

USE SUPPLEMENTS
USE SUPPLEMENTS
INCREASE PN MAGNESIUM
CONECT ALKALOSIS
MAINTAIN CONSTANT PN, INCREASE
POTASSIUM
REDUCE SUPPLEMENTS
EVALUATE ALKALOSIS, TREAT WITH PN
ACETATE SALT AND DECREASE
POTASSIUM
EVALUATE PATIENT AND ADJUST PN AS
INDICATED
INCREASE CALCIUM

INCREASE CALCIUM
SUPPLEMENT CALCIUM
REDUCE OR ELIMINATE CALCIUM

SUPPLEMENT MAGNESIUM
SUPPLEMENT MAGNESIUM
SUPPLEMENT PHOSPHORUS

USE SUPPLEMENTS
REDUCE PHOSPHORUS

REDUCE AMINO ACID LEVEL BUT CON-
SIDER RENAL REPLACEMENT THERAPY
IF CANNOT PROVIDE 1 G PROTEIN PER
KG FOR PROLONGED PERIODS

NOTE: PN, PARENTERAL NUTRITION.
CHAPTER 73 ENTERAL AND PARENTERAL NUTRITION THERAPY

THIRDS OF THE TOTAL 24-H AMOUNT CAN BE ADDED TO THE NEXT DAY’S ORDER, WITH SUBCUTANEOUS INSULIN SUPPLEMENTS AS NEEDED. ADVANCES IN TPN CONCENTRATION SHOULD BE MADE WHEN REASONABLE GLUCOSE CONTROL IS ESTABLISHED, AND THE INSULIN DOSE ADJUSTED PROPORTIONATELY TO THE CALORIES ADDED AS GLUCOSE AND AMINO ACIDS. THESE ARE GENERAL RULES, AND THEY ARE CONSERVATIVE. GIVEN THE ADVERSE CLINICAL IMPACT OF HYPERGLYCEMIA, IT MAY BE NECESSARY TO USE CONTINUOUS INSULIN THERAPY AS A SEPARATE INFUSION WITH A STANDARD PROTOCOL TO INITIALLY ESTABLISH CONTROL. ONCE ESTABLISHED, THIS INSULIN DOSE CAN BE ADDED TO THE PN FORMULA. ACID-BASE IMBALANCE IS ALSO COMMON DURING PN THERAPY. AMINO ACID FORMULAS ARE BUFFERED, BUT CRITICALLY ILL PATIENTS ARE PRONE TO METABOLIC ACIDOSIS, OFTEN DUE TO RENAL TUBULAR IMPAIRMENT. THE USE OF SODIUM AND POTASSIUM ACETATE SALTS IN THE PN FORMULA MAY ADDRESS THIS PROBLEM. BICARBONATE SALTS SHOULD NOT BE USED BECAUSE THEY ARE INCOMPATIBLE WITH TPN FORMULATIONS. NASOGASTRIC DRAINAGE PRODUCES A HYPOCHLOREMIC ALKALOSIS THAT CAN BE MANAGED BY ATTENTION TO CHLORIDE BALANCE. OCCASIONALLY, HYDROCHLORIC ACID MAY BE REQUIRED FOR A MORE RAPID RESPONSE OR WHEN DIURETIC THERAPY LIMITS THE ABILITY TO PROVIDE SUBSTANTIAL SODIUM CHLORIDE. UP TO 100 MEQ/L AND UP TO 150 MEQ OF HYDROCHLORIC ACID PER DAY MAY BE PLACED IN A FAT-FREE PN FORMULA.

INFECTIOUS INFECTIONS OF THE CENTRAL ACCESS CATHETER RARELY OCCUR IN THE FIRST 72 H. FEVER DURING THIS PERIOD IS USUALLY FROM INFECTION ELSEWHERE OR ANOTHER CAUSE. FEVER THAT DEVELOPS DURING PN CAN BE ADDRESSED BY CHECKING THE CATHETER SITE AND, IF THE SITE LOOKS CLEAN, EXCHANGING THE CATHETER OVER A WIRE WITH CULTURES TAKEN THROUGH THE CATHETER AND AT THE CATHETER TIP. IF THESE CULTURES ARE NEGATIVE, AS THEY ARE MOST OF THE TIME, THE NEW CATHETER CAN CONTINUE TO BE USED. IF A CULTURE IS POSITIVE FOR A RELATIVELY NONPATHOGENIC BACTERIA LIKE STAPHYLOCOCCUS EPIDERMIDIS, CONSIDER A SECOND EXCHANGE OVER A WIRE WITH REPEAT CULTURES OR REPLACE THE CATHETER DEPENDING ON THE CLINICAL CIRCUMSTANCES. IF CULTURES ARE POSITIVE FOR MORE PATHOGENIC BACTERIA, OR FOR FUNGI LIKE CANDIDA ALBICANS, IT IS GENERALLY BEST TO REPLACE THE CATHETER AT A NEW SITE. WHETHER ANTIBIOTIC TREATMENT IS REQUIRED IS A CLINICAL DECISION, BUT C. ALBICANS GROWN FROM THE BLOOD CULTURE IN A PATIENT RECEIVING PN SHOULD ALWAYS BE TREATED BECAUSE THE CONSEQUENCES OF FAILURE TO TREAT CAN BE DIRE. CATHETER INFECTIONS CAN BE MINIMIZED BY DEDICATING THE FEEDING CATHETER TO PN, WITHOUT BLOOD SAMPLING OR MEDICATION ADMINISTRATION.
CENTRAL CATHETER INFECTIONS ARE A SERIOUS COMPLICATION WITH AN ATTRIBUTED MORTALITY OF 12-25%.
INFECTIONS IN CENTRAL VENOUS CATHETERS DEDICATED TO FEEDING SHOULD OCCUR LESS FREQUENTLY THAN 3 PER 1000 CATHETER-DAYS. HOME PN CATHETERS THAT BECOME INFECTED MAY BE TREATED THROUGH THE CATHETER WITHOUT REMOVAL OF THE CATHETER, PARTICULARLY IF THE OFFENDING ORGANISM IS *S. EPIDERMIDIS*. CLEARING OF THE BIOFILM AND FIBRIN SHEATH BY LOCAL TREATMENT OF THE CATHETER WITH INDWELLING ALTEPLASE MAY INCREASE THE LIKELIHOOD OF ERADICATION. ANTIBIOTIC LOCK THERAPY WITH HIGH CONCENTRATIONS OF ANTIBIOTIC, WITH OR WITHOUT HEPARIN IN ADDITION TO SYSTEMIC THERAPY, MAY IMPROVE EFFICACY. SEPSIS WITH HYPOTENSION SHOULD PRECIPITATE CATHETER REMOVAL IN EITHER THE TEMPORARY OR PERMANENT PN SETTING.

ENTERAL NUTRITION
TUBE PLACEMENT AND PATIENT MONITORING

THE TYPES OF ENTERAL FEEDING TUBES, METHODS OF INSERTION, THEIR CLINICAL USES, AND POTENTIAL COMPLICATIONS ARE OUTLINED IN TABLE 73-9. THE DIFFERENT TYPES OF ENTERAL FORMULAS ARE LISTED IN TABLE 73-10. PATIENTS RECEIVING EN ARE AT RISK FOR MANY OF THE SAME METABOLIC COMPLICATIONS AS THOSE WHO RECEIVE PN AND SHOULD BE MONITORED IN THE SAME MANNER. EN CAN BE A SOURCE OF SIMILAR PROBLEMS, BUT NOT TO THE SAME DEGREE, BECAUSE THE INSULIN RESPONSE TO EN IS ABOUT HALF OF THAT SEEN WITH PN. ENTERAL FEEDING FORMULAS HAVE FIXED ELECTROLYTE COMPOSITIONS THAT ARE GENERALLY MODEST IN SODIUM AND SOMEWHAT HIGHER IN POTASSIUM CONTENT. ACID-BASE DISTURBANCES CAN BE ADDRESSED TO A MORE LIMITED EXTENT WITH EN. ACETATE SALTS CAN BE ADDED TO THE FORMULA TO TREAT CHRONIC METABOLIC ACIDOSIS. CALCIUM CHLORIDE CAN BE ADDED TO TREAT MILD CHRONIC METABOLIC ALKALOSIS. MEDICATIONS AND OTHER ADDITIVES TO ENTERAL FEEDING FORMULAS CAN CLOG THE TUBES (E.G., CALCIUM CHLORIDE MAY INTERACT WITH CASEIN-BASED FORMULAS TO PRODUCE INSOLUBLE CALCIUM CASEINATE PRODUCTS) AND MAY REDUCE THE EFFICACY OF SOME DRUGS (E.G., PHENYTOIN). SINCE SMALL-BORE TUBES ARE EASILY DISPLACED, TUBE POSITION SHOULD BE CHECKED AT INTERVALS BY ASPIRATING AND MEASURING THE PH OF THE GUT FLUID (<4 IN THE STOMACH, >6 IN THE JEJUNUM).

COMPLICATIONS
**ASPIRATION**  The debilitated patient with poor gastric emptying and impairment of swallowing and cough is at risk for aspiration; this is particularly true for those who are mechanically ventilated. Tracheal suctioning induces coughing and gastric regurgitation, and cuffs on endotracheal or tracheostomy tubes seldom protect against aspiration. Preventive measures include elevating the head of the bed to 30 degrees.

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**TABLE 73-9 ENTERAL FEEDING TUBES**

**TYPE/INSERTION TECHNIQUE**

**NASOGASTRIC TUBE**

External measurement: nostril, ear, xiphisternum, tube stiffened by ice water or stylet; position verified by injecting air and auscultating, or by X-ray.

**NASODUODENAL TUBE**

External measurement: nostril, ear, anterior superior iliac spine; tube stiffened by stylet and passed through pylorus under fluoroscopy or with endoscopic loop.

**GASTROSTOMY TUBE**

Percutaneous placement endoscopically, radiologically, or surgically; after tract established, can be converted to a gastric “button”.

**JEJUNOSTOMY TUBE**

Percutaneous placement endoscopically, or radiologically, via pylorus or endoscopically or surgically directly into the jejunum.

**COMBINED GASTROJEJUNOSTOMY TUBE**

Percutaneous placement endoscopically, radiologically, or surgically; intragastric arm for continuous or intermittent gastric suction; jejunal arm.
FOR ENTERAL FEEDING

CLINICAL USES

SHORT-TERM CLINICAL SITUATION (WEEKS) OR LONGER PERIODS WITH INTERMITTENT INSERTION; BOLUS FEEDING SIMPLER, BUT CONTINUOUS DRIP WITH PUMP BETTER TOLERATED

SHORT-TERM CLINICAL SITUATIONS WHERE GASTRIC EMPTYING IMPAIRED OR PROXIMAL LEAK SUSPECTED; REQUIRES CONTINUOUS DRIP WITH PUMP

LONG-TERM CLINICAL SITUATIONS, SWALLOWING DISORDERS, OR IMPAIRED SMALL-BOWEL ABSORPTION REQUIRING CONTINUOUS DRIP

LONG-TERM CLINICAL SITUATIONS WHERE GASTRIC EMPTYING IMPAIRED; REQUIRES CONTINUOUS DRIP WITH PUMP; DIRECT ENDOSCOPIC PLACEMENT (PEJ) IS THE MOST COMFORTABLE FOR PATIENT

USED FOR PATIENTS WITH IMPAIRED GASTRIC EMPTYING AND AT HIGH RISK FOR ASPIRATION OR PATIENTS WITH ACUTE PANCREATITIS OR PROXIMAL LEAKS

POTENTIAL COMPLICATIONS

ASPIRATION; ULCERATION OF NASAL AND ESOPHAGEAL TISSUES, LEADING TO STRicture

SPONTANEOUS PULLING BACK INTO STOMACH (POSITION VERIFIED BY ASPIRATING CONTENT, PH > 6); DIARRHEA COMMON, FIBER-CONTAINING FORMULAS MAY HELP

ASPIRATION; IRRITATION AROUND
TUBE EXIT SITE, PERITONEAL LEAK; BALLOON MIGRATION AND OBSTRUCTION OF PYLORUS

CLOGGING OR DISPLACEMENT OF TUBE; JEJUNAL FISTULA IF LARGE-BORE TUBE USED; DIARRHEA FROM DUMPING; IRRITATION OF SURGICAL ANCHORING SUTURE

CLOGGING ESPECIALLY OF SMALL BORE JEJUNAL TUBE

*NOTE*: ALL SMALL TUBES ARE AT RISK FOR CLOGGING, ESPECIALLY IF USED FOR CRUSHED MEDICATIONS. IN LONG-TERM ENTERAL PATIENTS, GASTROSTOMY AND JEJUNOSTOMY TUBES CAN BE EXCHANGED FOR A LOW-PROFILE “BUTTON” ONCE THE TRACT IS ESTABLISHED.

*SOURCE*: ADAPTED FROM CHAPTER IN *HARRISON’S PRINCIPLES OF INTERNAL MEDICINE*, 16E, BY LYN HOWARD, MD.

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PAGE NO. 105

462 PART 5: NUTRITION

TABLE 73-10 ENTERAL FORMULAS

COMPOSITION CHARACTERISTICS

STANDARD ENTERAL FORMULA

1. COMPLETE DIETARY PRODUCTS (+)###A
   A. CALORIC DENSITY 1 KCAL/ML
   B. PROTEIN ~14% CALS, CASEINATES, SOY, LACTALBUMIN
   C. CHO ~60% CALS, HYDROLYZED CORN STARCH, MALTODEXTRIN, SUCROSE
   D. FAT ~30% CALS, CORN, SOY, SAFFLOWER OILS
   E. RECOMMENDED DAILY INTAKE OF ALL MINERALS AND VITAMINS IN >1500 KCAL/D
   F. OSMOLALITY (MOSMOL/KG): ~300

MODIFIED ENTERAL FORMULAS

1. CALORIC DENSITY 15-2 KCAL/ML (+)
2. A. HIGH PROTEIN ~20-25% PROTEIN (+)
   B. HYDROLYZED PROTEIN TO SMALL PEPTIDES (+)
   C. *ARGININE, GLUTAMINE, NUCLEOTIDES, *3 FAT (+++)
   D. *BRANCHED-CHAIN AMINO ACIDS, *AROMATIC AMINO ACIDS (+++)
   E. LOW PROTEIN OF HIGH BIOLOGIC VALUE
3. A. LOW FAT, PARTIAL MCT SUBSTITUTION (+)
B. * FAT >40% CALS (++)

C. * FAT FROM MUFA (++)

D. * FAT FROM *3 AND * *6 LINOLEIC ACID (+++)

4. FIBER PROVIDED AS SOY POLYSACCHARIDE (+)

**CLINICAL INDICATIONS**

SUITABLE FOR MOST PATIENTS REQUIRING TUBE FEEDING; SOME CAN BE USED ORALLY

FLUID-RESTRICTED PATIENTS
CRITICALLY ILL PATIENTS
IMPAIRED ABSORPTION
IMMUNE-ENHANCING DIETS
LIVER FAILURE PATIENTS INTOLERANT OF 0.8 G/KG PROTEIN

RENAL FAILURE PATIENT FOR BRIEF PERIODS IF CRITICALLY ILL
FAT MALABSORPTION
PULMONARY FAILURE WITH CO####2 RETENTION ON STANDARD FORMULA, LIMITED UTILITY
IMPROVEMENT IN GLYCEMIC INDEX CONTROL IN DIABETES
IMPROVED VENTILATION IN ARDS
IMPROVED LAXATION

COST: + INEXPENSIVE, ++ MODERATELY EXPENSIVE; +++ VERY EXPENSIVE.

**NOTE:** ARDS, ACUTE RESPIRATORY DISTRESS SYNDROME, CHO, CARBOHYDRATE; MCT, MEDIUM-CHAIN TRIGLYCERIDE; MUFA, MO-NOUNSATURATED FATTY ACIDS; *3 OR *6, POLYUNSATURATED FAT WITH FIRST DOUBLE BOND AT CARBON 3 (FISH OILS) OR CARTON 6 (VEGETABLE OILS).

**SOURCE:** ADAPTED FROM CHAPTER IN **HARRISON'S PRINCIPLES OF INTERNAL MEDICINE**, 16E, BY LYN HOWARD, MD.

USING NURSE-DIRECTED ALGORITHMS FOR FORMULA ADVANCEMENT, COMBINING ENTERAL WITH PARENTERAL FEEDING, AND USING POST-LIGAMENT OF TREITZ FEEDING. TUBE FEEDING SHOULD NOT BE DISCONTINUED FOR GASTRIC RESIDUALS OF <300 ML UNLESS THERE ARE OTHER SIGNS OF GASTROINTESTINAL INTOLERANCE SUCH AS NAUSEA, VOMITING, OR ABDOMINAL DISTENTION. CONTINUOUS FEEDING USING PUMPS IS BETTER TOLERATED INTRAGASTRICALLY AND IS ESSENTIAL FOR FEEDING INTO THE JEJUNUM. FOR SMALL-BOWEL FEEDING, RESIDUALS ARE NOT ASSESSED BUT ABDOMINAL PAIN AND DISTENTION SHOULD BE MONITORED.

**DIARRHEA** ENTERAL FEEDING OFTEN LEADS TO DIARRHEA, ESPECIALLY IF BOWEL FUNCTION IS COMPROMISED BY DISEASE OR DRUGS, PARTICULARLY BROAD-SPECTRUM ANTIBIOTICS. DIARRHEA MAY BE CONTROLLED BY THE USE OF A CONTIN-
IOUS DRIP, WITH A FIBER-CONTAINING FORMULA, OR BY ADDING AN ANTIDIARRHEAL AGENT TO THE FORMULA. HOWEVER, CLOSTRIDIUM DIFFICILE, WHICH IS A COMMON CAUSE OF DIARRHEA IN PATIENTS BEING TUBE FED, SHOULD BE RULED OUT BEFORE USING ANTIDIARRHEAL AGENTS. H2 BLOCKERS MAY ALSO ASSIST IN REDUCING THE NET FLUID PRESENTED TO THE COLON. DIARRHEA ASSOCIATED WITH ENTERAL FEEDING DOES NOT NECESSARILY IMPLY INADEQUATE ABSORPTION OF NUTRIENTS OTHER THAN WATER AND ELECTROLYTES. AMINO ACIDS AND GLUCOSE ARE PARTICULARLY WELL ABSORBED IN THE UPPER SMALL BOWEL EXCEPT IN THE MOST DISEASED OR SHORTEST BOWEL. SINCE LUMINAL NUTRIENTS EXERT TROPHIC EFFECTS ON THE GUT MUCOSA, IT IS OFTEN APPROPRIATE TO PERSIST WITH TUBE FEEDING, DESPITE THE DIARRHEA, EVEN WHEN THIS NECESSITATES SUPPLEMENTAL PARENTERAL FLUID SUPPORT.

DEFINITION AND MEASUREMENT

OBESITY IS A STATE OF EXCESS ADIPOSE TISSUE MASS. ALTHOUGH OFTEN VIEWED AS EQUIVALENT TO INCREASED BODY WEIGHT, THIS NEED NOT BE THE CASE-LEAN

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BUT VERY MUSCULAR INDIVIDUALS MAY BE OVERWEIGHT BY NUMERICAL STANDARDS WITHOUT HAVING INCREASED ADIPOSITY. BODY WEIGHTS ARE DISTRIBUTED CONTINUOUSLY IN POPULATIONS, SO THAT CHOICE OF A MEDICALLY MEANINGFUL DISTINCTION BETWEEN LEAN AND OBESE IS SOMEWHAT ARBITRARY. OBESITY IS THEREFORE MORE EFFECTIVELY DEFINED BY ASSESSING ITS LINKAGE TO MORBIDITY OR MORTALITY.

