Volume 2

GRADWOHL'S Clinical laboratory methods and diagnosis

A textbook on laboratory procedures and their interpretation

SEVENTH EDITION

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Preface

The goals for the seventh edition of *Gradwohl's Clinical Laboratory Methods and Diagnosis* are essentially the same as those for the sixth edition, the prime one being that this work serve as reference source, textbook, and laboratory manual. Major changes have been made, however, in material both added and deleted. Additions, for the most part, fall within the category of tests or technics to update the collection of methods. Deletions include obsolete material as well as subject matter considered inappropriate in a text of laboratory methods. In previous editions some highly specialized material was included that was not essential in a text of this nature. As laboratory and clinical criteria changed and the amount of essential material increased, it became necessary to consider the merit of each topic in light of space vs. current value. For this reason material thought to be too specialized, such as "hair in police technics" and "preservation of museum specimens," was eliminated to gain space needed for subjects more closely allied to the modern clinical laboratory. In a similar vein, clinical discussions of subjects such as electrocardiography and basal metabolism were omitted so that more emphasis could be placed on laboratory technics and pertinent discussions. Overall, the elimination of this material has resulted in some reduction in size, and yet all the subject matter necessary to produce a modern, current, and complete clinical laboratory text has been retained.

In **Clinical Chemistry** there have been many changes. A good deal of emphasis has been placed on enzymology. Several of the technics have been modified or changed, and additions have been made to include those of interest in urine examination and those determined by heat treatment. In other chapters deletions include many illustrations, all visual colorimetry calculations, and several methods no longer of clinical value. All the chapters concerning protein-bound iodine, lipids, steroid and catecholamine assays, toxicology, and automation have been completely rewritten.

Investigators who are leaders in their respective fields were selected to make the alterations and additions. Dr. Sidney Goldenberg, past president of the St. Louis Clinical Diabetes Society and program chairman for the St. Louis Diabetes Association, has expanded and updated the discussion of carbohydrate metabolism and current concepts of glucose tolerance tests. The chapters Protein-bound Iodine and Blood Lipids have been written jointly by Dr. Bennie Zak and Dr. Eugene S. Baginski, who have long been known for their creative work in many endeavors relating to analytical methods. Dr. Emanuel Epstein and Dr. John Lucas, both of whom have made significant contributions in their field, collaborated in preparing the chapters on steroids and catecholamines. Mr. Louis A. Williams, writing the chapter Toxicology, has had many years of experience in conceiving and adapting toxicologic technics to practical laboratory use and has published extensively. Dr. Gerald Kessler, extremely well known for his significant work in the field of automation, has expanded this chapter considerably to keep pace with modern laboratory trends. And in the area of rapid tests in urine analysis there is no one more qualified than Dr. Alfred H. Free, who has been instrumental in the development of many of the tests.

The section on **Hematology** (Bauer) has been written with a different emphasis from that of previous editions. Clinical hematology has been deemphasized, and the laboratory aspects have been expanded. This section now represents one of the most complete compilations of hematologic laboratory tests.

Hemoglobin is exhaustively discussed, including synthesis, catabolism, and detailed descriptions of the various abnormal forms.

The usual staining technics are described as well as the cytochemical stains used to demonstrate alkaline and acid phosphatases,

peroxidase, other enzymes, and nucleic acids.

Coagulation and hemorrhagic diseases are discussed with emphasis on the detailed description of the laboratory tests employed in the investigation of coagulation defects.

Normal blood findings in domesticated animals are once again included, but this material has also been rewritten and brought up to date.

The section on **Blood Groups, Transfusion, and Medicolegal Applications** (Erskine) has been completely revised, and many portions have been rewritten. The present edition contains discussions of such subjects as prevention of Rh isosensitization in pregnancy, coding of blood groups, projection of thoughts for future computerizing of the blood grouping reactions, the **LW** factor (discovered by Dr. Philip Levine), amniocentesis and possible intrauterine transfusion of the fetus in suspected erythroblastosis fetalis, immunoglobulins (especially in relation to antibodies), the zeta potential explanation of agglutination of erythrocytes, and added blood group factors (especially $\mathbf{Rh_{null}}$).

