LABORATORY TECHNIQUE IN BIOLOGY AND MEDICINE

BY

E. V. COWDRY

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PREFACE TO THE THIRD EDITION

The request by The Williams & Wilkins Company to prepare a Third Edition so soon after the Second Edition was published (1948) was unexpected. It would have been a simple matter for them simply to reprint the Second Edition in whatever number they thought that they could sell within a reasonable period. This, however, would have been a short-sighted policy for there have been great advances in laboratory technique since the material for the Second Edition was assembled in 1947 prior to publication in 1948, which advances should be included. In fact the advances made, or reported, in the four years 1948, 1949, 1950 and 1951 are probably greater than those accomplished in any previous 10 year period. Though some of the modifications of standard techniques that proved useful during the war as well as altogether new techniques were published before 1948, many, while known to specialists in various fields, had not been gathered together in convenient form from which laboratory workers as a whole could select those most likely to be helpful in their particular problems. It is hardly necessary to note that the vast amount of new work in the four years mentioned, reflecting the appreciation of the value of research in medicine and biology gained in the war, and implemented by an unprecedented outpouring of funds for research, has resulted in the discovery of new and better means of revealing the structure of organisms from the highest to the lowest in health and disease. The electron microscope has found its way into about 300 laboratories. Satisfactory methods for cutting the extremely thin sections required have been devised. There is a rapprochement between what one can see at very high magnification and increasing knowledge of molecular structure and orientation determined by several methods. The phase microscope likewise has been produced in quantity in this country. The quality of moving pictures of living cells has thereby been greatly improved. Thanks to the Atomic Energy Commission radioactive isotopes have been made available. Plastics have been introduced in great variety. Microscopic localization of enzyme activities has been advanced. Microchemistry has leaped ahead. Quantitative analyses of extremely small amounts of material reduce the gap between chemistry and microscopy. The separation and collection en masse in a condition suitable for analysis of certain cellular components has been most helpful. And so on almost without end.

Obviously no single individual can authoritatively present these new techniques, as well as myriads of others of great value, because he cannot have personal experience with all of them. Partly to compensate for such limitations I have included descriptions of some of the key techniques written by the investigators who introduced them, or by others who have had extensive experience in their use. Most of these descriptions are new while others are revisions of accounts given in previous editions of this book. The name of each contributor

is given followed by the address (so that questions can be asked if desired) and the date (so that it can be seen at a glance when it was last checked for improvements). To all of these friends I am very grateful. Proofs of their contributions have not been submitted to them for corrections, because there are so many such accounts—most of them very brief—and the material presented by them was clear and concise. For typographical errors the responsibility is to be shared by me and the publisher. Most of the text I have written or revised and in doing so I have relied heavily on the reference books listed on page xxxi.

It is hardly necessary to explain that the policy is to provide brief accounts of techniques, and leads to others, and to make this information quickly available by alphabetical arrangement. It is better to give some data not required by experts than it is to write mainly for well-informed but limited groups. Obviously the said experts must approach fields other than their own as beginners handicapped by unfamiliarity with specialized technique and equipment. Because the several biological and medical sciences have so much to offer each other in the way of laboratory technique this exposure of opportunities may facilitate inter-specialty cooperation. My thanks are due to Mrs. Theresa Bresnahan for help in preparing the manuscript.

E. V. Cowdry

St. Louis

COOPERATION

My friends have generously contributed techniques written by them as follows:

G. Adolph Ackerman, Hinsman Hall, Ohio State University, Columbia 10, Ohio, May 24, 1951.

Auer Bodies.

Paul M. Aggeler, University of California Medical Center, San Francisco 22, California, November 15, 1951.

Blood Platelets.

Glenn H. Algire, U. S. Public Health Service, Bethesda Maryland, June 15, 1950.

Transparent Chamber Techniques.

James W. Bartholomew, Department of Bacteriology, University of Southern California, Los Angeles 7, California, July 9, 1951.

Gram Stains Mechanism.

H. W. Beams, Department of Zoology, University of Iowa, Iowa City, Iowa, September 27 1951.

Ultracentrifuges.

R. Bogoroch, Department of Anatomy, McGill University, Montreal, Canada, September 12, 1951.

Radioautographic Technique (With C. P. Leblond).

L. R. Boling, Department of Anatomy, Washington University School of Dentistry, St. Louis 10, Missouri, December 16, 1950.

Teeth Blood Vessels.

Teeth Decalcification.

Geoffrey Bourne, Department of Histology, London Hospital Medical School, London, England, November 5, 1951.