ALTHOUGH NOT A DIRECT MEASURE OF ADIPOSITY, THE MOST WIDELY USED METHOD TO GAUGE OBESITY IS THE BODY MASS INDEX (BMI), WHICH IS EQUAL TO WEIGHT/HEIGHT###2 (IN KG/M###2) (FIG. 74-1). OTHER APPROACHES TO QUANTIFYING OBESITY INCLUDE ANTHROPOMETRY (SKIN-FOLD THICKNESS), DENSITOMETRY (UNDERWATER WEIGHING), CT OR MRI, AND ELECTRICAL IMPEDANCE. USING DATA FROM THE METROPOLITAN LIFE TABLES, BMIS FOR THE MIDPOINT OF ALL HEIGHTS AND FRAMES AMONG BOTH MEN AND WOMEN RANGE FROM 19-26 KG/M###2; AT A SIMILAR BMI, WOMEN HAVE MORE BODY FAT THAN MEN. BASED ON DATA OF SUBSTANTIAL MORBIDITY, A BMI OF 30 IS MOST COMMONLY USED AS A THRESHOLD FOR OBESITY IN BOTH MEN AND WOMEN. LARGE-SCALE EPIDEMIOLOGIC STUDIES SUGGEST THAT ALL-CAUSE, METABOLIC, CANCER, AND CARDIOVASCULAR MORBIDITY BEGIN TO RISE (ALBEIT AT A SLOW RATE) WHEN BMIS ARE > 25, SUGGESTING THAT THE CUT-OFF FOR OBESITY SHOULD BE LOW-
**CHAPTER 74 BIOLOGY OF OBESITY**

**FIGURE 74-1 NOMOGRAM FOR DETERMINING BODY MASS INDEX.** To use this nomogram, place a ruler or other straight edge between the body weight (without clothes) in kilograms or pounds located on the left-hand line and the height (without shoes) in centimeters or inches located on the right-hand line. The body mass index is read from the middle of the scale and is in metric units.

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ERED. Most authorities use the term OVERWEIGHT (rather than obese) to describe individuals with BMIs between 25 and 30. A BMI between 25 and 30 should be viewed as medically significant and worthy of therapeutic intervention, especially in the presence of risk factors that are influenced by adiposity, such as hypertension and glucose intolerance.

The distribution of adipose tissue in different anatomic depots also has substantial implications for morbidity. Specifically, intraabdominal and abdominal subcutaneous fat have more significance than subcutaneous fat present in the buttocks and lower extremities. This distinction is most easily made clinically by determining the waist-to-hip ratio, with a ratio >0.9 in women and >1.0 in men being abnormal. Many of the most important complications of obesity, such as insulin resistance, diabetes, hypertension, hyperlipidemia, and hyperandrogenism in women, are linked more strongly to intraabdominal and/or upper body fat than to overall adiposity (Chap. 236). The mechanism underlying this association is unknown but may relate to the fact that intraabdominal adipocytes are more lipolytically active than those from other depots.

Release of free fatty acids into the portal circulation has adverse metabolic actions, especially on the liver. Whether adipokines and cytokines secreted by visceral adipocytes play an additional role in systemic complications of obesity is an area of active investigation.

**PREVALENCE**

Data from the National Health and Nutrition Examination Surveys (NHANES) show that the percent of the American adult population with obesity (BMI > 30) has increased from 14.5% (between 1976 and 1980) to 30.5% (between 1999 and 2000). As many as 64% of U.S. adults 20 years of age were overweight (defined as BMI > 25).
BETWEEN THE YEARS OF 1999 AND 2000, EXTREME OBESITY (BMI *40) HAS ALSO INCREASED AND AFFECTS 4.7% OF THE POPULATION. THE INCREASING PREVALENCE OF MEDICALLY SIGNIFICANT OBESITY RAISES GREAT CONCERN. OBESITY IS MORE COMMON AMONG WOMEN AND IN THE POOR; THE PREVALENCE IN CHILDREN IS ALSO RISING AT A WORRISOME RATE.

PHYSIOLOGIC REGULATION OF ENERGY BALANCE

SUBSTANTIAL EVIDENCE SUGGESTS THAT BODY WEIGHT IS REGULATED BY BOTH ENDOCRINE AND NEURAL COMPONENTS THAT ULTIMATELY INFLUENCE THE EFFECTOR ARMS OF ENERGY INTAKE AND EXPENDITURE. THIS COMPLEX REGULATORY SYSTEM IS NECESSARY BECAUSE EVEN SMALL IMBALANCES BETWEEN ENERGY INTAKE AND EXPENDITURE WILL ULTIMATELY HAVE LARGE EFFECTS ON BODY WEIGHT. FOR EXAMPLE, A 0.3% POSITIVE IMBALANCE OVER 30 YEARS WOULD RESULT IN A 9-KG (20-LB) WEIGHT GAIN. THIS EXQUISITE REGULATION OF ENERGY BALANCE CANNOT BE MONITORED EASILY BY CALORIE-COUNTING IN RELATION TO PHYSICAL ACTIVITY. RATHER, BODY WEIGHT REGULATION OR DYSREGULATION DEPENDS ON A COMPLEX INTERPLAY OF HORMONAL AND NEURAL SIGNALS. ALTERATIONS IN STABLE WEIGHT BY FORCED OVERFEEDING OR FOOD DEPRIVATION INDUCE PHYSIOLOGIC CHANGES THAT RESIST THESE PERTURBATIONS: WITH WEIGHT LOSS, APPETITE INCREASES AND ENERGY EXPENDITURE FALLS; WITH OVERFEEDING, APPETITE FALLS AND ENERGY EXPENDITURE INCREASES. THIS LATTER COMPENSATORY MECHANISM FREQUENTLY FAILS, HOWEVER, PERMITTING OBESITY TO DEVELOP WHEN FOOD IS ABUNDANT AND PHYSICAL ACTIVITY IS LIMITED. A MAJOR REGULATOR OF THESE ADAPTIVE RESPONSES IS THE ADIPOCYTE-DERIVED HORMONE LEPTIN, WHICH ACTS THROUGH BRAIN CIRCUITS (PREDOMINANTLY IN THE HYPOTHALAMUS) TO INFLUENCE APPETITE, ENERGY EXPENDITURE, AND NEUROENDOCRINE FUNCTION (SEE BELOW). APPETITE IS INFLUENCED BY MANY FACTORS THAT ARE INTEGRATED BY THE BRAIN, MOST IMPORTANTLY WITHIN THE HYPOTHALAMUS (FIG. 74-2). SIGNALS THAT IMPINGE ON THE HYPOTHALAMIC CENTER INCLUDE NEURAL AFFERENTS, HORMONES, AND METABOLITES. VAGAL INPUTS ARE PARTICULARLY IMPORTANT, BRINGING INFORMATION FROM VISCERA, SUCH AS GUT DISTENTION. HORMONAL SIGNALS INCLUDE LEPTIN, INSULIN, CORTISOL, AND GUT PEPTIDES. AMONG THE LATTER ARE GHRELIN, WHICH IS MADE IN THE STOMACH AND STIMULATES FEEDING, AND PEPTIDE YY (PYY) AND CHOLECYSTOKININ, WHICH ARE MADE IN THE SMALL INTESTINE AND
SIGNAL TO THE BRAIN THROUGH DIRECT ACTION ON HYPOTHALAMIC CONTROL CENTERS AND/OR VIA THE VAGUS NERVE. METABOLITES, INCLUDING GLUCOSE, CAN INFLUENCE APPETITE, AS SEEN BY THE EFFECT OF HYPOGLYCEMIA TO INDUCE HUNGER; HOWEVER, GLUCOSE IS NOT NORMALLY A MAJOR REGULATOR OF APPETITE. THESE DIVERSE HORMONAL, METABOLIC, AND NEURAL SIGNALS ACT BY INFLUENCING THE EXPRESSION AND RELEASE OF VARIOUS HYPOTHALAMIC PEPTIDES [E.G., NEUROPEPTIDE Y (NPY), AGOUTI-RELATED PEPTIDE (AGRP), MELANOCYTE-STIMULATING HORMONE (*-MSH), AND MELANIN-CONCENTRATING HORMONE (MCH)] THAT ARE INTEGRATED WITH SEROTONERGIC, CATECHOLAMINERGIC, ENDOCANNABINOID,

PAGE NO. 107

464 PART 5: NUTRITION

FIGURE 74-2 THE FACTORS THAT REGULATE APPETITE THROUGH EFFECTS ON CENTRAL NEURAL CIRCUITS. SOME FACTORS THAT INCREASE OR DECREASE APPETITE ARE LISTED. NPY, NEUROPEPTIDE Y; MCH, MELANIN-CONCENTRATING HORMONE; AGRP, AGOUTI-RELATED PEPTIDE; MSH, MELANOCYTE-STIMULATING HORMONE; CART, COCAINE- AND AMPHETAMINE-RELATED TRANSCRIPT. GLP-1, GLUCAGON-RELATED PEPTIDE-1; CCK, CHOLECYSTOKININ.

AND OPIOID SIGNALING PATHWAYS (SEE BELOW). PSYCHOLOGICAL AND CULTURAL FACTORS ALSO PLAY A ROLE IN THE FINAL EXPRESSION OF APPETITE. APART FROM RARE GENETIC SYNDROMES INVOLVING LEPTIN, ITS RECEPTOR, AND THE MELANOCORTIN SYSTEM, SPECIFIC DEFECTS IN THIS COMPLEX APPETITE CONTROL NETWORK THAT INFLUENCE COMMON CASES OF OBESITY ARE NOT WELL DEFINED.

ENERGY EXPENDITURE INCLUDES THE FOLLOWING COMPONENTS: (1) RESTING OR BASAL METABOLIC RATE; (2) THE ENERGY COST OF METABOLIZING AND STORING FOOD; (3) THE THERMIC EFFECT OF EXERCISE; AND (4) ADAPTIVE THERMOGENESIS, WHICH VARIES IN RESPONSE TO CHRONIC CALORIC INTAKE (RISING WITH INCREASED INTAKE). BASAL METABOLIC RATE ACCOUNTS FOR ~70% OF DAILY ENERGY EXPENDITURE, WHEREAS ACTIVE PHYSICAL ACTIVITY CONtributes 5-10%. THUS, A SIGNIFICANT COMPONENT OF DAILY ENERGY CONSUMPTION IS FIXED. GENETIC MODELS IN MICE INDICATE THAT MUTATIONS IN CERTAIN GENES (E.G., TARGETED DELETION OF THE INSULIN RECEPTOR IN ADIPOSE TISSUE) PROTECT AGAINST OBESITY, APPARENTLY BY INCREASING ENERGY EXPENDITURE. ADAPTIVE THERMOGENESIS OCCURS IN BROWN ADIPOSE TISSUE (BAT), WHICH PLAYS AN IMPORTANT ROLE IN ENERGY METABOLISM IN MANY MAMMALS. IN CONTRAST TO WHITE ADIPOSE TISSUE, WHICH IS USED TO STORE ENERGY IN THE FORM OF LIPIDS, BAT EXPENDS STORED ENERGY AS HEAT. A MITOCHONDRIAL UNCOUPLING PROTEIN (UCP-1) IN BAT DISSIPATES THE HYDROGEN ION GRADI-
ENT IN THE OXIDATIVE RESPIRATION CHAIN AND RELEASES ENERGY AS HEAT. THE METABOLIC ACTIVITY OF BAT IS INCREASED BY A CENTRAL ACTION OF LEPTIN, ACTING THROUGH THE SYMPATHETIC NERVOUS SYSTEM, WHICH HEAVILY INNERVATES THIS TISSUE. IN RODENTS, BAT DEFICIENCY CAUSES OBESITY AND DIABETES; STIMULATION OF BAT WITH A SPECIFIC ADRENERGIC AGONIST (*3 AGONIST) PROTECTS AGAINST DIABETES AND OBESITY. ALTHOUGH BAT EXISTS IN HUMANS (ESPECIALLY NEONATES), ITS PHYSIOLOGIC ROLE IS NOT YET ESTABLISHED. HOMOLOGUES OF UCP-1 (UCP-2 AND -3) MAY MEDIATE UNCOUPLED MITOCHONDRIAL RESPIRATION IN OTHER TISSUES.

THE ADIPOCYTE AND ADIPOSE TISSUE

ADIPOSE TISSUE IS COMPOSED OF THE LIPID-STORING ADIPOSE CELL AND A Stromal/VASCULAR COMPARTMENT IN WHICH CELLS INCLUDING PREADIPOCYTES AND MACROPHAGES RESIDE. ADIPOSE MASS INCREASES BY ENLARGEMENT OF ADIPOSE CELLS THROUGH LIPID DEPOSITION, AS WELL AS BY AN INCREASE IN THE NUMBER OF ADIPOCYTES. OBESE ADIPOSE TISSUE IS ALSO CHARACTERIZED BY INCREASED NUMBERS OF INFILTRATING MACROPHAGES. THE PROCESS BY WHICH ADIPOSE CELLS ARE DERIVED FROM A MESENCHYMAL PREADIPOCYTE INVOLVES AN ORCHESTRATED SERIES OF DIFFERENTIATION STEPS MEDIATED BY A CASCADE OF SPECIFIC TRANSCRIPTION FACTORS. ONE OF THE KEY TRANSCRIPTION FACTORS IS PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR *(PPAR*), A NUCLEAR RECEPTOR THAT BINDS THE THIAZOLIDINEDIONE CLASS OF INSULIN-SENSITIZING DRUGS USED IN THE TREATMENT OF TYPE 2 DIABETES (CHAP. 338). ALTHOUGH THE ADIPOCYTE HAS GENERALLY BEEN REGARDED AS A STORAGE DEPOT FOR FAT, IT IS ALSO AN ENDOCRINE CELL THAT RELEASES NUMEROUS MOLECULES IN A REGULATED FASHION (FIG. 74-3). THESE INCLUDE THE ENERGY BALANCE-REGULATING HORMONE LEPTIN, CYTOKINES SUCH AS TUMOR NECROSIS FACTOR (TNF) * AND INTERLEUKIN (IL)-6, COMPLEMENT FACTORS SUCH AS FACTOR D (ALSO KNOWN AS ADIPSIN), PROTHROMBOTIC AGENTS SUCH AS PLASMINOGEN ACTIVATOR INHIBITOR I, AND A COMPONENT OF THE BLOOD PRESSURE REGULATING SYSTEM, ANGIOTENSINOGEN. ADIPONECTIN, AN ABUNDANT ADIPOSE-DERIVED PROTEIN WHOSE LEVELS ARE REDUCED IN OBESITY, ENHANCES INSULIN SENSITIVITY AND LIPID OXIDATION AND IT HAS VASCULAR PROTECTIVE EFFECTS, WHEREAS RESISTIN AND RBP4, WHOSE LEVELS ARE INCREASED IN OBESITY, MAY INDUCE INSULIN RESISTANCE. THESE FACTORS, AND OTHERS NOT YET IDENTIFIED, PLAY A ROLE IN THE PHYSIOLOGY OF LIPID HOMEOSTASIS, INSULIN SENSITIVITY, BLOOD PRESSURE CONTROL, COAGULATION, AND VASCULAR HEALTH, AND ARE LIKELY TO CONTRIBUTE TO OBESITY-RELATED PATHOLOGIES.

ETIOLOGY OF OBESITY

THOUGH THE MOLECULAR PATHWAYS REGULATING ENERGY BALANCE ARE BEGINNING TO BE ILLUMINATED, THE CAUSES OF OBESITY REMAIN ELUSIVE. IN PART,
THIS REFLECTS THE FACT THAT OBESITY IS A HETEROGENEOUS GROUP OF DISORDERS. AT ONE LEVEL, THE PATHOPHYSIOLOGY OF OBESITY SEEMS SIMPLE: A CHRONIC EXCESS OF NUTRIENT INTAKE RELATIVE TO THE LEVEL OF ENERGY EXPENDITURE. HOWEVER, DUE TO THE COMPLEXITY OF THE NEUROENDOCRINE AND METABOLIC SYSTEMS THAT REGULATE ENERGY INTAKE, STORAGE, AND EXPENDITURE, IT HAS BEEN DIFFICULT TO QUANTITATE ALL THE RELEVANT PARAMETERS (E.G., FOOD INTAKE AND ENERGY EXPENDITURE) OVER TIME IN HUMAN SUBJECTS.

ROLE OF GENES VERSUS ENVIRONMENT  OBESITY IS COMMONLY SEEN IN FAMILIES, AND THE HERITABILITY OF BODY WEIGHT IS SIMILAR TO THAT FOR HEIGHT. INHERITANCE IS USUALLY NOT MENDELIAN, HOWEVER, AND IT IS DIFFICULT TO DIS- TINGUISH THE ROLE OF GENES AND ENVIRONMENTAL FACTORS. ADOPTEES MORE CLOSELY RESEMBLE THEIR BIOLOGIC THAN ADOPTIVE PARENTS WITH RESPECT TO OBESITY, PROVIDING STRONG SUPPORT FOR GENETIC INFLUENCES. LIKewise, IDENTICAL TWINS HAVE VERY SIMILAR BMIS WHETHER REARED TOGETHER OR APART, AND THEIR BM IS ARE MUCH MORE STRONGLY CORRELATED THAN THOSE OF DIZYGOTIC TWINS. THESE GENETIC EFFECTS APPEAR TO RELATE TO BOTH ENERGY INTAKE AND EXPENDITURE.

WHATEVER THE ROLE OF GENES, IT IS CLEAR THAT THE ENVIRONMENT PLAYS A KEY ROLE IN OBESITY, AS EVIDENCED BY THE FACT THAT FAMINE PREVENTS OBESITY IN EVEN THE MOST OBESITY-PRONE INDIVIDUAL. IN ADDITION, THE RECENT INCREASE IN THE PREVALENCE OF OBESITY IN THE UNITED STATES IS FAR TOO RAPID TO BE DUE TO CHANGES IN THE GENE POOL. UNDOUBTedly, GENES INFLUENCE THE SUSCEPTIBILITY TO OBESITY IN RESPONSE TO SPECIFIC DIETS AND AVAILABILITY OF NUTRITION. CULTURAL FACTORS ARE ALSO IMPORTANT- THESE RELATE TO BOTH AVAILABILITY AND COMPOSITION OF THE DIET AND TO CHANGES IN THE LEVEL OF PHYSICAL ACTIVITY. IN INDUSTRIAL SOCIETIES, OBESITY IS MORE COMMON AMONG POOR WOMEN, WHEREAS IN UNDERDEVELOPED COUNTRIES, WEALTHIER WOMEN ARE MORE OFTEN OBESE. IN CHILDREN, OBESITY CORRELATES TO SOME DEGREE WITH TIME SPENT WATCHING TELEVISION. ALTHOUGH THE ROLE OF DIET COMPOSITION IN OBESITY CONTINUES TO GENERATE CONTROVERSY, IT APPEARS THAT HIGH-FAT DIETS MAY PROMOTE OBESITY, ESPECIALLY WHEN COMBINED WITH DIETS RICH IN SIMPLE (AS OPPOSED TO COMPLEX) CARBOHYDRATES. ADDITIONAL ENVIRONMENTAL FACTORS MAY CONTRIBUTE TO THE INCREASING OBESITY PREVALENCE. BOTH EPIDEMIOLOGIC CORRELATIONS AND EXPERIMENTAL DATA SUGGEST THAT SLEEP DEPRIVATION LEADS TO INCREASED OBESITY. LESS WELL.
FALLING LEPTIN LEVELS ACT THROUGH THE HYPOTHALAMUS TO INFLUENCE APPETITE, ENERGY EXPENDITURE, AND NEUROENDOCRINE FUNCTION AND THROUGH PERIPHERAL SITES TO INFLUENCE SYSTEMS SUCH AS THE IMMUNE SYSTEM.

SUPPORTED IN HUMANS ARE POTENTIAL CHANGES IN GUT FLORA WITH CAPACITY TO ALTER ENERGY BALANCE AND A POSSIBLE ROLE FOR OBESIGENIC VIRAL INFECTIONS.