The discussion of the Lewis blood group system has been completely rewritten, and nomenclature in other systems has been revised. The glossary of terms used in immunohematology has been enlarged.

Additional topics discussed include chemistry of the blood group substances and automation in blood grouping. The blood groups and blood group factors in nonhuman primates is included, since this subject bears some relationship to the possible use of animal tissue for transplantation to human beings at some time in the future.

Any technic in blood grouping or in the identification of antigens or antibodies that needed revision has been brought up to date, and new charts on heredity of the blood groups now replace the lengthy ones that appeared in the sixth edition.

The section on **Tissue Cutting and Staining** (Ahlvin) has been completely rewritten. Only those procedures used in a modern tissue laboratory have been included. The section has been condensed, and the more exotic stains have been deleted.

Exfoliative Cytology (Pharr) has been revised and expanded to include a discussion of the estrogen index of vaginal smears.

The **Bacteriology** section has been completely rewritten to allow for a comprehensive treatment of the subject. Considerable effort was expended throughout the section to update and revise the nomenclature and classification of bacteria to conform, as far as possible, to the decisions of the International Committee on Nomenclature of Bacteria (International Association of Microbiological Societies) promulgated in the last decade and the changes effected in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (to be published in 1970). Additions to the text include many new methods and procedures (each accompanied by critical evaluation, description of rationale, and interpretation) and numerous new tables to allow rapid scanning of essential characteristics and reactions useful in identification.

The scope of the section's revision and of its usefulness as a reference guide is illustrated, among other ways, by the expansion and updating of the references: The bacteriology section in the sixth edition contained approximately 600 references, whereas in this edition there are about 1200; of these, over 800 are new.

A new chapter General Considerations (Sonnenwirth) includes a discussion of taxonomy, classification and nomenclature, characteristics of bacteria and their variants (L-forms, spheroplasts, and protoplasts), the scope of clinical bacteriology, equipment, and the beginning of automation in the bacteriology laboratory. The chapter Bacteriologic Methods (Sonnenwirth) includes material previously described in two separate chapters (General Methods and Special Methods). This change allowed incorporation of considerable new material on sterilization and culture technics, including the use of membrane filtration for both, and addition of enlarged and updated discussions on anaerobic culture methods and fluorescence microscopy technics. The new material on quality control deals with a subject of increasing importance and concern, which is now part of the statutes of the federal, state, and other regulatory agencies.

New or improved methods added in the chapters Stains and Staining Procedures and Media, Tests, and Reagents (Sonnenwirth) are, among others, the auramine-rhodamine fluorescent stain for acid-fast bacteria; selective media for anaerobes; the β-D-galactosidase (ONPG), oxidation-fermentation (O-F), and deoxyribonuclease (DNase) tests; and the phenylketonuria (PKU) inhibition assay. A separate section is devoted to advances in rapid and micromethods used for characterization and identification tests.

In the chapter Collection and Culture of Specimens and Guides for Bacterial Identification (Sonnenwirth), additions include, among others, screening methods for bacteriuria, an efficacious method for postmortem blood cultures, microbiology of contact lenses, and an updated schema for differentiation of intestinal pathogens, including the use of lysine-iron agar (LIA). The "guide to the presumptive recognition of common groups of bacteria" included here has been completely revised, and it now incorporates a number of newly described (or renamed) genera and species. Following, in part, the work of the late Elizabeth King and the concepts of Cowan and Steel, the guide is based on a few key characteristics (such as cell morphology, staining characteristics, oxygen requirements, oxidase and catalase reactions, and nature of attack on carbohydrates, i.e., fermentative, oxidative, or neither) that allow preliminary and presumptive placement of an unknown isolate into a larger group (genus or family); it also indicates additional selected characteristics and reactions (such as decarboxylation of certain amino acids) most likely to be useful for identification of the unknown. The guide has been critically evaluated in the laboratory and should prove practical and useful in aiding the laboratory worker to make a rational choice, *after referring to the appropriate and indicated chapter,* for proceeding with a choice of tests that will lead to the identification of the isolate within a reasonable span of time and without undue expenditure of a large number of often irrelevant media and tests. This approach and concern, emphasized throughout the entire Bacteriology section, have not heretofore been stressed in textbooks of this nature.