Golgi Apparatus.

Mitochondria.

Marian Pfingsten Bryan (Mrs. William T. K. Bryan), Department of Otolaryngology, Washington University School of Medicine, St. Louis 10, Missouri, September 20, 1951.

Ear Cell Smears.

Nasal Cell Smears.

E. J. Carey, Department of Anatomy, Marquette University School of Medicine, Milwaukee, Wisconsin, 1942 (now deceased).

Carey's Method for Motor End Plates.

Christopher Carruthers, Division of Cancer Research, Washington University, St. Louis 10, Missouri, May 12, 1950.

Oxidation-Reduction Potential.

Vitamins.

Polarographic Technique.

JANE E. CASON, Department of Pathology, Medical College of Alabama, Birmingham, Alabama, January 27, 1951.

Mallory-Heidenhain Rapid One-step Stain for Connective Tissue.

ROBERT CHAMBERS, Department of Biology, Washington Square College of New York University, New York, New York, May 15, 1950.

Micromanipulation.

E. R. CLARK, Department of Anatomy, University of Pennsylvania, and the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, November 28, 1951.

Transparent Chamber Technique.

BARRY COMMONER, The Henry Shaw School of Botany, Washington University, St. Louis 5, Missouri, November 27, 1951.

Microspectrophotometry.

A. H. COONS, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts, August 31, 1951.

Antigen Localization.

W. P. COVELL, Departments of Anatomy and Otolaryngology, Washington University School of Medicine, St. Louis 10, Missouri, October 26, 1951.

Ear.

E. W. DEMPSEY, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, February 26, 1951.

Cholinesterase.

Dehydrogenase.

Enzymes.

Esterases.

Nucleases.

Phosphatases.

W. T. DEMPSTER, Department of Anatomy, University of Michigan, Ann Arbor, Michigan and R. C. WILLIAMS, Department of Biochemistry, University of California, Berkeley, California, June 9, 1950.

Shadow Casting.

O. H. DUGGINS, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, May 18, 1950.

Hairs (With Mildred Trotter).

F. DURAN-REYNALS, Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut, October 8, 1951.

Spreading Factors.

W. R. EARLE, National Cancer Institute, Bethesda, Maryland, July 10, 1951.

Tissue Culture.

LÁRUS EINARSON, Normal-Anatomisk Institut, Aarhus Universitet, Aarhus, Denmark, February 27, 1951.

Gallocyanin-Chromalum Staining of Basophilic Cell Structures.

JOSEPH A. FALZONE, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, October 26, 1951.

Desoxyribose Nucleic Acid, Method for Determination In Isolated Hepatic Nuclei.

Differential Centrifugation of Cell Particulates.

HONOR B. FELL, Strangeways Research Laboratory, Cambridge, England, June 8, 1951.

Organ Culture in Vitro.

F. H. J. FIGGE, Department of Anatomy, University of Maryland Medical School, Baltimore, Maryland, October 10, 1951.

Porphyrins.

Hematoporphyrin.

H. I. FIRMINGER, Pathology Section, National Cancer Institute, Bethesda, Maryland, February 9, 1951.

Carbowax Embedding.

E. D. GARDNER, Department of Anatomy, Wayne University School of Medicine, Detroit, Michigan, June 15, 1950.

Articular Nerve Terminals.

DAVID GLICK, Department of Physiological Chemistry, University of Minnesota Medical School, Minneapolis 14, Minnesota, October 17, 1951.

Linderstrøm-Lang, Kaj. U., and Holter Heinz, Histochemical Advances.

MORRIS GOLDMAN, Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, January 29, 1951.

Iron Hematoxylin Single Stain.

G. GOMORI, Department of Medicine, University of Chicago, Chicago, Illinois, May 7, 1950.

Gomori's Method for Reticulum and Acid Phosphatase.

A. R. GOPAL-AYENGAR, Barnard Free Skin and Cancer Hospital, St. Louis, Missouri, September 10, 1946 (now Tata Memorial Hospital, Bombay).

Chromosomes.

Hyaluronic Acid.

H. S. N. GREENE, Laboratory of Pathology, Yale University School of Medicine, New Haven, Connecticut, September 11, 1951.

Anterior Chamber Transplantation.

JOAN HABERMAN, Parkland, Washington, March 10, 1951.

Anethole Clearing Agent.

J. D. HAMILTON, Department of Medical Research, University of Western Ontario, London, Canada, February 13, 1951.

Cell Measurement, The Elliptometer.