SPECIFIC GENETIC SYNDROMES  FOR MANY YEARS OBESITY IN RODENTS HAS BEEN KNOWN TO BE CAUSED BY A NUMBER OF DISTINCT MUTATIONS DISTRIBUTED THROUGH THE GENOME. MOST OF THESE SINGLE-GENE MUTATIONS CAUSE BOTH HYPERPHAGIA AND DIMINISHED ENERGY EXPENDITURE, SUGGESTING A PHYSIOLOGIC LINK BETWEEN THESE TWO PARAMETERS OF ENERGY HOMEOSTASIS. IDENTIFICATION OF THE \textit{OB} GENE MUTATION IN GENETICALLY OBESE (OB/OB) MICE REPRESENTED A MAJOR BREAKTHROUGH IN THE FIELD. THE OB/OB MOUSE DEVELOPS SEVERE OBESITY, INSULIN RESISTANCE, AND HYPERPHAGIA, AS WELL AS EFFICIENT METABOLISM (E.G., IT GETS FAT EVEN WHEN INGESTING THE SAME NUMBER OF CALORIES AS LEAN LITTER MATES). THE PRODUCT OF THE \textit{OB} GENE IS THE PEPTIDE LEPTIN, A NAME DERIVED FROM THE GREEK ROOT \textit{LEPTOS}, MEANING THIN. LEPTIN IS SECRETED BY ADIPOSE CELLS AND ACTS PRIMARILY THROUGH THE HYPOTHALAMUS. ITS LEVEL OF PRODUCTION PROVIDES AN INDEX OF ADIPOSE ENERGY STORES (FIG. 74-4). HIGH LEPTIN LEVELS DECREASE FOOD INTAKE AND INCREASE ENERGY EXPENDITURE. ANOTHER MOUSE MUTANT, DB/DB, WHICH IS RESISTANT TO LEPTIN, HAS A MUTATION IN THE LEPTIN RECEPTOR AND DEVELOPS A SIMILAR SYNDROME. THE \textit{OB} GENE IS PRESENT IN HUMANS AND EXPRESSED IN FAT. SEVERAL FAMILIES WITH MORBID, EARLY-ONSET OBESITY CAUSED BY INACTIVATING MUTATIONS IN EITHER LEPTIN OR THE LEPTIN RECEPTOR HAVE BEEN DESCRIBED, THUS DEMONSTRATING THE BIOLOGIC RELEVANCE OF LEPTIN IN HUMANS. THE OBESITY IN THESE INDIVIDUALS BEGINS SHORTLY AFTER BIRTH, IS SEVERE, AND IS ACCOMPANIED BY NEUROENDOCRINE ABNORMALITIES. THE MOST PROMINENT OF THESE IS HYPOGONADOTROPIC HYPOGONADISM, WHICH IS REVERSED BY LEPTIN REPLACEMENT. CENTRAL HYPOTHYROIDISM AND GROWTH RETARDATION ARE SEEN IN THE MOUSE MODEL, BUT THEIR OCCURRENCE IN LEPTIN-DEFICIENT HUMANS IS LESS CLEAR. TO DATE, THERE IS NO EVIDENCE TO SUGGEST THAT MUTATIONS OR POLYMORPHISMS IN THE LEPTIN OR LEPTIN RECEPTOR GENES PLAY A PROMINENT ROLE IN COMMON FORMS OF OBESITY. MUTATIONS IN SEVERAL OTHER GENES CAUSE SEVERE OBESITY IN HUMANS (TABLE 74-1); EACH OF THESE SYNDROMES IS RARE. MUTATIONS IN THE GENES ENCODING PROOPIOMELANOCORTIN (POMC) CAUSE SEVERE OBESITY THROUGH...
FAILURE TO SYNTHESIZE *-MSH, A KEY NEUROPEPTIDE THAT INHIBITS APPETITE IN THE HYPOTHALAMUS. THE ABSENCE OF POMC ALSO CAUSES SECONDARY ADRENAL INSUFFICIENCY DUE TO ABSENCE OF ADRENOCORTICOTROPIC HORMONE (ACTH), AS WELL AS PALE SKIN AND RED HAIR DUE TO ABSENCE OF *-MSH. PROENZYME CONVERTASE 1 (PC-1) MUTATIONS ARE THOUGHT TO CAUSE OBESITY BY PREVENTING SYNTHESIS OF *-MSH FROM ITS PRECURSOR PEPTIDE, POMC. *-MSH BINDS TO THE TYPE 4 MELANOCORTIN RECEPTOR (MC4R), A KEY HYPOTHALAMIC RECEPTOR THAT INHIBITS EATING. HETEROZYGOUS LOSS-OF-FUNCTION MUTATIONS OF THIS RECEPTOR ACCOUNT FOR AS MUCH AS 5% OF SEVERE OBESITY. THESE FIVE GENETIC DEFECTS DEFINE A PATHWAY THROUGH WHICH LEPTIN (BY STIMULATING POMC AND INCREASING *-MSH) RESTRICTS FOOD INTAKE AND LIMITS WEIGHT (FIG. 74-5).

IN ADDITION TO THESE HUMAN OBESITY GENES, STUDIES IN RODENTS REVEAL SEVERAL OTHER MOLECULAR CANDIDATES FOR HYPOTHALAMIC MEDIATORS OF HUMAN OBESITY OR LEANNESS. THE TUB GENE ENCODES A HYPOTHALAMIC PEPTIDE OF UNKNOWN FUNCTION; MUTATION OF THIS GENE CAUSES LATE-ONSET OBESITY. THE FAT GENE ENCODES CARBOXYPEPTIDASE E, A PEPTIDE-PROCESSING ENZYME; MUTATION OF THIS GENE IS THOUGHT TO CAUSE OBESITY BY DISRUPTING PRODUCTION OF ONE OR MORE NEUROPEPTIDES. AGRP IS COEXPRESSED WITH NPY IN ARCUATE NUCLEUS NEURONS. AGRP ANTAGONIZES *-MSH ACTION AT MC4 RECEPTORS, AND ITS OVEREXPRESSION INDUCES OBESITY. IN CONTRAST, A MOUSE DEFICIENT IN THE PEPTIDE MCH, WHOSE ADMINISTRATION CAUSES FEEDING, IS LEAN.

A NUMBER OF COMPLEX HUMAN SYNDROMES WITH DEFINED INHERITANCE ARE ASSOCIATED WITH OBESITY (TABLE 74-2). ALTHOUGH SPECIFIC GENES ARE UNDEFINED AT PRESENT, THEIR IDENTIFICATION WILL LIKELY ENHANCE OUR UNDERSTANDING OF MORE COMMON FORMS OF HUMAN OBESITY. IN THE PRADER-WILLI SYNDROME, OBESITY COEXISTS WITH SHORT STATURE, MENTAL RETARDATION, HYPOGONADOTROPIC HYPOGONADISM, HYPTONIA, SMALL HANDS AND FEET, FISH-SHAPED MOUTH, AND HYPERPHAGIA. MOST PATIENTS HAVE A CHROMOSOME 15 DELETION, AND REDUCED EXPRESSION OF THE SIGNALING PROTEIN NECDIN MAY BE AN IMPORTANT CAUSE OF DEFECTIVE HYPOTHALAMIC NEURAL DEVELOPMENT IN THIS DISORDER (CHAP. 63). BARDET-BIEDL SYNDROME

| TABLE 74-1 SOME OBESITY GENES IN HUMANS AND MICE |

**GENE**

**LEP (OB)**

**LEPR (DB)**

**POMC**

**MC4R**

**AGRP**
LEPTIN, a fat-derived hormone

LEPTIN RECEPTOR
PROOPIOMELANOCORTIN, a precursor of several hormones and neuropeptides
TYPE 4 RECEPTOR FOR MSH

AGOUTI-RELATED PEPTIDE, a neuropeptide expressed in the hypothalamus
PROHORMONE CONVERTASE 1, a processing enzyme
CARBOXYPEPTIDASE E, a processing enzyme
TUB, a hypothalamic protein of unknown function
TRKB, a neurotrophin receptor

MECHANISM OF OBESITY

MUTATION PREVENTS LEPTIN FROM DELIVERING SATIETY SIGNAL; BRAIN PERCEIVES STARVATION SAME AS ABOVE
MUTATION PREVENTS SYNTHESIS OF MELANOCYTE-STIMULATING HORMONE (MSH), A SATIETY SIGNAL
MUTATION PREVENTS RECEPTION OF SATIETY SIGNAL FROM MSH
OVEREXPRESSION INHIBITS SIGNAL THROUGH MC4R

MUTATION PREVENTS SYNTHESIS OF NEUROPEPTIDE,
PROBABLY MSH
SAME AS ABOVE

HYPOTHALAMIC DYSFUNCTION

HYPERPHAGIA DUE TO
UNCHARACTERIZED
HYPOTHALAMIC DEFECT

IN HUMAN

YES

YES

YES

NO

YES

NO

NO

YES

IN RODENT

YES

YES

YES

YES

NO
FIGURE 74-5 A CENTRAL PATHWAY THROUGH WHICH LEPTIN ACTS TO REGULATE APPETITE AND BODY WEIGHT. LEPTIN SIGNALS THROUGH PRO-OPIO-MELANOCORTIN (POMC) NEURONS IN THE HYPOTHALAMUS TO INDUCE INCREASED PRODUCTION OF *-MELANOCYTE-STIMULATING HORMONE (*-MSH), REQUIRING THE PROCESSING ENZYME PC-1 (PROENZYME CONVERTASE 1). *-MSH ACTS AS AN AGONIST ON MELANOCORTIN-4 RECEPTORS TO INHIBIT APPETITE, AND THE NEUROPEPTIDE AGRP (AGOUTI-RELATED PEPTIDE) ACTS AS AN ANTAGONIST OF THIS RECEPTOR. MUTATIONS THAT CAUSE OBESITY IN HUMANS ARE INDICATED BY THE SOLID GREEN ARROWS.

(BBS) IS A GENETICALLY HETEROGENEOUS DISORDER CHARACTERIZED BY OBESITY, MENTAL RETARDATION, RETINITIS PIGMENTOSA, RENAL AND CARDIAC MALFORMATIONS, POLYDACTYLY, AND HYPOGONADOTROPIC HYPOGONADISM. AT LEAST EIGHT GENETIC LOCI HAVE BEEN IDENTIFIED, AND BBS MAY INVOLVE DEFECTS IN CILIARY FUNCTION.

OTHER SPECIFIC SYNDROMES ASSOCIATED WITH OBESITY * CUSHING’S SYNDROME ALTHOUGH OBESE PATIENTS COMMONLY HAVE CENTRAL OBESITY, HYPERTENSION, AND GLUCOSE INTOLERANCE, THEY LACK OTHER SPECIFIC STIGMATA OF CUSHING’S SYNDROME (CHAP. 336). NONETHELESS, A POTENTIAL DIAGNOSIS OF CUSHING’S SYNDROME IS OFTEN ENTERTAINED. CORTISOL PRODUCTION AND URINARY METABOLITES (170H STEROIDS) MAY BE INCREASED IN SIMPLE OBESITY. UNLIKE IN CUSHING’S SYNDROME, HOWEVER, CORTISOL LEVELS IN BLOOD AND URINE IN THE BASAL STATE AND IN RESPONSE TO CORTICOTROPIN-RELEASING HORMONE (CRH) OR ACTH ARE NORMAL; THE OVERNIGHT 1-MG DEXAMETHASONE SUPPRESSION TEST IS NORMAL IN 90%, WITH THE REMAINDER BEING NORMAL ON A STANDARD 2-DAY LOW-DOSE DEXAMETHASONE SUPPRESSION TEST. OBESITY MAY BE ASSOCIATED WITH EXCESSIVE LOCAL REACTIVATION OF CORTISOL IN FAT BY 11*-HYDROXSTEROID DEHYDROGENASE 1, AN ENZYME THAT CONVERTS INACTIVE CORTISONE TO CORTISOL.

HYPOTHYROIDISM THE POSSIBILITY OF HYPOTHYROIDISM SHOULD BE CONSIDERED, BUT IT IS AN UNCOMMON CAUSE OF OBESITY; HYPOTHYROIDISM IS EASILY RULED OUT BY MEASURING THYROID-STIMULATING HORMONE (TSH). MUCH OF THE WEIGHT GAIN THAT OCCURS IN HYPOTHYROIDISM IS DUE TO MYX-
EDEMA (CHAP. 335).

**INSULINOMA** Patients with insulinoma often gain weight as a result of overeating to avoid hypoglycemic symptoms (CHAP. 339). The increased substrate plus high insulin levels promote energy storage in fat. This can be marked in some individuals but is modest in most.

**CRANIOPHARYNGIOMA AND OTHER DISORDERS INVOLVING THE HYPOTHALAMUS** Whether through tumors, trauma, or inflammation, hypothalamic dysfunction of systems controlling satiety, hunger, and energy expenditure can cause varying degrees of obesity (CHAP. 333). It is uncommon to identify a discrete anatomic basis for these disorders. Subtle hypothalamic dysfunction is probably a more common cause of obesity than can be documented using currently available imaging techniques. Growth hormone (GH), which exerts lipolytic activity, is diminished in obesity and is increased with weight loss. Despite low GH levels, insulin-like growth factor (IGF) I (somatomedin) production is normal, suggesting that GH suppression is a compensatory response to increased nutritional supply.

**PATHOGENESIS OF COMMON OBESITY** Obesity can result from increased energy intake, decreased energy expenditure, or a combination of the two. Thus, identifying the etiology of obesity should involve measurements of both parameters. However, it is nearly impossible to perform direct and accurate measurements of energy intake in free-living individuals, and the obese, in particular, often underreport intake. Measurements of chronic energy expenditure have only recently become available using doubly labeled water or metabolic chamber/rooms. In subjects at stable weight and body composition, energy intake equals expenditure. Consequently, these techniques allow assessment of energy intake in free-living individuals. The level of energy expenditure differs in established obesity, during periods of weight gain or loss, and in the pre- or postobese state. Studies that fail to take note of this phenomenon are not easily interpreted.

**TABLE 74-2 A COMPARISON OF SYNDROMES OF OBESITY - HYPOGONADISM AND MENTAL RETARDATION**

**FEATURE**

**INHERITANCE**

**STATURE**

**OBESITY**

**CRANIOFACIES**

**LIMBS**
REPRODUCTIVE
STATUS

OTHER FEATURES

MENTAL
RETARDATION

SYNDROME

PRADER-WILLI

SPORADIC; TWO-THIRDS HAVE
DEFECT
SHORT

GENERALIZED
MODERATE TO SEVERE
ONSET 1-3 YRS
NARROW BIFRONTAL DIAMETER
ALMOND-SHAPED EYES
STRABISMUS
V-SHAPED MOUTH
HIGH-ARCHED PALATE
SMALL HANDS AND FEET
HYPOTONIA

1###0 HYPOGONADISM

ENAMEL HYPOPLASIA
HYPERPHAGIA
TEMPER TANTRUMS
NASAL SPEECH
MILD TO MODERATE

LAURENCE- MOON-B*ED*

AUTOSOMAL RECESSIVE
NORMAL; INFREQUENTLY
SHORT
GENERALIZED
EARLY ONSET, 1-2 YRS

NOT DISTINCTIVE

POLYDACTYLY
HYPOGONADISM

AH*STROM

AUTOSOMAL RECESSIVE

NORMAL; INFREQUENTLY SHORT
TRUNCAL
EARLY ONSET, 2-5 YRS

NOT DISTINCTIVE

NO ABNORMALITIES

HYPOGONADISM IN MALES BUT NOT IN FEMALES
NORMAL INTELLIGENCE

COHEN

PROBABLY AUTOSOMAL RECESSIVE
SHORT OR TALL

TRUNCAL
MID-CHILDHOOD, AGE 5

HIGH NASAL BRIDGE
ARCHED PALATE
OPEN MOUTH
SHORT PHILTRUM

HYPOTONIA
NARROW HANDS AND FEET

NORMAL GONADAL FUNCTION OR HYPOGONADOTROPHIC
HYPOGONADISM
DYSPLASTIC EARS
DELAYED PUBERTY

MILD

CARPENTER
AUTOSOMAL RECESSIVE

NORMAL

TRUNCAL, GLUTEAL

ACROCEPHALY
FLAT NASAL BRIDGE
HIGH-ARCHED PALATE

POLYDACTYLY
SYNDACTYLY
GENU VALGUM
2###0 HYPOGONADISM

SLIGHT

467 CHAPTER 74 BIOLOGY OF OBESITY


WHAT IS THE STATUS OF FOOD INTAKE IN OBESITY? (DO THE OBESE EAT MORE THAN THE LEAN?) THIS QUESTION HAS STIMULATED MUCH DEBATE, DUE IN PART TO THE METHODOLOGIC DIFFICULTIES INHERENT IN DETERMINING FOOD INTAKE. MANY OBESE INDIVIDUALS BELIEVE THAT THEY EAT SMALL QUANTITIES OF FOOD, AND THIS CLAIM HAS OFTEN BEEN SUPPORTED BY THE RESULTS OF FOOD INTAKE QUESTIONNAIRES. HOWEVER, IT IS NOW ESTABLISHED THAT AVERAGE ENERGY EXPENDITURE INCREASES AS INDIVIDUALS GET MORE OBESE, DUE PRIMARILY TO
THE FACT THAT METABOLICALLY ACTIVE LEAN TISSUE MASS INCREASES WITH OBESITY. GIVEN THE LAWS OF THERMODYNAMICS, THE OBESE PERSON MUST THEREFORE EAT MORE THAN THE AVERAGE LEAN PERSON TO MAINTAIN THEIR INCREASED WEIGHT. IT MAY BE THE CASE, HOWEVER, THAT A SUBSET OF INDIVIDUALS WHO ARE PREDISPOSED TO OBESITY HAVE THE CAPACITY TO BECOME OBESE INITIALLY WITHOUT AN ABSOLUTE INCREASE IN CALORIC CONSUMPTION.

WHAT IS THE STATE OF ENERGY EXPENDITURE IN OBESITY? THE AVERAGE TOTAL DAILY ENERGY EXPENDITURE IS HIGHER IN OBESE THAN LEAN INDIVIDUALS WHEN MEASURED AT STABLE WEIGHT. HOWEVER, ENERGY EXPENDITURE FALLS AS WEIGHT IS LOST, DUE IN PART TO LOSS OF LEAN BODY MASS AND TO DECREASED SYMPATHETIC NERVE ACTIVITY. WHEN REDUCED TO NEAR-NORMAL WEIGHT AND MAINTAINED THERE FOR A WHILE, (SOME) OBESE INDIVIDUALS HAVE LOWER ENERGY EXPENDITURE THAN (SOME) LEAN INDIVIDUALS. THERE IS ALSO A TENDENCY FOR THOSE WHO WILL DEVELOP OBESITY AS INFANTS OR CHILDREN TO HAVE LOWER RESTING ENERGY EXPENDITURE RATES THAN THOSE WHO REMAIN LEAN. THE PHYSIOLOGIC BASIS FOR VARIABLE RATES OF ENERGY EXPENDITURE (AT A GIVEN BODY WEIGHT AND LEVEL OF ENERGY INTAKE) IS ESSENTIALLY UNKNOWN.

A MUTATION IN THE HUMAN *###3-ADRENERGIC RECEPTOR MAY BE ASSOCIATED WITH INCREASED RISK OF OBESITY AND/OR INSULIN RESISTANCE IN CERTAIN (BUT NOT ALL) POPULATIONS. HOMOLOGUES OF THE BAT UNCOUPLING PROTEIN, NAMED UCP-2 AND UCP-3, HAVE BEEN IDENTIFIED IN BOTH RODENTS AND HUMANS. UCP-2 IS EXPRESSED WIDELY, WHEREAS UCP-3 IS PRIMARILY EXPRESSED IN SKELETAL MUSCLE. THESE PROTEINS MAY PLAY A ROLE IN DISORDERED ENERGY BALANCE. ONE NEWLY DESCRIBED COMPONENT OF THERMOGENESIS, CALLED NONEXERCISE ACTIVITY THERMOGENESIS (NEAT), HAS BEEN LINKED TO OBESITY. IT IS THE THERMOGENESIS THAT ACCOMPANIES PHYSICAL ACTIVITIES OTHER THAN VOLITIONAL EXERCISE, SUCH AS THE ACTIVITIES OF DAILY LIVING, FIDGETING, SPONTANEOUS MUSCLE CONTRACTION, AND MAINTAINING POSTURE. NEAT ACCOUNTS FOR ABOUT TWO-THIRDS OF THE INCREASED DAILY ENERGY EXPENDITURE INDUCED BY OVERFEEDING. THE WIDE VARIATION IN FAT STORAGE SEEN IN OVERFED INDIVIDUALS IS PREDICTED BY THE DEGREE TO WHICH NEAT IS INDUCED. THE MOLECULAR BASIS FOR NEAT AND ITS REGULATION IS UNKNOWN.