New to this edition are discussions on *Neisseria lactamicus, Kurthia, Actinomyces naeslundii* and *A. eriksonii, Bifidobacterium, Eubacterium, Catenabacterium, Ramibacterium, Yersinia enterocolitica, Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium,* and *Comamonas.* Considerable effort was expended on the nomenclature, description, and identification of nonfermentative aerobic gram-negative rods (oxidizers and nonoxidizers), which are becoming increasingly implicated in clinical infections (both hospital- and nonhospital-acquired) in man and also as contaminants of the hospital environment. Thus the sections on *Moraxella, Flavobacterium, Achromobacter (Acinetobacter;* Mima-Herellea), *Pseudomonas, Chromobacterium,* and *Actinobacillus* were completely rewritten and greatly expanded (Sonnenwirth). The section on *Vibrio,* as well as the chapters Gram-positive and Gram-negative Cocci, Gram-positive Bacilli (Sonnenwirth), and The Mycobacteria (Tarshis) similarly underwent extensive revision.

The discussion of Enterobacteriaceae was revised to include the nomenclatural system of W. H. Ewing (as emended in 1967), discussion of the genus *Edwardsiella,* and numerous newer tests and media useful in speciation.

The chapter Gram-negative Anaerobic Rods—Bacteroidaceae, which also includes a discussion of their role in pathophysiologic states and infectious processes, newly characterized forms, and a critical evaluation of all presently used technics for their isolation and identification, is a completely new contribution by Dr. Sydney M. Finegold, a leading authority in the field. Immunofluorescence identification of *Treponema pallidum* in lesions is incorporated in the chapter The Spirochetes (Sonnenwirth). The chapter Mycoplasma, completely rewritten and updated by Dr. Thomas R. Cate, deals with procedures for culture and identification of mycoplasma as well as with diseases caused by them.

The chapters The Bacteriophage and Bacterial Sensitivity Testing were completely revised by Dr. Lewis J. Griffith, well known for his contributions in these areas. The latter chapter includes the description and latest revision of the Kirby-Bauer single disk diffusion method. An enlarged section on hospital epidemiology was incorporated in the chapter Sanitary Bacteriology (Sonnenwirth), whereas Disinfectants, Sanitizers, and Antiseptics, a new chapter by Dr. Norman E. Dewar, who has had many years of experience in their development and evaluation, treats these subjects in considerable detail.

The section on **Serology** has undergone considerable revision. The chapter General Considerations now includes new material on the nomenclature and classes of immunoglobulins, antigen-antibody reactions, microtechnics, and automation in serology. In the chapter Tests for Syphilis the Hinton, Mazzini, Kline, and fluorescent treponemal antibody-200 (FTA-200) tests were eliminated, and the Rapid Plasma Reagin (RPR) (circle) Card Test and the fluorescent treponemal antibody-absorption (FTA-ABS) test were added (Son-

nenwirth). The chapter Bacterial Hemagglutination Tests, not available in other textbooks of this nature, was updated and revised by Dr. Erwin Neter who was instrumental in conceiving and developing many of the tests.

Additions to the chapter Miscellaneous Serologic Tests consist of greatly expanded sections on tests used in the diagnosis of rheumatoid arthritis, the detection of thyroid antibodies, and the detection of antinuclear antibodies (including immunofluorescence). A new chapter Tests for Pregnancy describes various qualitative and quantitative immunologic technics as well as biologic tests for detection of human chorionic gonadotropin (Sonnenwirth).

The gain in knowledge of the biochemical, biophysical, and biologic properties of viruses and the accompanying advances in the laboratory diagnosis of viral diseases are reflected in the extensive revision of the section on **Viral and Rickettsial Diagnostic Procedures** by Dr. J. Mehsen Joseph.

In the chapter Classification of Viruses and General Considerations, viruses are now classified on the basis of their chemical composition and architecture of the virion, eliminating an earlier classification scheme that was based arbitrarily on tissue affinities and clinical symptomatology. Discussion of infectious hepatitis (IH) and serum hepatitis (SH) viruses, Australia antigen, papovaviruses, and the Epstein-Barr (EB) virus has been added, and the material on Coxsackie viruses, echoviruses, adenoviruses, and reoviruses has been brought up to date.