LEPTIN IN TYPICAL OBESITY THE VAST MAJORITY OF OBESE PERSONS HAVE INCREASED LEPTIN LEVELS BUT DO NOT HAVE MUTATIONS OF EITHER LEPTIN OR ITS RECEPTOR. THEY APPEAR, THEREFORE, TO HAVE A FORM OF FUNCTIONAL “LEPTIN RESISTANCE.” DATA SUGGESTING THAT SOME INDIVIDUALS PRODUCE LESS LEPTIN PER UNIT FAT MASS THAN OTHERS OR HAVE A FORM OF RELATIVE LEPTIN DEFICIENCY THAT PREDISPOSES TO OBESITY ARE AT PRESENT CONTRADICTORY AND UNSETTLED. THE MECHANISM FOR LEPTIN RESISTANCE, AND WHETHER IT CAN BE OVERCOME BY RAISING LEPTIN LEVELS, IS NOT YET ESTABLISHED. SOME DATA SUGGEST THAT
LEPTIN MAY NOT EFFECTIVELY CROSS THE BLOOD-BRAIN BARRIER AS LEVELS RISE. IT IS ALSO APPARENT FROM ANIMAL STUDIES THAT LEPTIN SIGNALING INHIBITORS, SUCH AS SOCS3 AND PTP1B, ARE INVOLVED IN THE LEPTIN-RESISTANT STATE.

PATHOLOGIC CONSEQUENCES OF OBESITY

(SEE ALSO CHAP. 75) OBESITY HAS MAJOR ADVERSE EFFECTS ON HEALTH. OBESITY IS ASSOCIATED WITH AN INCREASE IN MORTALITY, WITH A 50-100% INCREASED RISK OF DEATH FROM ALL CAUSES COMPARED TO NORMAL-WEIGHT INDIVIDUALS, MOSTLY DUE TO CARDIOVASCULAR CAUSES. OBESITY AND OVERWEIGHT TOGETHER ARE THE SECOND LEADING CAUSE OF PREVENTABLE DEATH IN THE UNITED STATES, ACCOUNTING FOR 300,000 DEATHS PER YEAR. MORTALITY RATES RISE AS OBESITY INCREASES, PARTICULARLY WHEN OBESITY IS ASSOCIATED WITH INCREASED INTRABDOMINAL FAT (SEE ABOVE). LIFE EXPECTANCY OF A MODERATELY OBESE INDIVIDUAL COULD BE SHORTENED BY 2-5 YEARS, AND A 20- TO 30-YEAR-OLD MALE WITH A BMI > 45 MAY LOSE 13 YEARS OF LIFE. IT IS ALSO APPARENT THAT THE DEGREE TO WHICH OBESITY AFFECTS PARTICULAR ORGAN SYSTEMS IS INFLUENCED BY SUSCEPTIBILITY GENES THAT VARY IN THE POPULATION.

INSULIN RESISTANCE AND TYPE 2 DIABETES MELLITUS HYPERINSULINEMIA AND INSULIN RESISTANCE ARE PERVASIVE FEATURES OF OBESITY, INCREASING WITH WEIGHT GAIN AND DIMINISHING WITH WEIGHT LOSS (CHAP. 236). INSULIN RESISTANCE IS MORE STRONGLY LINKED TO INTRABDOMINAL FAT THAN TO FAT IN OTHER DEPOTS. THE MOLECULAR LINK BETWEEN OBESITY AND INSULIN RESISTANCE IN TISSUES SUCH AS FAT, MUSCLE, AND LIVER HAS BEEN SOUGHT FOR MANY YEARS. MAJOR FACTORS UNDER INVESTIGATION INCLUDE: (1) INSULIN ITSELF, BY INDUCING RECEPTOR DOWNREGULATION; (2) FREE FATTY ACIDS, KNOWN TO BE INCREASED AND CAPABLE OF IMPAIRING INSULIN ACTION; (3) INTRACELLULAR LIPID ACCUMULATION; AND (4) VARIOUS CIRCULATING PEPTIDES PRODUCED BY ADIPOCYTES, INCLUDING THE CYTOKINES TNF-*, IL-6, RBP4, AND THE “ADIPOKINES” ADIPOnectin AND Resistin, WHICH ARE PRODUCED BY ADIPOCYTES, HAVE ALTERED EXPRESSION IN OBESE ADIPOCYTES, AND ARE CAPABLE OF MODIFYING INSULIN ACTION. DESPITE NEARLY UNIVERSAL INSULIN RESISTANCE, MOST OBESE INDIVIDUALS DO NOT DEVELOP DIABETES, SUGGESTING THAT THE ONSET OF DIABETES REQUIRES AN INTERACTION BETWEEN OBESITY-INDUCED INSULIN RESISTANCE AND OTHER FACTORS THAT PREDISPOSE TO DIABETES, SUCH AS IMPAIRED INSULIN SECRETION (CHAP. 338). OBESITY, HOWEVER, IS A MAJOR RISK FACTOR FOR DIABETES, AND AS MANY AS 80% OF PATIENTS WITH TYPE 2 DIABETES MELLITUS ARE OBESE. WEIGHT LOSS AND EXERCISE, EVEN OF MODEST DEGREE, ARE ASSOCIATED WITH INCREASED INSULIN SENSITIVITY AND OFTEN IMPROVE GLUCOSE CONTROL IN DIABETES.

REPRODUCTIVE DISORDERS DISORDERS THAT AFFECT THE REPRODUCTIVE AXIS ARE
ASSOCIATED WITH OBESITY IN BOTH MEN AND WOMEN. MALE HYPOGONADISM IS ASSOCIATED WITH INCREASED ADIPOSE TISSUE, OFTEN DISTRIBUTED IN A PATTERN MORE TYPICAL OF FEMALES. IN MEN >160% IDEAL BODY WEIGHT, PLASMA TESTOSTERONE AND SEX HORMONE-BINDING GLOBULIN (SHBG) ARE OFTEN REDUCED, AND ESTROGEN LEVELS (DERIVED FROM CONVERSION OF ADRENAL ANDROGENS IN ADIPOSE TISSUE) ARE INCREASED (CHAP. 340). GYNECOMASTIA MAY BE SEEN. HOWEVER, MASCULINIZATION, LIBIDO, POTENCY, AND SPERMATOGENESIS ARE PRE-SERVED IN MOST OF THESE INDIVIDUALS. FREE TESTOSTERONE MAY BE DECREASED IN MORBIDLY OBESE MEN WHOSE WEIGHT IS >200% IDEAL BODY WEIGHT. OBESITY HAS LONG BEEN ASSOCIATED WITH MENSTRUAL ABNORMALITIES IN WOMEN, PARTICULARLY IN WOMEN WITH UPPER BODY OBESITY (CHAP. 341). COMMON FINDINGS ARE INCREASED ANDROGEN PRODUCTION, DECREASED SHBG, AND INCREASED PERIPHERAL CONVERSION OF ANDROGEN TO ESTROGEN. MOST OBESE WOMEN WITH OLIGOMENORRHEA HAVE THE POLYCYSTIC OVARIAN SYNDROME (PCOS), WITH ITS ASSOCIATED ANOVULATION AND OVARIAN HYPER-ANDROGENISM; 40% OF WOMEN WITH PCOS ARE OBESE. MOST NONOBESE WOMEN WITH PCOS ARE ALSO INSULIN-RESISTANT, SUGGESTING THAT INSULIN RESISTANCE, HYPERINSULINEMIA, OR THE COMBINATION OF THE TWO ARE CAUSATIVE OR CONTRIBUTE TO THE OVARIAN PATHOPHYSIOLOGY IN PCOS IN BOTH OBESE AND LEAN INDIVIDUALS. IN OBESE WOMEN WITH PCOS, WEIGHT LOSS OR TREATMENT WITH INSULIN-SENSITIZING DRUGS OFTEN RESTORES NORMAL MENSES. THE INCREASED CONVERSION OF ANDROSTENEDIONE TO ESTROGEN, WHICH OCCURS TO A GREATER DEGREE IN WOMEN WITH LOWER BODY OBESITY, MAY CONTRIBUTE TO THE INCREASED INCIDENCE OF UTERINE CANCER IN POSTMENOPAUSAL WOMEN WITH OBESITY.

CARDIOVASCULAR DISEASE THE FRAMINGHAM STUDY REVEALED THAT OBESITY WAS AN INDEPENDENT RISK FACTOR FOR THE 26-YEAR INCIDENCE OF CARDIOVASCULAR DISEASE IN MEN AND WOMEN [INCLUDING CORONARY DISEASE, STROKE, AND CONGESTIVE HEART FAILURE (CHF)]. THE WAIST/HIP RATIO MAY BE THE BEST PREDICTOR OF THESE RISKS. WHEN THE ADDITIONAL EFFECTS OF HYPERTENSION AND Glucose INTOLERANCE ASSOCIATED WITH OBESITY ARE INCLUDED, THE ADVERSE IMPACT OF OBESITY IS EVEN MORE EVIDENT. THE EFFECT OF OBESITY ON CARDIOVASCULAR MORTALITY IN WOMEN MAY BE SEEN AT BMIS AS LOW AS 25. OBESITY, ESPECIALLY ABDOMINAL OBESITY, IS ASSOCIATED WITH AN ATEROGENIC LIPID PROFILE: WITH INCREASED LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL, VERY LOW DENSITY LIPOPROTEIN, AND TRIGLYCERIDE; AND WITH DECREASED HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND DECREASED LEVELS OF

PAGE NO. 111

468 PART 5: NUTRITION

ON CARDIOVASCULAR MORTALITY IN WOMEN MAY BE SEEN AT BMIS AS LOW AS 25. OBESITY, ESPECIALLY ABDOMINAL OBESITY, IS ASSOCIATED WITH AN ATEROGENIC LIPID PROFILE: WITH INCREASED LOW-DENSITY LIPOPROTEIN (LDL) CHOLESTEROL, VERY LOW DENSITY LIPOPROTEIN, AND TRIGLYCERIDE; AND WITH DECREASED HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND DECREASED LEVELS OF
THE VASCULAR PROTECTIVE ADIPOKINE ADIPONECTIN (CHAP. 350). OBESITY IS ALSO ASSOCIATED WITH HYPERTENSION. MEASUREMENT OF BLOOD PRESSURE IN THE OBESE REQUIRES USE OF A LARGER CUFF SIZE TO AVOID ARTIFACTUAL INCREASES.

OBESITY-INDUCED HYPERTENSION IS ASSOCIATED WITH INCREASED PERIPHERAL RESISTANCE AND CARDIAC OUTPUT, INCREASED SYMPATHETIC NERVOUS SYSTEM TONE, INCREASED SALT SENSITIVITY, AND INSULIN-MEDIATED SALT RETENTION; IT IS OFTEN RESPONSIVE TO MODEST WEIGHT LOSS.

PULMONARY DISEASE OBESITY MAY BE ASSOCIATED WITH A NUMBER OF PULMONARY ABNORMALITIES. THESE INCLUDE REDUCED CHEST WALL COMPLIANCE, INCREASED WORK OF BREATHING, INCREASED MINUTE VENTILATION DUE TO INCREASED METABOLIC RATE, AND DECREASED FUNCTIONAL RESIDUAL CAPACITY AND EXPIRATORY RESERVE VOLUME (CHAP. 246). SEVERE OBESITY MAY BE ASSOCIATED WITH OBSTRUCTIVE SLEEP APNEA AND THE “OBESITY HYPVENTILATION SYNDROME” WITH ATTENUATED HYPOXIC AND HYPERCAPNIC VENTILATORY RESPONSES (CHAP. 258). SLEEP APNEA CAN BE OBSTRUCTIVE (MOST COMMON), CENTRAL, OR MIXED AND IS ASSOCIATED WITH HYPERTENSION. WEIGHT LOSS (10-20 KG) CAN BRING SUBSTANTIAL IMPROVEMENT, AS CAN MAJOR WEIGHT LOSS FOLLOWING GASTRIC BYPASS OR RESTRICTIVE SURGERY. CONTINUOUS POSITIVE AIRWAY PRESSURE HAS BEEN USED WITH SOME SUCCESS.

GALLSTONES OBESITY IS ASSOCIATED WITH ENHANCED BILIARY SECRETION OF CHOLESTEROL, SUPERSATURATION OF BILE, AND A HIGHER INCIDENCE OF GALLSTONES, PARTICULARLY CHOLESTEROL GALLSTONES (CHAP. 305). A PERSON 50% ABOVE IDEAL BODY WEIGHT HAS ABOUT A SIXFOLD INCREASED INCIDENCE OF SYMPTOMATIC GALLSTONES. PARADOXICALLY, FASTING INCREASES SUPERSATURATION OF BILE BY DECREASING THE PHOSPHOLIPID COMPONENT. FASTING-INDUCED CHOLECYSTITIS IS A COMPLICATION OF EXTREME DIETS.

CANCER OBESITY IN MALES IS ASSOCIATED WITH HIGHER MORTALITY FROM CANCER, INCLUDING CANCER OF THE ESOPHAGUS, COLON, RECTUM, PANCREAS,

75 EVALUATION AND MANAGEMENT OF OBESITY ROBERT F. KUSHNER

OVER 66% OF U.S. ADULTS ARE CURRENTLY CATEGORIZED AS OVERWEIGHT OR OBESE, AND THE PREVALENCE OF OBESITY IS INCREASING RAPIDLY THROUGHOUT MOST OF THE INDUSTRIALIZED WORLD. BASED ON STATISTICS FROM THE WORLD HEALTH ORGANIZATION, OVERWEIGHT AND OBESITY MAY SOON REPLACE MORE TRADITIONAL PUBLIC HEALTH CONCERNS SUCH AS UNDERNUTRITION AND INFECTIOUS DISEASES AS THE MOST SIGNIFICANT CONTRIBUTORS TO ILL HEALTH. CHILDREN AND ADOLESCENTS ARE ALSO BECOMING MORE OBESE, INDICATING THAT THE CURRENT TRENDS WILL ACCELERATE OVER TIME. OBESITY IS ASSOCIATED WITH AN INCREASED RISK OF MULTIPLE HEALTH PROBLEMS, INCLUDING HYPERTENSION, TYPE 2 DIABETES, DYSLIPIDEMIA, DEGENERATIVE JOINT DISEASE, AND SOME MALIGNANCIES. THUS, IT IS IMPORTANT FOR PHYSICIANS TO ROUTINELY IDENTIFY, EVALUATE, AND
TREAT PATIENTS FOR OBESITY AND ASSOCIATED COMORBID CONDITIONS.

EVALUATION

THE U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDS THAT PHYSICIANS SCREEN ALL ADULT PATIENTS FOR OBESITY AND OFFER INTENSIVE COUNSELING AND BEHAVIORAL INTERVENTIONS TO PROMOTE SUSTAINED WEIGHT LOSS. THIS RECOMMENDATION IS CONSISTENT WITH PREVIOUSLY RELEASED GUIDELINES FROM THE NATIONAL HEART, LUNG, AND BLOOD INSTITUTE (NHLBI) AND A NUMBER OF MEDICAL SOCIETIES. THE FIVE MAIN STEPS IN THE EVALUATION OF OBESITY ARE DESCRIBED BELOW AND INCLUDE (1) FOCUSED OBESITY-RELATED HISTORY, (2) PHYSICAL EXAMINATION TO DETERMINE THE DEGREE AND TYPE OF OBESITY, LIVER, AND PROSTATE; OBESITY IN FEMALES IS ASSOCIATED WITH HIGHER MORTALITY FROM CANCER OF THE GALLBLADDER, BILE DUCTS, BREASTS, ENDOMETRIUM, CERVIX, AND OVARIES. SOME OF THE LATTER MAY BE DUE TO INCREASED RATES OF CONVERSION OF ANDROSTENEDIONE TO ESTRONE IN ADIPOSE TISSUE OF OBESE INDIVIDUALS. IT WAS RECENTLY ESTIMATED THAT OBESITY ACCOUNTS FOR 14% OF CANCER DEATHS IN MEN AND 20% IN WOMEN IN THE UNITED STATES.

BONE, JOINT, AND CUTANEOUS DISEASE OBESITY IS ASSOCIATED WITH AN INCREASED RISK OF OSTEOARTHRITIS, NO DOUBT PARTLY DUE TO THE TRAUMA OF ADDED WEIGHT BEARING AND JOINT MALALIGNMENT. THE PREVALENCE OF GOUT MAY ALSO BE INCREASED (CHAP. 327). AMONG THE SKIN PROBLEMS ASSOCIATED WITH OBESITY IS ACANTHOSIS NIGRICANS, MANIFESTED BY DARKENING AND THICKENING OF THE SKIN FOLDS ON THE NECK, ELBOWS, AND DORSAL INTERPHALANGEAL SPACES. ACANTHOSIS REFLECTS THE SEVERITY OF UNDERLYING INSULIN RESISTANCE AND DIMINISHES WITH WEIGHT LOSS. FRIABILITY OF SKIN MAY BE INCREASED, ESPECIALLY IN SKIN FOLDS, ENHANCING THE RISK OF FUNGAL AND YEAST INFECTIONS. FINALLY, VENOUS STASIS IS INCREASED IN THE OBESE.

FURTHER READINGS

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COMORBID CONDITIONS, (4) FITNESS LEVEL, AND (5) THE PATIENT'S READINESS TO ADOPT LIFESTYLE CHANGES.

THE OBESITY-FOCUSED HISTORY INFORMATION FROM THE HISTORY SHOULD AD-
DRESS THE FOLLOWING SIX QUESTIONS:

- WHAT FACTORS CONTRIBUTE TO THE PATIENT’S OBESITY?
- HOW IS THE OBESITY AFFECTING THE PATIENT’S HEALTH?
- WHAT IS THE PATIENT’S LEVEL OF RISK FROM OBESITY?
- WHAT ARE THE PATIENT’S GOALS AND EXPECTATIONS?
- IS THE PATIENT MOTIVATED TO BEGIN A WEIGHT MANAGEMENT PROGRAM?
- WHAT KIND OF HELP DOES THE PATIENT NEED?

ALTHOUGH THE VAST MAJORITY OF OBESITY CAN BE ATTRIBUTED TO BEHAVIORAL FEATURES THAT AFFECT DIET AND PHYSICAL ACTIVITY PATTERNS, THE HISTORY MAY SUGGEST SECONDARY CAUSES THAT MERIT FURTHER EVALUATION. DISORDERS TO CONSIDER INCLUDE POLYCYSTIC OVARIAN SYNDROME, HYPOTHYROIDISM, CUSHING’S SYNDROME, AND HYPOTHALAMIC DISEASE. DRUG-INDUCED WEIGHT GAIN SHOULD ALSO TO BE CONSIDERED. COMMON CAUSES INCLUDE ANTIDIABETES AGENTS (INSULIN, SULFONYLUREAS, THIAZOLIDINEDIONES); STEROID HORMONES; PSYCHOTROPIC AGENTS; MOOD STABILIZERS (LITHIUM); ANTIDEPRESSANTS (TRICYCLICS, MONOAMINE OXIDASE INHIBITORS, PARAXETINE, MIRTAZAPINE); AND ANTIEPILEPTIC DRUGS (VALPROATE, GABAPENTIN, CARBAMAZAPINE). OTHER MEDICATIONS SUCH AS NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND CALCIUM-CHANNEL BLOCKERS MAY CAUSE PERIPHERAL EDEMA, BUT THEY DO NOT INCREASE BODY FAT.

THE PATIENT’S CURRENT DIET AND PHYSICAL ACTIVITY PATTERNS MAY REVEAL FACTORS THAT CONTRIBUTE TO THE DEVELOPMENT OF OBESITY IN ADDITION TO IDENTIFYING BEHAVIORS TO TARGET FOR TREATMENT. THIS TYPE OF HISTORICAL INFORMATION IS BEST OBTAINED BY USING A QUESTIONNAIRE IN COMBINATION WITH AN INTERVIEW.
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BMI

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183
BMI AND WAIST CIRCUMFERENCE  THREE KEY ANTHROPOMETRIC MEASUREMENTS ARE IMPORTANT TO EVALUATE THE DEGREE OF OBESITY—WEIGHT, HEIGHT, AND WAIST CIRCUMFERENCE. THE BODY MASS INDEX (BMI), CALCULATED AS WEIGHT (KG)/HEIGHT (M)², OR AS WEIGHT (LBS)/HEIGHT (INCHES)² X 703, IS USED TO CLASSIFY WEIGHT STATUS AND RISK OF DISEASE. (TABLES 75-1 AND 75-2). BMI IS USED SINCE IT PROVIDES AN ESTIMATE OF BODY FAT AND IS RELATED TO RISK OF DISEASE. LOWER BMI THRESHOLDS FOR OVERWEIGHT AND OBESITY HAVE BEEN PROPOSED FOR THE ASIA-PACIFIC REGION SINCE THIS POPULATION APPEARS TO BE AT-RISK AT LOWER BODY WEIGHTS FOR GLUCOSE AND LIPID ABNORMALITIES. EXCESS ABDOMINAL FAT, ASSESSED BY MEASUREMENT OF WAIST CIRCUMFERENCE OR WAIST-TO-HIP RATIO, IS INDEPENDENTLY ASSOCIATED WITH HIGHER RISK

TABLE 75-2 CLASSIFICATION OF WEIGHT STATUS AND RISK OF DISEASE

UNDERWEIGHT
HEALTHY WEIGHT
OVERWEIGHT
OBESITY
OBESITY
EXTREME OBESITY

BMI (KG/M²)

<18.5
18.5-24.9
25.0-29.9
30.0-34.9
35.0-39.9
*40

OBESITY CLASS

I
II
III

RISK OF DISEASE

INCREASED
HIGH
VERY HIGH
EXTREMELY HIGH


FOR DIABETES MELLITUS AND CARDIOVASCULAR DISEASE. MEASUREMENT OF THE WAIST CIRCUMFERENCE IS A SURROGATE FOR VISCERAL ADIPOSE TISSUE AND SHOULD BE PERFORMED IN THE HORIZONTAL PLANE ABOVE THE ILIAC CREST. CUT POINTS THAT DEFINE HIGHER RISK FOR MEN AND WOMEN BASED ON ETHNICITY HAVE BEEN PROPOSED BY THE INTERNATIONAL DIABETES FEDERATION (*TABLE 75-3*).

**TABLE 75-3** ETHNIC-SPECIFIC VALUES FOR WAIST CIRCUMFERENCE

**ETHNIC GROUP**

EUROPEANS
- MEN
- WOMEN

SOUTH ASIANS AND CHINESE
- MEN
- WOMEN

JAPANESE
- MEN
- WOMEN

ETHNIC SOUTH AND CENTRAL AMERICANS

SUB-SAHARAN AFRICANS

EASTERN MEDITERRANEAN AND MIDDLE EAST (ARAB) POPULATIONS

**WAIST CIRCUMFERENCE**

>94CM (37 IN)
>80 CM (31.5 IN)

>90 CM (35 IN)
>80CM (31.5 IN)

>85 CM (33.5 IN)
>90CM (35 IN)

USE SOUTH ASIAN RECOMMENDATIONS UNTIL MORE SPECIFIC DATA ARE AVAILABLE.

USE EUROPEAN DATA UNTIL MORE
SPECIFIC DATA ARE AVAILABLE.

USE EUROPEAN DATA UNTIL MORE SPECIFIC DATA ARE AVAILABLE.


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PAGE NO. 113

**470 PART 5: NUTRITION**

**PHYSICAL FITNESS** SEVERAL PROSPECTIVE STUDIES HAVE DEMONSTRATED THAT PHYSICAL FITNESS, REPORTED BY QUESTIONNAIRE OR MEASURED BY A MAXIMAL TREADMILL EXERCISE TEST, IS AN IMPORTANT PREDICTOR OF ALL-CAUSE MORTALITY INDEPENDENT OF BMI AND BODY COMPOSITION. THESE OBSERVATIONS HIGHLIGHT THE IMPORTANCE OF TAKING AN EXERCISE HISTORY DURING EXAMINATION AS WELL AS EMPHASIZING PHYSICAL ACTIVITY AS A TREATMENT APPROACH.

**OBESITY-ASSOCIATED COMORBID CONDITIONS** THE EVALUATION OF COMORBID CONDITIONS SHOULD BE BASED ON PRESENTATION OF SYMPTOMS, RISK FACTORS, AND INDEX OF SUSPICION. ALL PATIENTS SHOULD HAVE A FASTING LIPID PANEL (TOTAL, LDL, AND HDL CHOLESTEROL AND TRIGLYCERIDE LEVELS) AND BLOOD GLUCOSE MEASURED AT PRESENTATION ALONG WITH BLOOD PRESSURE DETERMINATION. SYMPTOMS AND DISEASES THAT ARE DIRECTLY OR INDIRECTLY RELATED TO OBESITY ARE LISTED IN TABLE 75-4. ALTHOUGH INDIVIDUALS VARY, THE NUMBER AND SEVERITY OF ORGAN-SPECIFIC COMORBID CONDITIONS USUALLY RISE WITH INCREASING LEVELS OF OBESITY. PATIENTS AT VERY HIGH ABSOLUTE RISK INCLUDE THE FOLLOWING: ESTABLISHED CORONARY HEART DISEASE; PRESENCE OF OTHER ATHEROSCLEROTIC DISEASES SUCH AS PERIPHERAL ARTERIAL DISEASE, ABDOMINAL AORTIC ANEURYSM, AND SYMPTOMATIC CAROTID ARTERY DISEASE; TYPE 2 DIABETES; AND SLEEP APNEA.

**ASSESSING THE PATIENT’S READINESS TO CHANGE** AN ATTEMPT TO INITIATE LIFESTYLE CHANGES WHEN THE PATIENT IS NOT READY USUALLY LEADS TO FRUSTRATION AND MAY HAMPER FUTURE WEIGHT-LOSS EFFORTS. ASSESSMENT INCLUDES PATIENT MOTIVATION AND SUPPORT, STRESSFUL LIFE EVENTS, PSYCHIATRIC STATUS, TIME AVAILABILITY AND CONSTRAINTS, AND APPROPRIATENESS OF GOALS AND EX-
PECTATIONS. READINESS CAN BE VIEWED AS THE BALANCE OF TWO OPPOSING FORCES: (1) MOTIVATION, OR THE PATIENT’S DESIRE TO CHANGE; AND (2) RESISTANCE, OR THE PATIENT’S RESISTANCE TO CHANGE. A HELPFUL METHOD TO BEGIN A READINESS ASSESSMENT IS TO “ANCHOR” THE PATIENT’S INTEREST AND CONFIDENCE TO CHANGE ON A NUMERICAL SCALE. USING THIS TECHNIQUE, THE PATIENT IS ASKED TO RATE HIS OR HER LEVEL OF INTEREST AND CONFIDENCE ON A SCALE FROM 0 TO 10, WITH 0 BEING NOT SO IMPORTANT (OR CONFIDENT) AND 10 BEING VERY IMPORTANT (OR CONFIDENT) TO LOSE WEIGHT AT THIS TIME. THIS EXERCISE HELPS TO ESTABLISH READINESS TO CHANGE AND ALSO SERVES AS A BASIS FOR FURTHER DIALOGUE.

OBESITY

THE GOAL OF THERAPY THE PRIMARY GOAL OF TREATMENT IS TO IMPROVE OBESITY-RELATED COMORBID CONDITIONS AND REDUCE THE RISK OF DEVELOPING FUTURE COMORBIDITIES. INFORMATION OBTAINED FROM THE HISTORY, PHYSICAL EXAMINATION, AND DIAGNOSTIC TESTS IS USED TO DETERMINE RISK AND DEVELOP A TREATMENT PLAN (FIG. 75-1). THE DECISION OF HOW AGGRESSIVELY TO TREAT THE PATIENT, AND WHICH MODALITIES TO USE, IS DETERMINED BY THE PATIENT’S RISK STATUS, EXPECTATIONS, AND AVAILABLE RESOURCES. THERAPY FOR OBESITY ALWAYS BEGINS WITH LIFESTYLE MANAGEMENT AND MAY INCLUDE PHARMACOTHERAPY OR SURGERY, DEPENDING ON BMI RISK CATEGORY (TABLE 75-5). SETTING AN INITIAL WEIGHT-LOSS GOAL OF 10% OVER 6 MONTHS IS A REALISTIC TARGET.

LIFESTYLE MANAGEMENT OBESITY CARE INVOLVES ATTENTION TO THREE ESSENTIAL ELEMENTS OF LIFE STYLE: DIETARY HABITS, PHYSICAL ACTIVITY, AND BEHAVIOR MODIFICATION. BECAUSE OBESITY IS FUNDAMENTALLY A DISEASE OF ENERGY IMBALANCE, ALL PATIENTS MUST LEARN HOW AND WHEN ENERGY IS CONSUMED (DIET), HOW AND WHEN ENERGY IS EXPENDED (PHYSICAL ACTIVITY), AND HOW TO INCORPORATE THIS INFORMATION INTO THEIR DAILY LIFE (BEHAVIOR THERAPY). LIFESTYLE MANAGEMENT HAS BEEN SHOWN TO RESULT IN A MODEST (TYPICALLY 3-5 KG) WEIGHT LOSS COMPARED TO NO TREATMENT OR USUAL CARE.

DIET THERAPY THE PRIMARY FOCUS OF DIET THERAPY IS TO REDUCE OVERALL CALORIE CONSUMPTION. THE NHLBI GUIDELINES RECOMMEND INITIATING TREATMENT WITH A CALORIE DEFICIT OF 500-1000 KCAL/D COMPARED TO THE PATIENT’S HABITUAL DIET. THIS REDUCTION IS CONSISTENT WITH A GOAL OF LOSING APPROXIMATELY 1-2 LB PER WEEK. THIS CALORIE DEFICIT CAN BE ACCOMPLISHED BY SUGGESTING SUBSTITUTIONS OR ALTERNATIVES TO THE DIET. EXAMPLES INCLUDE CHOOSING SMALLER PORTIONS, EATING MORE FRUITS AND VEGETABLES, CONSUMING MORE WHOLE-GRAIN CEREALS, SELECTING LEANER CUTS OF MEAT AND SKIMMED DAIRY PRODUCTS, REDUCING FRIED FOODS AND OTHER ADDED FATS AND OILS, AND DRINKING WATER INSTEAD OF CALORIC BEVERAGES. IT IS IMPORTANT THAT THE DIETARY COUNSELING REMAINS PATIENT-CENTERED AND THAT THE GOALS ARE PRACTICAL, REALISTIC, AND ACHIEVABLE.

The macronutrient composition of the diet will vary depending on the patient’s preference and medical condition. The 2005 U.S. Department of

TABLE 75-4 OBESITY-RELATED ORGAN SYSTEMS REVIEW

CARDIOVASCULAR
HYPERTENSION
CONGESTIVE HEART FAILURE
COR PULMONALE
VARICOSE VEINS
PULMONARY EMBOLISM
CORONARY ARTERY DISEASE

ENDOCRINE

METABOLIC SYNDROME
TYPE 2 DIABETES
DYSLIPIDEMIA
POLYCYSTIC OVARIAN SYNDROME

MUSCULOSKELETAL

HYPERURICEMIA AND GOUT
IMMOBILITY
OSTEOARTHRITIS (KNEES AND HIPS)
LOW BACK PAIN
CARPAL TUNNEL SYNDROME

PSYCHOLOGICAL

DEPRESSION/LOW SELF-ESTEEM
BODY IMAGE DISTURBANCE
SOCIAL STIGMATIZATION

INTEGUMENT

STRIAE DISTENSAE
STASIS PIGMENTATION OF LEGS
LYMPHEDEMA
CELLULITIS
INTERTRIGO, CARBUNCLES
ACANTHOSIS NIGRICANS
ACROCHORDON (SKIN TAGS)
HIDRADENITIS SUPPURATIVA

RESPIRATORY

DYSPNEA
OBSTRUCTIVE SLEEP APNEA
HYPOVENTILATION SYNDROME
PICKWICKIAN SYNDROME
ASTHMA

GASTROINTESTINAL

GASTROESOPHAGEAL REFLUX DISEASE
NONALCOHOLIC FATTY LIVER DISEASE
CHOLELITHIASIS
HERNIAS
COLON CANCER
Agriculture dietary guidelines for Americans (Chap. 70), which focus on health promotion and risk reduction, can be applied to treatment of the overweight or obese patient. The recommendations include maintaining a diet rich in whole grains, fruits, vegetables, and dietary fiber, consuming two servings (8 oz) of fish high in omega 3 fatty acids per week; decreasing sodium to <2300 mg/d; consuming 3 cups of milk (or equivalent low-fat or fat-free dairy products) per day; limiting cholesterol to <300 mg/d, and keeping total fat between 20 and 35% of daily calories and saturated fats to <10% of daily calories. Application of these guidelines to specific calorie goals can be found on the website www.mypyramid.gov. The revised dietary reference intakes for macronutrients released by the Institute of Medicine recommends 45-65% of calories from carbohydrates, 20-35% from fat, and 10-35% from protein. The guidelines also recommend daily fiber intake of 38 g (men) and 25 g (women) for persons over 50 years of age and 30 g (men) and 21 g (women) for those under 50.

Since portion control is one of the most difficult strategies for patients to manage, the use of pre-prepared products, such as meal replacements, is a simple and convenient suggestion. Examples include frozen entrees, canned beverages and bars. Use of meal replacements in the diet has been shown to result in a 7-8% weight loss.

A current area of controversy is the use of low-carbohydrate, high-protein diets for weight loss. These diets are based on the concept that carbohydrates are the primary cause of obesity and lead to insulin resistance most low-carbohydrate diets (e.g., South Beach, Zone, and Sugar Busters!) recommend a carbohydrate level of approximately 40-46% of energy. The Atkins diet contains 5-15% carbohydrate, depending on the phase of the diet. Several randomized, controlled trials of these low-carbohydrate diets have demonstrated greater weight loss at 6 months with improvement in coronary heart disease risk factors, including an increase in HDL cholesterol and a decrease in triglyceride levels. Weight loss between groups did not remain statistically significant at 1 year; however, low-carbohydrate diets appear to be at least as effective as low-fat diets in inducing weight loss for up to 1 year.

Another dietary approach to consider is the concept of energy density,
WHICH REFERS TO THE NUMBER OF CALORIES (ENERGY) A FOOD CONTAINS PER UNIT OF WEIGHT. PEOPLE TEND TO INGEST A CONSTANT VOLUME OF FOOD, REGARDLESS OF CAL- LORIC OR MACRONUTRIENT CONTENT. ADDING WATER OR FIBER TO A FOOD DECREASES ITS ENERGY DENSITY BY INCREASING WEIGHT WITHOUT AFFECTING CALORIC CONTENT. EXAMPLES OF FOODS WITH LOW-ENERGY DENSITY INCLUDE SOUPS, FRUITS, VEGETA- BLES, OATMEAL, AND LEAN MEATS. DRY FOODS AND HIGH-FAT FOODS SUCH AS PRET-

471 CHAPTER 75 EVALUATION AND MANAGEMENT OF OBESITY

FIGURE 75-1 TREATMENT ALGORITHM. THIS ALGORITHM APPLIES ONLY TO THE ASSESSMENT FOR OVERWEIGHT AND OBESITY AND SUBSEQUENT DECISIONS ON THAT ASSESSMENT. IT DOES NOT REFLECT ANY INITIAL OVERALL ASSESSMENT FOR OTHER CONDITIONS THAT THE PHYSICIAN MAY WISH TO PERFORM. HT, HEIGHT; HX, HISTORY; WT, WEIGHT. (FROM NOTIONAL, HEART, LUNG, AND BLOOD INSTITUTE: CLINICAL GUIDELINES ON THE IDENTIFICATION, EVALUATION, AND TREATMENT OF OVERWEIGHT AND OBESITY IN ADULTS: THE EVIDENCE REPORT. WASHINGTON, DC, US DEPARTMENT OF HEALTH AND HUMAN SERVICES, 1998.)

ZELS, CHEESE, EGG YOLKS, POTATO CHIPS, AND RED MEAT HAVE A HIGH-ENERGY DENSITY. DIETS CONTAINING LOW-ENERGY DENSE FOODS HAVE BEEN SHOWN TO CONTROL HUNGER AND RESULT IN DECREASED CALORIC INTAKE AND WEIGHT LOSS. OCCASIONALLY, VERY-LOW-CALORIE DIETS (VLCDs) ARE PRESCRIBED AS A FORM OF AGGRESSIVE DIETARY THERAPY. THE PRIMARY PURPOSE OF A VLCD IS TO PRO-

TABLE 75-5 A GUIDE TO SELECTING TREATMENT

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BMI CATEGORY</th>
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<tbody>
<tr>
<td>DIET, EXERCISE,</td>
<td>25-26.9</td>
</tr>
<tr>
<td>BEHAVIOR THERAPY</td>
<td>WITH COMORBIDITIES</td>
</tr>
<tr>
<td>PHARMACOTHERAPY</td>
<td>27-29.9</td>
</tr>
<tr>
<td>SURGERY</td>
<td>WITH COMORBIDITIES</td>
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<tr>
<td></td>
<td>WITH COMORBIDITIES</td>
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<tr>
<td></td>
<td>30-35</td>
</tr>
</tbody>
</table>
WITH COMORBIDITIES

*40


MOTE A RAPID AND SIGNIFICANT (1 3-23 KG) SHORT-TERM WEIGHT LOSS OVER A 3-6 MONTH PERIOD. THESE PROPRIETY FORMULAS TYPICALLY SUPPLY *800 KCAL, 50-80 G PROTEIN, AND 100% OF THE RECOMMENDED DAILY INTAKE FOR VITAMINS AND MINERALS. ACCORDING TO A REVIEW BY THE NATIONAL TASK FORCE ON THE PREVENTION AND TREATMENT OF OBESITY, INDICATIONS FOR INITIATING A VLCD INCLUDE WELL-MOTIVATED INDIVIDUALS WHO ARE MODERATELY TO SEVERELY OBESE (BMI >30), HAVE FAILED AT MORE CONSERVATIVE APPROACHES TO WEIGHT LOSS, AND HAVE A MEDICAL CONDITION THAT WOULD BE IMMEDIATELY IMPROVED WITH RAPID WEIGHT LOSS. THESE CONDITIONS INCLUDE POORLY CONTROLLED TYPE 1 DIABETES, HYPERTRIGLYCERIDEMIA, OBSTRUCTIVE SLEEP APNEA, AND SYMPTOMATIC PERIPHERAL EDEMA. THE RISK FOR GALLSTONE FORMATION INCREASES EXPONENTIALY AT RATES OF WEIGHT LOSS > 1.5 KG/WEEK (3.3 LB/WEEK). PROPHYLAXIS AGAINST GALLSTONE FORMATION WITH URSODEOXYCHOLIC ACID, 600 MG/D, IS EFFECTIVE IN REDUCING THIS RISK. BECAUSE OF THE

472 PART 5: NUTRITION

NEED FOR CLOSE METABOLIC MONITORING, THESE DIETS ARE USUALLY PRESCRIBED BY PHYSICIANS SPECIALIZING IN OBESITY CARE.

PHYSICAL ACTIVITY THERAPY ALTHOUGH EXERCISE ALONE IS ONLY MODERATELY EFFECTIVE FOR WEIGHT LOSS, THE COMBINATION OF DIETARY MODIFICATION AND EXERCISE IS THE MOST EFFECTIVE BEHAVIORAL APPROACH FOR THE TREATMENT OF OBESITY. THE MOST IMPORTANT ROLE OF EXERCISE APPEARS TO BE IN THE MAINTENANCE OF THE WEIGHT LOSS. CURRENTLY, THE MINIMUM PUBLIC HEALTH RECOMMENDATION FOR PHYSICAL ACTIVITY IS 30 MIN OF MODERATE INTENSITY PHYSICAL ACTIVITY ON
MOST, AND PREFERABLY ALL, DAYS OF THE WEEK. FOCUSING ON SIMPLE WAYS TO ADD PHYSICAL ACTIVITY INTO THE NORMAL DAILY ROUTINE THROUGH LEISURE ACTIVITIES, TRAVEL, AND DOMESTIC WORK SHOULD BE SUGGESTED. EXAMPLES INCLUDE WALKING, USING THE STAIRS, DOING HOME AND YARD WORK, AND ENGAGING IN SPORT ACTIVITIES. ASKING THE PATIENT TO WEAR A PEDOMETER TO MONITOR TOTAL ACCUMULATION OF STEPS AS PART OF THE ACTIVITIES OF DAILY LIVING IS A USEFUL STRATEGY. STEP COUNTS ARE HIGHLY CORRELATED WITH ACTIVITY LEVEL. STUDIES HAVE DEMONSTRATED THAT LIFESTYLE ACTIVITIES ARE AS EFFECTIVE AS STRUCTURED EXERCISE PROGRAMS FOR IMPROVING CARDIORESPIRATORY FITNESS AND WEIGHT LOSS. THE DIETARY GUIDELINES FOR AMERICANS 2005 SUMMARIZES COMPELLING EVIDENCE THAT AT LEAST 60-90 MIN OF DAILY MODERATE-INTENSITY PHYSICAL ACTIVITY (420-630 MIN PER WEEK) IS NEEDED TO SUSTAIN WEIGHT LOSS. (HTTP://WWW.HEALTH.GOV/DIETARYGUIDELINES/DGA2005/). THE AMERICAN COLLEGE OF SPORTS MEDICINE RECOMMENDS THAT OVERWEIGHT AND OBESE INDIVIDUALS PROGRESSIVELY INCREASE TO A MINIMUM OF 150 MIN OF MODERATE INTENSITY PHYSICAL ACTIVITY PER WEEK AS A FIRST GOAL. HOWEVER, FOR LONG-TERM WEIGHT LOSS, A HIGHER LEVEL OF EXERCISE (E.G., 200-300 MIN OR *2000 KCAL PER WEEK) IS NEEDED. THESE RECOMMENDATIONS ARE DAUNTING TO MOST PATIENTS AND NEED TO BE IMPLEMENTED GRADUALLY. CONSULTATION WITH AN EXERCISE PHYSIOLOGIST OR PERSONAL TRAINER MAY BE HELPFUL.