Procedures on the preparation and propagation of human amnion cells, monkey kidney tissue, and chick embryo fibroblast cultures were added to the chapter Tissue Culture and Chick Embryo Technics. A new section is devoted to technics of detection of mycoplasma contamination in tissue cell culture and its eradication and to adventitious agents in tissue culture.

In the chapter Routine Procedures for Isolation and Identification of Viruses, the discussion on the viral spectrum of tissue cell cultures now includes several continuous heteroploid and also diploid cell lines; the completely revised, detailed, tabular directions on clinical materials to be submitted for diagnosis of viral and related diseases should be of considerable value. Identification of echoviruses by the intersecting serum schema, typing of adenoviruses and enteroviruses by hemagglutination-inhibition tests, and the plaque technic for isolation of viruses represent further additions.

The chapter Serologic Diagnosis of Viral Infections now incorporates the rubella and rubeola hemagglutination-inhibition technics and the indirect fluorescent antibody (IFA) test for titration of rubella antibodies. Detailed instructions for the fluorescent antibody (FA) technic for diagnosis of rabies were added to the chapter Cytologic and Cytochemical Technics for the Study of Viral Infections.

The section on **Medical Parasitology,** extensively revised by Dr. Michael H. Ivey, treats both protozoa and helminths in complete detail, with methods of transmission, host-parasite relationships, morphology, and laboratory diagnosis presented for each parasite. New illustrations have been added to the chapter Arthropods.

The inclusion of *Entamoeba hartmanni* and the discussion of amoebic meningoencephalitis and *Naegleria, Hartmannella,* and *Acanthamoeba* in the chapter Phylum Protozoa are examples of the completeness of this section.

Of invaluable aid will be the completely rewritten chapter Laboratory Procedures in Parasitology. Among the considerable number of technical procedures added are the trichrome staining technic, a method for permanent mounting of helminth eggs, the Cleveland-Collier medium for *Entamoeba histolytica,* Stoll procedure for egg count, the Tobie and Weinman media for cultivation of trypanosomes, examination of spinal fluid for detection of *Toxoplasma* and soil amebae *(Naegleria),* and directions for examination of duodenal, bile, liver, splenic, and proctoscopic aspirates and for various biopsy materials, lung lesions, and skin scrapings.

Two valuable, concise aids incorporated are a "specimen-parasite summary," which lists the frequency of use of a particular specimen in detection of specified parasites, and the "examination procedure summary," which indicates for each parasite the proper specimen and examination technic to be used—features not usually found in textbooks of laboratory medicine. The immunodiagnostic tests are updated with added discussions of various fluorescent antibody, hemagglutination, and hemagglutination-inhibition technics.

The section on **Medical Mycology** has been completely rewritten by Dr. George S. Kobayashi and Dr. Demosthenes Pappagianis, both of whom have made significant contributions in their field. It is an up-to-date, advanced

guide to laboratory technics and a concise description of mycotic diseases. Emphasis has been placed on methods for study of medically important fungi by inclusion of numerous new tables and an extraordinarily large number of new photomicrographs and other illustrations to facilitate the handling and examination of clinical specimens suspected of containing fungus organisms and their identification on culture.

Because of the apparent increase in the incidence of opportunistic fungal infections caused by ubiquitous saprobic fungi, the importance of a working knowledge of these organisms is discussed, along with suggested methods for maintenance of stock culture collections.

The chapter The Mycoses has been organized according to the tissue levels representing the primary focus of infection, i.e., superficial, cutaneous, subcutaneous, and systemic infections. An exception is the material on yeastlike fungi of the genera *Candida* and *Cryptococcus,* since these organisms are handled in the laboratory in a manner different from that used for the filamentous fungi.