BEHAVIORAL THERAPY COGNITIVE BEHAVIORAL THERAPY IS USED TO HELP CHANGE AND REINFORCE NEW DIETARY AND PHYSICAL ACTIVITY BEHAVIORS. STRATEGIES INCLUDE SELF-MONITORING TECHNIQUES (E.G., JOURNALING, WEIGHING, AND MEASURING FOOD AND ACTIVITY); STRESS MANAGEMENT; STIMULUS CONTROL (E.G., USING SMALLER PLATES, NOT EATING IN FRONT OF THE TELEVISION OR IN THE CAR); SOCIAL SUPPORT; PROBLEM SOLVING; AND COGNITIVE RESTRUCTURING TO HELP PATIENTS DEVELOP MORE POSITIVE AND REALISTIC THOUGHTS ABOUT THEMSELVES. WHEN RECOMMENDING ANY BEHAVIORAL LIFESTYLE CHANGE, HAVE THE PATIENT IDENTIFY WHAT, WHEN, WHERE, AND HOW THE BEHAVIORAL CHANGE WILL BE PERFORMED THE PATIENT SHOULD KEEP A RECORD OF THE ANTICIPATED BEHAVIORAL CHANGE SO THAT PROGRESS CAN BE REVIEWED AT THE NEXT OFFICE VISIT. BECAUSE THESE TECHNIQUES ARE TIME-CONSUMING TO IMPLEMENT, THEY ARE OFTEN PROVIDED BY ANCILLARY OFFICE STAFF SUCH AS A NURSE CLINICIAN OR REGISTERED DIETITIAN.

PHARMACOTHERAPY ADJUVANT PHARMACOLOGIC TREATMENTS SHOULD BE CONSIDERED FOR PATIENTS WITH A BMI >30 KG/M###2 OR WITH A BMI >27 KG/M###2 WHO ALSO HAVE CONCOMITANT OBESITY-RELATED DISEASES AND FOR WHOM DIETARY AND PHYSICAL ACTIVITY THERAPY HAS NOT BEEN SUCCESSFUL. WHEN PRESCRIBING AN ANTI OBESITY MEDICATION, PATIENTS SHOULD BE ACTIVELY ENGAGED IN A LIFESTYLE PROGRAM THAT PROVIDES THE STRATEGIES AND SKILLS NEEDED TO EFFECTIVELY USE THE DRUG SINCE THIS SUPPORT INCREASES TOTAL WEIGHT LOSS. THERE ARE SEVERAL POTENTIAL TARGETS OF PHARMACOLOGIC THERAPY FOR OBESITY. THE MOST THOROUGHLY EXPLORRED TREATMENT IS SUPPRESSION OF APPETITE VIA CENTRALLY ACTIVE MEDICATIONS THAT ALTER MONOAMINE NEUROTRANSMITTERS. A SECOND STRATEGY IS TO REDUCE THE ABSORPTION OF SELECTIVE MACRONUTRIENTS FROM THE GASTROINTESTINAL (GL) TRACT, SUCH AS FAT. THESE TWO MECHANISMS FORM THE BASIS FOR ALL CURRENTLY PRESCRIBED ANTI OBESITY AGENTS. A THIRD TARGET, SELECTIVE BLOCKING OF THE ENDOCANNABINOID SYSTEM, HAS RECENTLY BEEN IDENTIFIED.
Centrally acting anorexiant medications, appetite-suppressing drugs, or anorexiant, affect satiety—the absence of hunger after eating—and hunger—a biologic sensation that initiates eating. By increasing satiety and decreasing hunger, these agents help patients reduce caloric intake without a sense of deprivation. The target site for the actions of anorexiants is the ventromedial and lateral hypothalamic regions in the central nervous system (Chap. 74). Their biological effect on appetite regulation is produced by augmenting the neurotransmission of three monoamines: norepinephrine, serotonin [5-hydroxytryptamine (5-HT)]; and, to a lesser degree, dopamine. The classic sympathomimetic adrenergic agents (benzphetamine, phendimetrazine, diethylpropion, mazindol, and phentermine) function by stimulating norepinephrine release or by blocking its reuptake. In contrast, sibutramine (Meridia) functions as a serotonin and norepinephrine reuptake inhibitor. Unlike other previously used anorexiants, sibutramine is not pharmacologically related to amphetamine and has no addictive potential.

Sibutramine is the only anorexiant that is currently approved by the Food and Drug Administration (FDA) for long-term use. It produces an average loss of about 5-9% of initial body weight at 12 months. Sibutramine has been demonstrated to maintain weight loss for up to 2 years. The most commonly reported adverse events of sibutramine are headache, dry mouth, insomnia, and constipation. These are generally mild and well-tolerated. The principal concern is a dose-related increase in blood pressure and heart rate that may require discontinuation of the medication. A dose of 10-15 mg/d causes an average increase in systolic and diastolic blood pressure of 2-4 mmHg and an increase in heart rate of 4-6 beats/min. For this reason, all patients should be monitored closely and evaluated within 1 month after initiating therapy. The risk of adverse effects on blood pressure are no greater in patients with controlled hypertension than in those who do not have hypertension. And the drug does not appear to cause cardiac valve dysfunction. Contraindications to sibutramine use include uncontrolled hypertension, congestive heart failure, symptomatic coronary heart disease, arrhythmias, or history of stroke. Similar to other antiobesity medications, weight reduction is enhanced when the drug is used along with behavioral therapy, and body weight increases when the medication is discontinued.

Peripherally acting medications Orlistat (Xenical) is a synthetic hydrogenated derivative of a naturally occurring lipase inhibitor, lipostatin, produced by the mold Streptomyces toxytricini. Orlistat is a potent, slowly reversible inhibitor of pancreatic, gastric, and carboxylester lipases and phospholipase A2, which are required for the hydrolysis of dietary fat into fatty ac-
IDS AND MONOACYLGLYCEROLS THE DRUG ACTS IN THE LUMEN OF THE STOMACH AND SMALL INTESTINE BY FORMING A COVALENT BOND WITH THE ACTIVE SITE OF THESE LIPASES. TAKEN AT A THERAPEUTIC DOSE OF 120 MG TID, ORLISTAT BLOCKS THE DIGESTION AND ABSORPTION OF ABOUT 30% OF DIETARY FAT. AFTER DISCONTINUATION OF THE DRUG, FECAL FAT USUALLY RETURNSTO NORMAL CONCENTRATIONS WITHIN 48-72 H.

MULTIPLE RANDOMIZED, 1-2 YEAR DOUBLE-BLIND, PLACEBO-CONTROLLED STUDIES HAVE SHOWN THAT AFTER ONE YEAR, ORLISTAT PRODUCES A WEIGHT LOSS OF ABOUT 9-10%, COMPARED WITH A 4-6% WEIGHT LOSS IN THE PLACEBO-TREATED GROUPS. BECAUSE ORLISTAT IS MINIMALLY (<1%) ABSORBED FROM THE GI TRACT, IT HAS NO SYSTEMIC SIDE EFFECTS. TOLERABILITY TO THE DRUG IS RELATED TO THE MALABSORPTION OF DIETARY FAT AND SUBSEQUENT PASSAGE OF FAT IN THE FECES. GI TRACT ADVERSE EFFECTS ARE REPORTED IN AT LEAST 10% OF ORLISTAT-TREATED PATIENTS THESE INCLUDE FLATUS WITH DISCHARGE, FECAL URGENCY, FATTY/OILY STOOL, AND INCREASED DEFECATION. THESE SIDE EFFECTS ARE GENERALLY EXPERIENCED EARLY, DIMINISH AS PATIENTS CONTROL THEIR DIETARY FAT INTAKE, AND INFREQUENTLY CAUSE PATIENTS TO WITHDRAW FROM CLINICAL TRIALS. PSYLLIUM MUCILLOID IS HELPFUL IN CONTROLLING THE ORLISTAT-INDUCED GI SIDE EFFECTS WHEN TAKEN CONCOMITANTLY WITH THE MEDICATION. SERUM CONCENTRATIONS OF THE FAT-SOLUBLE VITAMINS D AND E AND *-CAROTENE MAY BE REDUCED, AND VITAMIN SUPPLEMENTS ARE RECOMMENDED TO PREVENT POTENTIAL DEFICIENCIES. ORLISTAT WAS APPROVED FOR OTHER-THE-COUNTER USE IN 2007.

THE ENDOCANNABINOID SYSTEM CANNABINOIDS AND THEIR ENDGENOUS LIGANDS HAVE BEEN IMPLICATED IN A VARIETY OF PHYSIOLOGIC FUNCTIONS, INCLUDING FEEDING, MODULATION OF PAIN, EMOTIONAL BEHAVIOR, AND PERIPHERAL LIPID METABOLISM CANNABIS AND ITS MAIN INGREDIENT, *-TETRAHYDROCANNABINOL (THC), IS AN EXOGENOUS CANNABINOID COMPOUND. TWO ENDOCANNABINOIDS HAVE BEEN IDENTIFIED, ANANDAMIDE AND 2-ARACHIDONYL GLYCERIDE. TWO CANNABINOID RECEPTORS HAVE BEEN IDENTIFIED: CB###1 (ABUNDANT IN THE BRAIN) AND CB###2 (PRESENT IN IMMUNE CELLS). THE BRAIN ENDOCANNABINOID SYSTEM IS THOUGHT TO CONTROL FOOD INTAKE THROUGH REINFORCING MOTIVATION TO FIND AND CONSUME FOODS WITH HIGH INCENTIVE VALUE AND TO REGULATE ACTIONS OF OTHER MEDIATORS OF APPETITE. THE FIRST SELECTIVE CANNABINOID CB###1 RECEPTOR ANTAGONIST, RIMONABANT, WAS DISCOVERED IN 1994. THE MEDICATION ANTAGONIZES THE OREXIGENIC EFFECT OF THC AND SUPPRESSES APPETITE WHEN GIVEN ALONE IN ANIMAL MODELS. SEVERAL LARGE PROSPECTIVE, RANDOMIZED CONTROLLED TRIALS HAVE DEMONSTRATED THE EFFICACITY OF RIMONABANT AS A WEIGHT-LOSS AGENT. TAKEN AS A 20 MG DOSE, SUBJECTS LOST AN AVERAGE OF 6.5 KG (14.32 LB) COMPARED TO 1.5 KG (3.3 LB) FOR PLACEBO AT 1 YEAR. CONCOMITANT IMPROVEMENTS WERE SEEN IN WAIST CIRCUMFERENCE AND CARDIOVASCULAR RISK FACTORS. THE MOST COMMON REPORTED SIDE EFFECTS INCLUDE DEPRESSION, ANXIETY, AND NAUSEA. FDA APPROVAL OF RIMONABANT IS STILL PENDING.
BARIATRIC SURGERY CAN BE CONSIDERED FOR PATIENTS WITH SEVERE OBESITY (BMI ≥ 40 kg/m²) OR THOSE WITH MODERATE OBESITY (BMI ≥ 35 kg/m²) ASSOCIATED WITH A SERIOUS MEDICAL CONDITION. SURGICAL WEIGHT LOSS FUNCTIONS BY REDUCING CALORIC INTAKE AND, DEPENDING ON THE PROCEDURE, MACRONUTRIENT ABSORPTION. WEIGHT-LOSS SURGERIES FALL INTO ONE OF TWO CATEGORIES: RESTRICTIVE AND RESTRICTIVE-MALABSORPTIVE (FIG. 75-2). RESTRICTIVE SURGERIES LIMIT THE AMOUNT OF FOOD THE STOMACH CAN HOLD AND SLOW THE RATE OF GASTRIC EMPTYING. THE VERTICAL BANDED GASTROPLASTY (VBG) IS THE PROTOTYPE OF THIS CATEGORY BUT IS CURRENTLY PERFORMED ON A VERY LIMITED BASIS DUE TO LACK OF EFFECTIVENESS IN LONG-TERM TRIALS. LAPAROSCOPIC ADJUSTABLE SILICONE GASTRIC BANDING (LASGB) HAS REPLACED THE VBG AS THE MOST COMMONLY PERFORMED RESTRICTIVE OPERATION THE FIRST BANDING DEVICE, THE LAP-BAND, WAS APPROVED FOR USE IN THE UNITED STATES IN 2001. IN CONTRAST TO PREVIOUS DEVICES, THE DIAMETER OF THIS BAND IS ADJUSTABLE BY WAY OF ITS CONNECTION TO A RESERVOIR THAT IS IMPLANTED UNDER THE SKIN INJECTION OR REMOVAL OF SALINE INTO THE RESERVOIR TIGHTENS OR LOOSENS THE BAND’S INTERNAL DIAMETER, THUS CHANGING THE SIZE OF THE GASTRIC OPENING. THE THREE RESTRICTIVE-MALABSORPTIVE BYPASS PROCEDURES COMBINE THE ELEMENTS OF GASTRIC RESTRICTION AND SELECTIVE MALABSORPTION. THESE PROCEDURES INCLUDE ROUX-EN-Y GASTRIC BYPASS (RYGB), BILIOPANCREATIC DIVERSION (BPD), AND BILIOPANCREATIC DIVERSION WITH DUODENAL SWITCH (BPDDS) (FIG. 75-2). RYGB IS THE MOST COMMONLY PERFORMED AND ACCEPTED BYPASS PROCEDURE. IT MAY BE PERFORMED WITH AN OPEN INCISION OR LAPAROSCOPICALLY. ALTHOUGH NO RECENT RANDOMIZED CONTROLLED TRIALS COMPARE WEIGHT LOSS AFTER SURGICAL AND NONSURGICAL INTERVENTIONS, DATA FROM META-ANALYSES AND LARGE DATABASES, PRIMARILY OBTAINED FROM OBSERVATIONAL STUDIES, SUGGEST THAT BARIATRIC SURGERY IS THE MOST EFFECTIVE WEIGHT-LOSS THERAPY FOR THOSE WITH CLINICALLY SEVERE OBESITY. THESE PROCEDURES GENERALLY PRODUCE A 30-35% AVERAGE TOTAL BODY WEIGHT LOSS.
ANOREXIA NERVOSA AND BULIMIA NERVOSA ARE CHARACTERIZED BY SEVERE DISTURBANCES OF EATING BEHAVIOR. THE SALIENT FEATURE OF ANOREXIA NERVOSA (AN) IS A REFUSAL TO MAINTAIN A MINIMALLY NORMAL BODY WEIGHT. BULIMIA NERVOSA (BN) IS CHARACTERIZED BY RECURRENT EPISODES OF BINGE EATING FOLLOWED BY ABNORMAL COMPENSATORY BEHAVIORS, SUCH AS SELF-INDUCED VOMITING. AN AND BN ARE DISTINCT CLINICAL SYNDROMES BUT SHARE CERTAIN FEATURES IN COMMON. BOTH DISORDERS OCCUR PRIMARILY AMONG PREVIOUSLY HEALTHY YOUNG WOMEN WHO BECOME OVERLY CONCERNED WITH BODY SHAPE AND WEIGHT. MANY PATIENTS WITH BN HAVE WEIGHT LOSS THAT IS MAINTAINED IN NEARLY 60% OF PATIENTS AT 5 YEARS. IN GENERAL, MEAN WEIGHT LOSS IS GREATER AFTER THE COMBINED RESTRICTIVE-MALABSORPTIVE PROCEDURES COMPARED TO THE RESTRICTIVE PROCEDURES. AN ABUNDANCE OF DATA SUPPORTS THE POSITIVE IMPACT OF BARIATRIC SURGERY ON OBESITY-RELATED MORBID CONDITIONS, INCLUDING DIABETES MELLITUS, HYPERTENSION, OBSTRUCTIVE SLEEP APNEA, DYSLIPIDEMIA, AND NONALCOHOLIC FATTY LIVER DISEASE. SURGICAL MORTALITY FROM BARIATRIC SURGERY IS GENERALLY <1% BUT VARIES WITH THE PROCEDURE, PATIENT'S AGE AND COMORBID CONDITIONS, AND EXPERIENCE OF THE SURGICAL TEAM. THE MOST COMMON SURGICAL COMPLICATIONS INCLUDE STOMAL STENOSIS OR MARGINAL ULCERS (OCCURRING IN 5-15% OF PATIENTS) THAT PRESENT AS PROLONGED NAUSEA AND VOMITING AFTER EATING OR INABILITY TO ADVANCE THE DIET TO SOLID FOODS. THESE COMPLICATIONS ARE TYPICALLY TREATED BY ENDOSCOPIC BALLOON DILATATION AND ACID SUPPRESSION THERAPY, RESPECTIVELY. FOR PATIENTS WHO UNDERGO LASGB, THERE ARE NO INTESTINAL ABSORPTIVE ABNORMALITIES OTHER THAN MECHANICAL REDUCTION IN GASTRIC SIZE AND OUTFLOW. THEREFORE, SELECTIVE DEFiciencies OCCUR UNCOMMONLY UNLESS EATING HABITS BECOME UNBALANCED. IN CONTRAST, THE RESTRICTIVE-MALABSORPTIVE PROCEDURES INCREASE RISK FOR MICRONUTRIENT DEFiciencies OF VITAMIN B###12, IRON, FOLATE, CALCIUM, AND VITAMIN D. PATIENTS WITH RESTRICTIVE-MALABSORPTIVE PROCEDURES REQUIRE LIFELONG SUPPLEMENTATION WITH THESE MICRONUTRIENTS.

FURTHER READINGS

BINGE EATING DISORDER (BED) IS A MORE RECENTLY DESCRIBED SYNDROME CHARACTERIZED BY REPEATED EPISODES OF BINGE EATING, SIMILAR TO THOSE OF BN, IN THE ABSENCE OF INAPPROPRIATE COMPENSATORY BEHAVIOR. PATIENTS WITH BED ARE TYPICALLY MIDDLE-AGED MEN OR WOMEN WITH SIGNIFICANT OBESITY. THEY HAVE AN INCREASED FREQUENCY OF ANXIETY AND DEPRESSION COMPARED TO SIMILARLY OBESE PATIENTS WITHOUT BED. IT IS NOT ESTABLISHED THAT PATIENTS WITH BED ARE AT INCREASED RISK FOR MEDICAL COMPLICATIONS OR THAT THEY REQUIRE SPECIFIC TREATMENT INTERVENTIONS.
APPROXIMATELY 1%. AN IS MUCH LESS COMMON IN MALES. AN IS MORE PREVALENT IN CULTURES WHERE FOOD IS PLENTIFUL AND IN WHICH BEING THIN IS ASSOCIATED WITH ATTRACTIVENESS. INDIVIDUALS WHO PURSUE INTERESTS THAT PLACE A PREMIUM ON THINNESS, SUCH AS BALLET AND MODELING, ARE AT GREATER RISK. THE INCIDENCE OF AN HAS INCREASED IN RECENT DECADES.

ETIOLOGY

THE ETIOLOGY OF AN IS UNKNOWN BUT APPEARS TO INVOLVE A COMBINATION OF PSYCHOLOGICAL, BIOLOGIC, AND CULTURAL RISK FACTORS. RISK FACTORS, SUCH AS SEXUAL OR PHYSICAL ABUSE AND A FAMILY HISTORY OF MOOD DISTURBANCE, ARE BEST VIEWED AS NONSPECIFIC RISK FACTORS THAT INCREASE VULNERABILITY TO A RANGE OF PSYCHIATRIC DISORDERS, INCLUDING AN. PATIENTS WHO DEVELOP AN ARE INCLINED TO BE MORE OBSESSIONAL AND PERFECTIONIST THAN THEIR PEERS. THE DISORDER OFTEN BEGINS AS A DIET NOT DISTINGUISHABLE AT THE OUTSET FROM THOSE UNDERTAKEN BY MANY ADOLESCENTS AND YOUNG WOMEN. AS WEIGHT LOSS PROGRESSES, THE FEAR OF GAINING WEIGHT GROWS; DIETING BECOMES STRICTER; AND PSYCHOLOGICAL, BEHAVIORAL, AND MEDICAL ABERRATIONS INCREASE. EATING DISORDERS, INCLUDING AN, MAY DEVELOP AMONG INDIVIDUALS WITH TYPE 1 DIABETES MELLITUS AND ARE ASSOCIATED WITH POORER GLYCEMIC CONTROL AND AN INCREASED FREQUENCY OF COMPLICATIONS (CHAP. 338).

NUMEROUS PHYSIOLOGIC DISTURBANCES, INCLUDING ABNORMALITIES IN A VARIETY OF NEUROTRANSMITTER SYSTEMS, HAVE BEEN DESCRIBED IN AN (SEE BELOW). IT IS DIFFICULT TO DISTINGUISH NEUROCHEMICAL, METABOLIC, AND HORMONAL CHANGES THAT MAY HAVE A ROLE IN THE INITIATION OR PERPETUATION OF THE SYNDROME FROM THOSE THAT ARE SECONDARY TO THE DISORDER. THE RESOLUTION OF MOST OF THESE ABNORMALITIES WITH WEIGHT RESTORATION ARGUES AGAINST AN ETIOLOGIC ROLE. GENETIC FACTORS CONTRIBUTE TO THE RISK OF DEVELOPMENT OF AN, AS ITS INCIDENCE IS GREATER IN FAMILIES WITH ONE AFFECTED MEMBER AND THE CONCORDANCE IN MONOZYGOTIC TWINS IS GREATER THAN IN DIZYGOTIC TWINS. HOWEVER, SPECIFIC GENES HAVE NOT BEEN IDENTIFIED.

CLINICAL FEATURES

AN TYPICALLY BEGINS IN MID TO LATE ADOLESCENCE, SOMETIMES IN ASSOCIATION WITH A STRESSFUL LIFE EVENT SUCH AS LEAVING HOME FOR SCHOOL (TABLE 76-1). THE DISORDER OCCASIONALLY DEVELOPS IN EARLY PUBERTY, BEFORE MENARCHE, BUT SELDOM BEGINS AFTER AGE 40. DESPITE BEING UNDERWEIGHT, PATIENTS WITH AN ARE IRRATIONALY AFRAID OF GAINING WEIGHT, OFTEN OUT OF A CONCERN THAT WEIGHT GAIN WILL GET “OUT OF CONTROL.” THEY ALSO EX-
HIBIT A DISTORTION OF BODY IMAGE, WHICH MAY EXPRESS ITSELF IN SEVERAL WAYS. FOR EXAMPLE, DESPITE BEING EMACIATED, PATIENTS WITH AN MAY BELIEVE THAT THEIR BODY AS A WHOLE, OR SOME PART OF THEIR BODY, IS TOO FAT. FURTHER WEIGHT LOSS IS VIEWED BY THE PATIENT AS A FULFILLING ACCOMPLISHMENT, WHILE WEIGHT GAIN IS SEEN AS A PERSONAL FAILURE. PATIENTS WITH AN RARELY COMPLAIN OF HUNGER OR FATIGUE AND OFTEN EXERCISE EXTENSIVELY. DESPITE THE DENIAL OF HUNGER, ONE-QUARTER TO ONE-HALF OF PATIENTS WITH AN ENGAGE IN EATING BINGES. PATIENTS TEND TO BECOME SOCIALLY DRAWDN AND INCREASINGLY COMMITTED TO WORK OR STUDY, DIETING, AND EXERCISE. AS WEIGHT LOSS PROGRESSES, THOUGHTS OF FOOD DOMINATE MENTAL LIFE AND IDIOSYNCRATIC RULES DEVELOP AROUND EATING. PATIENTS WITH AN MAY OBSESSIVELY COLLECT COOKBOOKS AND RECIPES AND BE DRAWN TO FOOD-RELATED OCCUPATIONS.

PHYSICAL FEATURES PATIENTS WITH AN TYPICALLY HAVE FEW PHYSICAL COMPLAINTS BUT MAY NOTE COLD INTOLERANCE. GASTROINTESTINAL MOTILITY IS DIMINISHED, LEADING TO REDUCED GASTRIC EMPTYING AND CONSTIPATION. SOME WOMEN WHO DEVELOP AN AFTER MENARCHE REPORT THAT THEIR MENSES CEASED BEFORE SIGNIFICANT WEIGHT LOSS OCCURRED. WEIGHT AND HEIGHT SHOULD BE MEASURED TO ALLOW CALCULATION OF BODY MASS INDEX (BMI; KG/M###2). VITAL SIGNS MAY REVEAL BRADYCARDIA, HYPOTENSION, AND MILD HYPOTHERMIA. SOFT, DOWNY HAIR GROWTH (LANUGO) SOMETIMES OCCURS, AND ALOPECIA MAY BE SEEN. SALIVARY GLAND ENLARGEMENT, WHICH IS ASSOCIATED WITH STARVATION AS WELL AS WITH BINGE EATING AND VOMITING, MAY MAKE THE FACE APPEAR SURPRISINGLY FULL IN CONTRAST TO THE MARKED GENERAL WASTING. ACROCYANOSIS OF THE DIGITS IS COMMON, AND PERIPHERAL EDEMA CAN BE SEEN IN THE ABSENCE OF HYPOALBUMINEMIA, PARTICULARLY WHEN THE PATIENT BEGINS TO REGAIN WEIGHT. CONSUMPTION OF LARGE AMOUNTS OF VEGETABLES CONTAINING VITAMIN A CAN RESULT IN A YELLOW TINT TO THE SKIN (HYPERCAROTERTEMIA), WHICH IS ESPECIALLY NOTABLE ON THE PALMS.

LABORATORY ABNORMALITIES MILD NORMOCHROMIC, NORMOCYTIC ANEMIA IS FREQUENT, AS IS MILD TO MODERATE LEUKOPENIA, WITH A DISPROPORTIONATE REDUCTION OF POLYMORPHONUCLEAR LEUKOCYTES. DEHYDRATION MAY RESULT IN SLIGHTLY INCREASED LEVELS OF BLOOD UREA NITROGEN AND CREATININE. SE- RUM TRANSAMINASE LEVELS MAY INCREASE, ESPECIALLY DURING THE EARLY PHASES OF REFEEDING. THE LEVEL OF SERUM PROTEINS IS USUALLY NORMAL. BLOOD SUGAR IS OFTEN LOW AND SERUM CHOLESTEROL MAY BE MODERATELY ELEVATED. HYPOKALEMIC ALKALOSIS SUGGESTS SELF-INDUCED VOMITING OR THE USE
TABLE 76-1 COMMON CHARACTERISTICS OF ANOREXIA NERVOSA AND BULIMIA NERVOSA

CLINICAL CHARACTERISTICS

ONSET
FEMALE:MALE
LIFETIME PREVALENCE IN WOMEN
WEIGHT
MENSTRUATION
BINGE EATING
MORTALITY

PHYSICAL AND LABORATORY FINDINGS

SKIN/EXTREMITIES
CARDIOVASCULAR
GASTROINTESTINAL
HEMATOPOIETIC
FLUID/ELECTROLYTE
ENDOCRINE

BONE

ANOREXIA NERVOSA

MID-adolescence
10:1
1%
Markedly decreased
Absent
25-50%
~5% per decade

Lanugo
Acrocyanosis
Edema
Bradycardia
Hypotension
Salivary gland enlargement
Slow gastric emptying
Constipation
Elevated liver enzymes
Normochromic, normocytic anemia
Leukopenia
Increased BUN, creatinine
Hypokalemia
Hypoglycemia
LOW ESTROGEN OR TESTOSTERONE
LOW LH AND FSH
LOW-NORMAL THYROXINE
NORMAL TSH
INCREASED CORTISOL
OSTEOPENIA

BULIMIA NERVO萨

LATE ADOLESCENCE/EARLY ADULTHOOD
10:1
1-3%
USUALLY NORMAL
USUALLY NORMAL
REQUIRED FOR DIAGNOSIS
LOW

SALIVARY GLAND ENLARGEMENT
DENTAL EROSION

HYPOKALEMIA
HYPOCHLOREMIA
ALKALOSIS

###APATIENTS WITH THE BINGE-EATING/PURGING SUBTYPE OF ANOREXIA NERVOサ MAY ALSO EXHIBIT THE PHYSICAL AND LABORATORY FINDINGS ASSOCIATED WITH BULIMIA NERVOサ.

ABBREVIATIONS: BUN, BLOOD UREA NITROGEN; LH, LUTEINIZING HORMONE; TSH, FOLLICLE STIMULATING HORMONE, TSH, THYROID STIMULATING HORMONE.

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475 CHAPTER 76 EATING DISORDERS

OF DIURETICS. HYponatremiA is common and may result from excess fluid intake and disturbances in the secretion of antidiuretic hormone.

ENDOCRINE ABNORMALITIES THE REGULATION OF VIRTUALLY EVERY ENDOCRINE SYSTEM IS ALTERED IN AN, BUT THE MOST STRIKING CHANGES OCCUR IN THE REPRODUCTIVE SYSTEM. AMENORRHEA IS HYPOTHALAMIC IN ORIGIN AND REFLECTS DIMINISHED PRODUCTION OF GONADOTROPIN-RELEASING HORMONE (GNRH). WHEN EXOGENOUS GNRH IS ADMINISTERED IN A PULSATILE MANNER, PITUITARY RESPONSES OF LUTEINIZING HORMONE (LH) AND FOLLICLE-STIMULATING HORMONE (FSH) ARE NORMALIZED, INDICATING THE ABSENCE OF A PRIMARY PITUITARY ABNORMALITY. THE RESULTING GONADOTROPIN DEFICIENCY CAUSES LOW PLASMA ESTROGEN IN WOMEN AND REDUCED TESTOSTERONE IN MEN. THE HYPOTHALAMIC GNRH PULSE GENERATOR IS EXQUISITELY SENSITIVE, PARTICULARLY IN WOMEN, TO BODY WEIGHT, STRESS, AND EXERCISE, EACH OF WHICH MAY
CONTRIBUTE TO *HYPOTHALAMIC AMENORRHEA* IN AN (CHAP. 341). SERUM LEPTIN LEVELS ARE MARKEDLY REDUCED IN AN AS A RESULT OF UNDER-NUTRITION AND DECREASED BODY FAT MASS. THE REDUCTION IN LEPTIN APPEARS TO BE THE PRIMARY FACTOR RESPONSIBLE FOR THE DISTURBANCES OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS, AND TO BE AN IMPORTANT MEDIATOR OF THE OTHER NEUROENDOCRINE ABNORMALITIES CHARACTERISTIC OF AN (CHAP. 74). SERUM CORTISOL AND 24-H URINE FREE CORTISOL LEVELS ARE GENERALLY ELEVATED BUT WITHOUT CHARACTERISTIC CLINICAL SIGNS OF CORTISOL EXCESS. THYROID FUNCTION TESTS RESEMBLE THE PATTERN SEEN IN EUTHYROID SICK SYNDROME (CHAP. 335). THYROXINE (T\#4) AND FREE T\#4 LEVELS ARE USUALLY IN THE LOW-NORMAL RANGE, TRIODOTHYRONINE (T\#3) LEVELS ARE REDUCED, AND REVERSE T\#3 (RT\#3) IS ELEVATED. THE LEVEL OF THYROID-STIMULATING HORMONE (TSH) IS NORMAL OR PARTIALLY SUPPRESSED. GROWTH HORMONE IS INCREASED, BUT INSULIN-LIKE GROWTH FACTOR 1 (IGF-1), WHICH IS PRODUCED MAINLY BY THE LIVER, IS REDUCED, AS IN OTHER CONDITIONS OF STARVATION. MINISHED BONE DENSITY IS ROUTINELY OBSERVED IN AN AND REFLECTS THE EFFECTS OF MULTIPLE NUTRITIONAL DEFICIENCIES, REDUCED GONADAL STEROIDS, AND INCREASED CORTISOL. THE DEGREE OF BONE DENSITY REDUCTION IS PROPORTIONAL TO THE LENGTH OF THE ILLNESS, AND PATIENTS ARE AT RISK FOR THE DEVELOPMENT OF SYMPTOMATIC FRACTURES. THE OCCURRENCE OF AN DURING ADOLESCENCE MAY LEAD TO THE PREMATURE CESSATION OF LINEAR BONE GROWTH AND A FAILURE TO ACHIEVE EXPECTED ADULT HEIGHT.

**CARDIAC ABNORMALITIES** CARDIAC OUTPUT IS REDUCED, AND CONGESTIVE HEART FAILURE OCCURS RARELY DURING RAPID REFEEDING. THE ELECTROCARDI-GRAM USUALLY SHOWS SINUS BRADYCARDIA, REDUCED QRS VOLTAGE, AND NON-SPECIFIC ST-T-WAVE ABNORMALITIES. SOME PATIENTS DEVELOP A PROLONGED QT\#C INTERVAL, WHICH MAY PREDISPOSE TO SERIOUS ARRHYTHMIAS, PARTICULARLY WHEN ELECTROLYTE ABNORMALITIES ALSO ARE PRESENT.

**DIAGNOSIS**

THE DIAGNOSIS OF AN IS BASED ON THE PRESENCE OF CHARACTERISTIC BEHAVIORAL, PSYCHOLOGICAL, AND PHYSICAL ATTRIBUTES (TABLE 76-2). WIDELY ACCEPTED DIAGNOSTIC CRITERIA ARE PROVIDED BY THE AMERICAN PSYCHIATRIC ASSOCIATION’S *DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS* (DSM-IV). THESE CRITERIA INCLUDE WEIGHT <85% OF THAT EXPECTED FOR AGE AND HEIGHT, WHICH IS ROUGHLY EQUIVALENT TO A BMI OF 18.5 KG/M\#2 FOR ADULT WOMEN. THIS WEIGHT CRITERION IS SOMEWHAT ARBITRARY, SO THAT A PATIENT WHO MEETS ALL OTHER DIAGNOSTIC CRITERIA BUT WEIGHS BETWEEN 85 AND 90% OF EXPECTED WOULD STIL SMERIT THE DIAGNOSIS OF AN. THE CURRENT DIAGNOSTIC CRITERIA REQUIRE THAT WOMEN WITH AN NOT HAVE SPONTANEOUS MENSES, BUT OCCASIONAL PATIENTS WITH THE CHARACTERISTICS AND COMPLICATIONS OF AN DESCRIBE REGULAR MENSTRUATION. TWO MUTUALLY EXCLUSIVE SUBTYPES OF AN ARE SPECIFIED IN DSM-IV. PATIENTS WHOSE WEIGHT LOSS
TABLE 76-2 DIAGNOSTIC FEATURES OF ANOREXIA NERVOSA

REFUSAL TO MAINTAIN BODY WEIGHT AT OR ABOVE A MINIMALLY NORMAL WEIGHT FOR AGE AND HEIGHT. (THIS INCLUDES A FAILURE TO ACHIEVE WEIGHT GAIN EXPECTED DURING A PERIOD OF GROWTH LEADING TO AN ABNORMALLY LOW BODY WEIGHT.) INTENSE FEAR OF WEIGHT GAIN OR BECOMING FAT. DISTORTION OF BODY IMAGE (E.G., FEELING FAT DESPITE AN OBJECTIVELY LOW WEIGHT OR MINIMIZING THE SERIOUSNESS OF LOW WEIGHT). AMENORRHEA. (THIS CRITERION IS MET IF MENSTRUAL PERIODS OCCUR ONLY FOLLOWING HORMONE-EG., ESTROGEN-ADMINISTRATION.)

IS MAINTAINED PRIMARILY BY CALORIC RESTRICTION, PERHAPS AUGMENTED BY EXCESSIVE EXERCISE, ARE CONSIDERED TO HAVE THE “RESTRICTING” SUBTYPE OF AN. THE “BINGE EATING/PURGING” SUBTYPE IS CHARACTERIZED BY BINGE EATING AND SELF-INDUCED VOMITING AND/OR LAXATIVE ABUSE. PATIENTS WITH THE BINGE/PURGE SUBTYPE ARE MORE PRONE TO DEVELOP ELECTROLYTE IMBALANCES, ARE MORE EMOTIONALLY LABILE, AND ARE MORE LIKELY TO HAVE OTHER PROBLEMS WITH IMPULSE CONTROL, SUCH AS DRUG ABUSE. THE DIAGNOSIS OF AN CAN USUALLY BE MADE CONFIDENTLY IN A PATIENT WITH A HISTORY OF WEIGHT LOSS ACCOMPLISHED BY RESTRICTIVE DIETING AND EXCESSIVE EXERCISE, ACCOMPANIED BY A MARKED RELUCTANCE TO GAIN WEIGHT. PATIENTS WITH AN OFTEN DENY THAT THEY HAVE A SERIOUS PROBLEM AND MAY BE BROUGHT TO MEDICAL ATTENTION BY CONCERED FAMILY OR FRIENDS. IN ATYPICAL PRESENTATIONS, OTHER CAUSES OF SIGNIFICANT WEIGHT LOSS IN PREVIOUSLY HEALTHY YOUNG PEOPLE SHOULD BE CONSIDERED, INCLUDING INFLAMMATORY BOWEL DISEASE, GASTRIC OUTLET OBSTRUCTION, DIABETES MELLITUS, CENTRAL NERVOUS SYSTEM (CNS) TUMORS, OR NEOPLASM (CHAP. 41).

PROGNOSIS

THE COURSE AND OUTCOME OF AN ARE HIGHLY VARIABLE. ONE-QUARTER TO ONE-HALF OF PATIENTS EVENTUALLY RECOVER FULLY, WITH FEW PSYCHOLOGICAL OR PHYSICAL SEQUELAE. HOWEVER, MANY PATIENTS HAVE PERSISTENT DIFFICULTIES WITH WEIGHT MAINTENANCE, DEPRESSION, AND EATING DISTURBANCES, INCLUDING BN. THE DEVELOPMENT OF OBESITY FOLLOWING AN IS RARE. THE LONG-TERM MORTALITY OF AN IS AMONG THE HIGHEST ASSOCIATED WITH ANY PSYCHIATRIC DISORDER. APPROXIMATELY 5% OF PATIENTS DIE PER DECADE OF FOLLOW-UP, PRIMARILY DUE TO THE PHYSICAL EFFECTS OF CHRONIC STARVATION OR BY SUICIDE. VIRTUALLY ALL OF THE PHYSIOLOGIC ABNORMALITIES ASSOCIATED WITH AN ARE OBSERVED IN OTHER FORMS OF STARVATION AND MARKEDLY IMPROVE OR DISAPPEAR WITH WEIGHT GAIN. A WORRISOME EXCEPTION IS THE REDUCTION IN BONE MASS, WHICH MAY NOT RECOVER FULLY, PARTICULARLY WHEN AN OCCURS DURING ADOLESCENCE WHEN PEAK BONE MASS IS NORMALLY ACHIEVED.
ANOREXIA NERVOSA

Because of the profound physiologic and psychological effects of starvation, there is a broad consensus that weight restoration to at least 90% of predicted weight is the primary goal in the treatment of AN. Unfortunately, because most patients resist this goal, the management of AN is often accompanied by frustration for the patient, the family, and the physician. Patients typically exaggerate their food intake and minimize their symptoms. Some patients resort to subterfuge to make their weights appear higher, for example, by water-loading before they are weighed. In attempting to engage the patient in treatment, it may be useful for the physician to elicit the patient’s physical concerns (e.g., about osteoporosis, weakness, or fertility) and, provide education about the importance of normalizing nutritional status in order to address those concerns. The physician should reassure the patient that weight gain will not be permitted to get but of contrarbut simultaneously emphasize that weight restoration is medically and psychologically imperative. The intensity of the initial treatment, including the need for hospitalization, is determined by the patient’s current weight, the rapidity of recent weight loss, and the severity of medical and psychological complications (FIG. 76-1). Hospitalization should be strongly considered for patients weighing <75% of expected, even if the results of routine blood studies are within normal limits. Acute medical problems, such as severe electrolyte imbalances, should be identified and addressed. Nutritional restoration can almost always be successfully accomplished by oral feeding, and parenteral methods are rarely required. For severely underweight patients, sufficient calories (approximately 1200-1800 kcal/d) should be provided initially in divided meals as food or liquid supplements to maintain weight and to permit stabilization of fluid and electrolyte balance. Calories can then be gradually increased to achieve a weight gain of 1-2 kg (2-4 lb) per week, typically requiring an intake of 3000-4000 kcal/d. Meals must be supervised, ideally by personnel who are firm regarding the necessity of food consumption, empathic regarding the challenges entailed, and reassuring about the patient’s eventual recovery. Patients have great psychological difficulty complying with the need for increased caloric consumption, and the assistance of psychiatrists or psychologists experienced in the treatment of AN is usually necessary. Less severely affected patients may be treated in a partial hospitalization program where medical and psychiatric supervision is available and

476 PART 5: NUTRITION

FIGURE 76-1 AN ALGORITHM FOR BASIC TREATMENT DECISIONS REGARD-
ING PATIENTS WITH ANOREXIA NERVOSA OR BULIMIA NERVOSA. BASED ON THE AMERICAN PSYCHIATRIC ASSOCIATIONS PRACTICE GUIDELINES FOR THE TREATMENT OF PATIENTS WITH EATING DISORDERS. *ALTHOUGH OUTPATIENT MANAGEMENT MAY BE CONSIDERED FOR PATIENTS WITH ANOREXIA NERVOSA WEIGHING MORE THAN 75% OF EXPECTED, THERE SHOULD BE A LOW THRESHOLD FOR USING MORE INTENSIVE INTERVENTIONS IF THE WEIGHT LOSS HAS BEEN RAPID OR IF CURRENT WEIGHT IS <80% OF EXPECTED.