Additions to the section include, among others, a new selective medium for the isolation of *Cryptococcus neoformans,* details of the fermentation and assimilation patterns of several *Candida* species, and a comprehensive, detailed tabular listing that indicates for each suspected mycotic disease the specimens needed and the recommended and preferred isolation technics and media. Available immunologic technics, i.e., skin tests and serologic procedures, including immunofluorescent methods wherever applicable, are discussed for each mycotic disease.

Once again the index has been expertly prepared by Mrs. Addine G. Erskine, and the extensive use of cross-references will enable the reader to locate any desired material rapidly and without frustration. It is only through the serious effort and the excellent cooperation of all the contributors that the preparation of this text was possible. We wish to express to them our sincere thanks and indebtedness.

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Contents

Volume 1

Part III HEMATOLOGY

Part IV BLOOD GROUPS, TRANSFUSION, AND MEDICOLEGAL APPLICATIONS

Color plates

PART VII

MICROBIOLOGY

Section one: Bacteriology

Section two: Serology

Section three: Viral and rickettsial diagnostic procedures

Section four: Medical parasitology

Section five: Medical mycology

Chapter 59

General considerations

Alex C. Sonnenwirth

CLASSIFICATION AND NOMENCLATURE

The taxonomic position of microorganisms has been the subject of continuous debate for many years. Bacteria, algae, and fungi were assigned many years ago to the plant kingdom and the protozoa to the animal kingdom. Although many biologists still adhere to this practice, it has become increasingly clear that these microorganisms cannot be assigned unequivocally to either of the 2 kingdoms, since their characteristics cut across the accepted definition of animals and plants. For example, many bacteria are motile (a characteristic of animals) but at the same time possess cell walls (a hallmark of plants). During their growth plants and animals develop highly differentiated tissue forms, consisting of specialized cells, whereas among microorganisms this does not occur; even among the multicellular forms, the cells are essentially alike. The establishment of a third kingdom, the *Protista* (Haeckel, 1866), has been proposed to accommodate these microorganisms characterized by relatively simple organization. Although not yet universally accepted, the concept of grouping bacteria, algae, fungi, and protozoa as protists has gained widespread support. Some protists are "plantlike" or "animal-like," and many share certain characteristics common to both animals and plants; however, all are distinguishable from higher animals and plants by virtue of their simple organization referred to above.[1]

The protists can be divided into 2 groups on the basis of their cellular structure,[1]—the higher protists (most algae, the fungi, and the protozoa) with **eucaryotic** cells and the lower protists (the blue-green algae and all bacteria) having **procaryotic** cells. The eucaryotic cells are like those of plants and animals—they possess a nuclear membrane, a mitotic apparatus, more than 1 chromosome, and mitochondria. The procaryotic cells are smaller, have no nuclear membrane and no mitotic apparatus, and have but a single chromosome (or, in different terms, the nuclear material is not organized into individual chromosomes). One group of infectious agents, the viruses, cannot be discussed in this framework, since their structure (the complete viral particle, the virion) is not comparable with that of a cell and their mode of multiplication (replication) is fundamentally different from that of cellular organisms in its total dependence on the synthetic system (enzymes and precursors) of the host cell.

It is beyond the scope of this introduction to discuss the difficulties inherent in creating a universally acceptable and applicable system for the classification of bacteria. It should suffice to point out that the classification of higher animals and plants is based on a phylogenetic, natural system in which the organisms are grouped according to the degree of their genetic relatedness and evolutionary relationships, whereas until very recently little or no evidence of such relationships was available for bacteria beyond their procaryotic cell nature. Thus systems of bacterial classification had to be constructed as more or less arbitrary descriptive keys based on a mixture of known structural and physiologic characteristics of bacteria.[1]

Recently the chemical study of deoxyribonucleic acid (DNA, the carrier of the genetic code) has shown a promising way of determining, to some degree, at least, the genetic relatedness of bacteria. The DNA molecule is a duplex structure; it consists of 2 strands wound around each other helically. The strands carry sequences of the 4 nucleotide bases adenine, guanine, thymine, and cytosine. Adenine is always opposite thymine, and guanine is opposite cytosine. The study of DNA base composition, expressed as the mean molar guanine + cytosine content (mole% G + C) has opened up a new approach to establishment of intrageneric and intergeneric relationships.[2] When