SEVERAL MEALS CAN BE MONITORED EACH DAY. OUTPATIENT TREATMENT MAY SUFFICE FOR MILDLY ILL PATIENTS. WEIGHT MUST BE MONITORED AT FREQUENT INTERVALS, AND EXPLICIT GOALS AGREED ON FOR WEIGHT GAIN, WITH THE UNDERSTANDING THAT MORE INTENSIVE TREATMENT WILL BE REQUIRED IF THE LEVEL OF CARE INITIALLY EMPLOYED IS NOT SUCCESSFUL. FOR YOUNGER PATIENTS, THE ACTIVE INVOLVEMENT OF THE FAMILY IN TREATMENT IS CRUCIAL REGARDLESS OF THE TREATMENT VENUE. PSYCHIATRIC TREATMENT FOCUSES PRIMARILY ON TWO ISSUES. FIRST, PATIENTS REQUIRE MUCH EMOTIONAL SUPPORT DURING THE PERIOD OF WEIGHT GAIN. PATIENTS OFTEN INTELLECTUALLY AGREE WITH THE NEED TO GAIN WEIGHT, BUT STRENUOUSLY RESIST INCREASES IN CALORIC INTAKE, AND OFTEN SURREPTITIOUSLY DISCARD FOOD THAT IS PROVIDED. SECOND, PATIENTS MUST LEARN TO BASE THEIR SELF-ESTEEM NOT ON THE ACHIEVEMENT OF AN INAPPROPRIATELY LOW WEIGHT, BUT ON THE DEVELOPMENT OF SATISFYING PERSONAL RELATIONSHIPS AND THE ATTAINMENT OF REASONABLE ACADEMIC AND OCCUPATIONAL GOALS. WHILE THIS IS OFTEN POSSIBLE, SOME PATIENTS WITH AN DEVELOP OTHER SERIOUS EMOTIONAL AND BEHAVIORAL SYMPTOMS SUCH AS DEPRESSION, SELF-MUTILATION, OBSESSIVE-COMPULSIVE BEHAVIOR, AND SUICIDAL IDEATION. THESE SYMPTOMS MAY REQUIRE ADDITIONAL THERAPEUTIC INTERVENTIONS, IN THE FORM OF PSYCHOTHERAPY, MEDICATION, OR HOSPITALIZATION.

MEDICAL COMPLICATIONS OCCASIONALLY OCCUR DURING REFEEDING. ESPECIALLY IN THE EARLY STAGES OF TREATMENT, SEVERELY MALNOURISHED PATIENTS MAY DEVELOP A ‘REFEEDING SYNDROME’ CHARACTERIZED BY HYPOPHOSPHATEMIA, HYPOMAGNESEMA, AND CARDIOVASCULAR INSTABILITY. ACUTE GASTRIC DILATATION HAS BEEN DESCRIBED WHEN REFEEDING IS RAPID. AS IN OTHER FORMS OF MALNUTRITION, FLUID RETENTION AND PERIPHERAL EDEMA MAY OCCUR, BUT THEY GENERALLY DO NOT REQUIRE SPECIFIC TREATMENT IN THE ABSENCE OF CARDIAC, RENAL, OR HEPATIC DYSFUNCTION. TRANSIENT MODEST ELEVATIONS IN SERUM LIVER ENZYME LEVELS OCCASIONALLY OCCUR. MULTIVITAMINS SHOULD BE GIVEN, AND AN ADEQUATE INTAKE OF VITAMIN D (400 IU/D) AND CALCIUM (1500 MG/D) SHOULD BE PROVIDED TO MINIMIZE BONE LOSS.

NO PSYCHOTROPIC MEDICATIONS ARE OF ESTABLISHED VALUE IN THE TREATMENT OF AN; TRICYCLIC ANTIDEPRESSANTS ARE CONTRAINDICATED WHEN THERE IS PROLONGATION OF THE QT INTERVAL. THE ALTERATIONS OF CORTISOL AND THYROID HORMONE METABOLISM DO NOT REQUIRE SPECIFIC TREATMENT AND ARE CORRECTED BY WEIGHT GAIN. EOS- TROGEN TREATMENT APPEARS TO HAVE MINIMAL IMPACT ON BONE DENSITY IN UNDERWEIGHT PATIENTS, AND THE SMALL BENEFIT OF BISPHOSPHONATE TREATMENT APPEARS TO BE OUTWEIGHED BY THE POTENTIAL RISKS OF SUCH AGENTS IN YOUNG WOMEN.
BULIMIA NERVOSA

EPIDEMIOLOGY

IN WOMEN, THE FULL SYNDROME OF BN OCCURS WITH A LIFETIME PREVALENCE OF 1-3%. VARIANTS OF THE DISORDER, SUCH AS OCCASIONAL BINGE EATING OR PURGING, ARE MUCH MORE COMMON AND OCCUR IN 5-10% OF YOUNG WOMEN. THE FREQUENCY OF BN AMONG MEN IS LESS THAN ONE-TENTH OF THAT AMONG WOMEN. THE PREVALENCE OF BN INCREASED DRAMATICALLY IN THE EARLY 1970S AND 1980S BUT MAY HAVE LEVELED OFF OR DECLINED SOMEWHAT IN RECENT YEARS.

ETIOLOGY

AS WITH AN, THE ETIOLOGY OF BN IS LIKELY TO BE MULTIFACTORIAL. PATIENTS WHO DEVELOP BN DESCRIBE A HIGHER-THAN-EXPECTED PREVALENCE OF CHILDHOOD AND PARENTAL OBESITY, SUGGESTING THAT A PREDISPOSITION TOWARD OBESITY MAY INCREASE VULNERABILITY TO THIS EATING DISORDER. THE MARKED INCREASE IN THE NUMBER OF CASES OF BN DURING THE PAST 25 YEARS AND THE RARITY OF BN IN UNDERDEVELOPED COUNTRIES SUGGEST THAT CULTURAL FACTORS ARE IMPORTANT. SEVERAL BIOLOGIC ABNORMALITIES IN PATIENTS WITH BN MAY PERPETUATE THIS DISORDER ONCE IT HAS BEGUN. THESE INCLUDE ABNORMALITIES OF CNS SEROTONERGIC FUNCTION, WHICH IS INVOLVED IN EATING BEHAVIOR, AND DISRUPTION OF PERIPHERAL SATIETY MECHANISMS, INCLUDING THE RELEASE OF CHOLECYSTOKININ (CCK) FROM THE SMALL INTESTINE.

CLINICAL FEATURES

THE TYPICAL PATIENT PRESENTING FOR TREATMENT OF BN IS A WOMAN OF NORMAL WEIGHT IN HER MID-TWENTIES WHO REPORTS BINGE EATING AND PURGING 5-10 TIMES A WEEK FOR 5-10 YEARS (TABLE 76-3). THE DISORDER USUALLY BEGINS IN LATE ADOLESCENCE OR EARLY ADULTHOOD DURING OR FOLLOWING A DIET, OFTEN IN ASSOCIATION WITH DEPRESSED MOOD. THE SELF-IMPOSED CALORIC RESTRICTION LEADS TO INCREASED HUNGER AND TO OVEREATING. IN AN ATTEMPT TO AVOID WEIGHT GAIN, THE PATIENT INDUCES VOMITING, TAKES LAXATIVES OR DIURETICS, OR ENGAGES IN SOME OTHER FORM OF COMPENSATORY BEHAVIOR. DURING BINGES, PATIENTS WITH THIS DISORDER TEND TO CONSUME LARGE AMOUNTS OF SWEET FOODS WITH A HIGH FAT CONTENT, SUCH AS DESSERT ITEMS. THE MOST FREQUENT COMPENSATORY BEHAVIORS ARE SELF-INDUCED VOMITING AND LAXATIVE ABUSE, BUT A WIDE VARIETY OF TECHNIQUES HAVE BEEN DESCRIBED, INCLUDING THE OMISSION OF INSULIN INJECTIONS BY INDIVIDUALS WITH TYPE 1 DIABETES MELLITUS. INITIALLY, PATIENTS MAY EXPERIENCE A SENSE OF SATISFACTION THAT APPEALING FOOD CAN BE EATEN WITHOUT WEIGHT GAIN. HOWEVER, AS THE DISORDER PROGRESSES, PATIENTS PERCEIVE DIMINISHED CONTROL OVER EATING. BINGES INCREASE IN SIZE AND FREQUENCY AND ARE PROVOKED BY A VARIETY
OF STIMULI, SUCH AS TRANSIENT DEPRESSION, ANXIETY, OR A SENSE THAT TOO MUCH FOOD HAS BEEN CONSUMED IN A NORMAL MEAL. BETWEEN BINGES, PATIENTS RESTRICT CALORIC INTAKE, WHICH INCREASES HUNGER AND SETS THE STAGE FOR THE NEXT BINGE. TYPICALLY, PATIENTS WITH BN ARE ASHAMED OF THEIR BEHAVIOR AND ENDEAVOR TO KEEP THEIR DISORDER HIDDEN FROM FAMILY AND FRIENDS. LIKE PATIENTS WITH AN, THOSE WITH BN PLACE AN UNUSUAL EMPHASIS ON WEIGHT AND SHAPE AS A BASIS FOR THEIR SELF-ESTEEM. MANY PATIENTS WITH BN HAVE MILD SYMPTOMS OF DEPRESSION. SOME PATIENTS EXHIBIT SERIOUS MOOD AND BEHAVIORAL DISTURBANCES, SUCH AS SUICIDE ATTEMPTS, SEXUAL PROMISCUITY, AND DRUG AND ALCOHOL ABUSE. ALTHOUGH VOMITING MAY BE

TABLE 76-3 DIAGNOSTIC FEATURES OF BULIMIA NERVOSA

RECURRENT EPISODES OF BINGE EATING, WHICH IS CHARACTERIZED BY THE CONSUMPTION OF A LARGE AMOUNT OF FOOD IN A SHORT PERIOD OF TIME AND A FEELING THAT THE EATING IS OUT OF CONTROL. RECURRENT INAPPROPRIATE BEHAVIOR TO COMPENSATE FOR THE BINGE EATING, SUCH AS SELF-INDUCED VOMITING. THE OCCURRENCE OF BOTH THE BINGE EATING AND THE INAPPROPRIATE COMPENSATORY BEHAVIOR AT LEAST TWICE WEEKLY, ON AVERAGE, FOR 3 MONTHS. OVERCONCERN WITH BODY SHAPE AND WEIGHT.

NOTE: IF THE DIAGNOSTIC CRITERIA FOR ANOREXIA NERVOSA ARE SIMULTANEOUSLY MET, ONLY THE DIAGNOSIS OF ANOREXIA NEIVOSA IS GIVEN.

PAGE NO. 120

477 CHAPTER 76 EATING DISORDERS

TRIGGERED INITIALLY BY MANUAL STIMULATION OF THE GAG REFLEX, MOST PATIENTS WITH BN DEVELOP THE ABILITY TO INDUCE VOMITING AT WILL. RARELY, PATIENTS RESORT TO THE REGULAR USE OF SYRUP OF IPECAC. LAXATIVES AND DIURETICS ARE FREQUENTLY TAKEN IN IMPRESSIVE QUANTITIES, SUCH AS 30 OR 60 LAXATIVE PILLS ON A SINGLE OCCASION. THE RESULTING FLUID LOSS PRODUCES DEHYDRATION AND A FEELING OF EMPTINESS BUT HAS LITTLE IMPACT ON CALORIC BALANCE. THE PHYSICAL ABNORMALITIES ASSOCIATED WITH BN PRIMARILY RESULT FROM THE PURGING BEHAVIOR. PAINLESS BILateral SALIVARY GLAND HYPTERTROPHY (SIALADENOSIS) MAY BE NOTED. A SCAR OR CALLUS ON THE DORSUM OF THE HAND MAY DEVELOP DUE TO REPEATED TRAUMA FROM THE TEETH AMONG PATIENTS WHO MANUALLY STIMULATE THE GAG REFLEX. RECURRENT VOMITING
AND
THE EXPOSURE OF THE LINGUAL SURFACES OF THE TEETH TO STOMACH ACID LEAD
TO
LOSS OF DENTAL ENAMEL AND EVENTUALLY TO CHIPPING AND EROSION OF THE
FRONT TEETH. LABORATORY ABNORMALITIES ARE SURPRISINGLY INFREQUENT, BUT
HYPOKALEMIA, HYPOCHLOREMIA, AND HYponATREMIA ARE OBSERVED OCCA-
SIONALLY. REPEATED VOMITING MAY LEAD TO ALKALOSIS, WHEREAS REPEATED
LAXATIVE ABUSE MAY PRODUCE A MILD METABOLIC ACIDOSIS. SERUM AMYLASE
MAY BE SLIGHTLY ELEVATED DUE TO AN INCREASE IN THE SALIVARY ISOENZYME.
SERIOUS PHYSICAL COMPLICATIONS RESULTING FROM BN ARE RARE. OLIGOMEN-
ORRHEA AND AMENORRHEA ARE MORE FREQUENT THAN AMONG WOMEN WITHOUT
EATING DISORDERS. ARRHYTHMIAS OCCASIONALLY OCCUR SECONDARY TO
ELECTROLYTE
DISTURBANCES. TEARING OF THE ESOPHAGUS AND RUPTURE OF THE STOMACH
HAVE
BEEN REPORTED AND CONSTITUTE LIFE-THREATENING EVENTS. SOME PATIENTS
WHO
CHRONICALLY ABUSE LAXATIVES OR DIURETICS DEVELOP TRANSIENT PERIPHERAL
EDE-
MA WHEN THIS BEHAVIOR CEASES, PRESUMABLY DUE TO HIGH LEVELS OF
ALDOSTER-
ONE SECONDARY TO PERSISTENT FLUID AND ELECTROLYTE DEPLETION.

DIAGNOSIS

THE CRITICAL DIAGNOSTIC FEATURES OF BN ARE REPEATED EPISODES OF BINGE
EAT-
ING FOLLOWED BY INAPPROPRIATE AND ABNORMAL BEHAVIORS AIMED AT AVOID-
ING WEIGHT GAIN (TABLE 76-3). THE DIAGNOSIS OF BN REQUIRES A CANDID
HISTORY FROM THE PATIENT DETAILING FREQUENT, LARGE EATING BINGES
FOLLOWED
BY THE PURPOSEFUL USE OF INAPPROPRIATE MECHANISMS TO AVOID WEIGHT
GAIN. MOST PATIENTS WITH BN WHO PRESENT FOR TREATMENT ARE DISTRESSED
BY
THEIR INABILITY TO CONTROL THEIR EATING BEHAVIOR BUT ARE ABLE TO
PROVIDE
SUCH DETAILS IF QUERIED IN A SUPPORTIVE AND NONJUDGMENTAL FASHION.
AS IN AN, THERE ARE TWO SUBTYPES OF BN. PATIENTS WITH THE “PURGING”
SUBTYPE UTILIZE COMPENSATORY BEHAVIORS THAT DIRECTLY RID THE BODY OF
CALORIES OR FLUIDS (E.G., SELF-INDUCED VOMITING, LAXATIVE, OR DIURETIC
ABUSE), WHEREAS THOSE WITH THE “NOnPURGING” SUBTYPE ATTEMPT TO COM-
PENSATE FOR BINGES BY FASTING OR BY EXCESSIVE EXERCISE. PATIENTS WITH
THE
NOnPURGING SUBTYPE TEND TO BE HEAVIER AND ARE LESS PRONE TO FLUID AND
ELECTROLYTE DISTURBANCES.

PROGNOSIS

THE PROGNOSIS OF BN IS MUCH MORE FAVORABLE THAN THAT OF AN. MORTAL-
ITY IS LOW, AND FULL RECOVERY OCCURS IN APPROXIMATELY 50% OF PATIENTS
WITHIN 10 YEARS. APPROXIMATELY 25% OF PATIENTS HAVE PERSISTENT SYMP-
TOMS OF BN OVER MANY YEARS. FEW PATIENTS PROGRESS FROM BN TO AN.
BULIMIA NERVOSA

BN CAN USUALLY BE TREATED ON AN OUTPATIENT BASIS (FIG. 76-1). COGNITIVE BEHAVIORAL THERAPY (CBT) IS A SHORT-TERM (4-6 MONTHS) PSYCHOLOGICAL TREATMENT THAT FOCUSES ON THE INTENSE CONCERN WITH SHAPE AND WEIGHT, THE PERSISTENT DIETING, AND THE BINGE EATING AND PURGING THAT CHARACTERIZE THIS DISORDER. PATIENTS ARE DIRECTED TO MONITOR THE CIRCUMSTANCES, THOUGHTS, AND EMOTIONS ASSOCIATED WITH BINGE/PURGE EPISODES, TO EAT REGULARLY, AND TO CHALLENGE THEIR ASSUMPTIONS LINKING WEIGHT TO SELF-ESTEEM. CBT PRODUCES SYMPTOMATIC REMISSION IN 25-50% OF PATIENTS. NUMEROUS DOUBLE-BLIND, PLACEBO-CONTROLLED TRIALS HAVE DOCUMENTED THAT ANTIDEPRESSANT MEDICATIONS ARE USEFUL IN THE TREATMENT OF BN BUT ARE PROBABLY SOMEWHAT LESS EFFECTIVE THAN CBT. ALTHOUGH EFFICACY HAS BEEN ESTABLISHED FOR VIRTUALLY ALL CHEMICAL CLASSES OF ANTIDEPRESSANTS, ONLY THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR FLUOXETINE (PROZAC) HAS BEEN APPROVED FOR USE IN BN BY THE U.S. FOOD AND DRUG ADMINISTRATION. ANTIDEPRESSANT MEDICATIONS ARE HELPFUL EVEN FOR PATIENTS WITH BN WHO ARE NOT DEPRESSED, AND THE DOSE OF FLUOXETINE RECOMMENDED FOR BN (60 MG/D) IS HIGHER THAN THAT TYPICALLY USED TO TREAT DEPRESSION. THESE OBSERVATIONS SUGGEST THAT DIFFERENT MECHANISMS MAY UNDERLIE THE UTILITY OF THESE MEDICATIONS IN BN AND IN DEPRESSION. A SUBSET OF PATIENTS DOES NOT RESPOND TO CBT, ANTIDEPRESSANT MEDICATION, OR THEIR COMBINATION. MORE INTENSIVE FORMS OF TREATMENT, INCLUDING HOSPITALIZATION, MAY BE REQUIRED.

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