N. L. HOERR, Department of Anatomy, School of Medicine, Western Reserve University, Cleveland 6, Ohio, November 28, 1951.

Frozen-Dehydration Method.

Isolation of Mitochondria.

R. D. HOTCHKISS, The Rockefeller Institute for Medical Research, New York 21, New York, November 14, 1951.

Polysaccharides.

M. H. KNISELY, Department of Anatomy, University of South Carolina, Charleston, South Carolina, June 27, 1950.

Quartz Rod Technique for Illuminating Living Organs.

N. B. KURNICK, Department of Medicine, Tulane University, New Orleans 12, Louisiana, January 31, 1951.

Aceto-Orcein-fast Green.

EDWARD L. KUFF, Department of Anatomy, Washington University School of Medicine, St. Louis, Missouri, December 18, 1951.

Nucleic Acid-Dye Interactions.

A. I. LANSING, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, October 5, 1951.

Collagenic Fibers.

Elastic Fibers.

A. LAZAROW, Department of Anatomy, Western Reserve University School of Medicine, Cleveland, Ohio, November 28, 1951.

Separation of Cell Components by Differential Centrifugation.

C. P. LEBLOND and R. BOGOROCH, Department of Anatomy, McGill University, Montreal, Canada, September 12, 1951.

Radioautographic Technique.

R. D. LILLIE, Division of Pathology, National Institute of Health, Bethesda, Maryland, May 3, 1950.

Azure or Toluidin Blue Eosin.

A. M. LUCAS, U. S. Regional Poultry Research Laboratory, East Lansing, Michigan, August 10, 1951.

Cilia (with M. S. Lucas).

M. S. LUCAS, Department of Biological Science, Michigan State College, East Lansing, Michigan, August 10, 1951.

Cilia (with A. M. Lucas).

Plastics.

B. J. LUYET, Department of Biology, St. Louis University, St. Louis, Missouri, January 15, 1951.

Revival after Ultra Rapid Cooling.

C. C. MACKLIN, Department of Anatomy, University of Western Ontario, London, Ontario, Canada, November 28, 1951.

Alveolar Epithelium.

Alveolar Fluid.

Alveolar Foam Cells.

Alveolar Pores.

Ammoniacal Silver.

Aquax.

Bronchiolar Epithelium.

Gash Irrigation Recovery Method for Lung Cells (G^1).

Heart Failure Cells.

Lungs, Uncollapsed, Fixation.

Pneumonocytes.

Silver Lineation on Pulmonary Alveolar Walls.

Carmine Dusting.
Dust Cells.
Tissue Phagocytes.
Vacuoloids.
Wash-out Recovery Method.

PAUL MASSON, Department of Pathology, University of Montreal, Montreal, Canada, October 24, 1951.
Masson's Trichrome Stain.

MORRIS MOORE, Barnard Free Skin and Cancer Hospital, St. Louis, Missouri, December 10, 1951.
Fungi.

NORMAN MOSKOWITZ, Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania, January 24, 1951.
Protein Silver for Staining Protozoa.

J. L. O'LEARY, Department of Neuropsychiatry, Washington University School of Medicine, St. Louis 10, Missouri, May 8, 1950.
Golgi-Cox Method.
Golgi Method, Quick.
O'Leary's Brazilin Method.

JEAN OLIVER, Department of Pathology, State University of New York, Brooklyn 2, New York, September 4, 1951.
Kidney.

DONALD L. OPDYKE, Department of Anatomy, Washington University School Medicine, St. Louis 10, Missouri, November 8, 1951.
Keratohyalin Granules, Separation and Analysis.

ROBERTSON PRATT, University of California, College of Pharmacy, San Francisco, January 29, 1951.
Triphenyltetrazolium Chloride.

EUGENE ROBERTS, Division of Cancer Research, Washington University, St. Louis 10, Missouri, July 15, 1951.
Paper Chromatography.

T. B. ROSENTHAL, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, June 6, 1951.
Fluorescence Microscopy.
Radioactive Isotopes.
Electron Microscopy.

C. H. SAWYER, Duke Hospital, Durham, North Carolina, December 16, 1950.
Safranin-Light Green.

FRANCIS O. SCHMITT, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, May 19, 1950.
Polarization Optical Method.

GORDON H. SCOTT, Department of Anatomy, Wayne University School of Medicine, Detroit, Michigan, January 16, 1951.
Altmann-Gersh Frozen Dehydration Method.
Microincineration.
Cryostat.

W. M. SHANKLIN, American University of Beirut, Beirut, Lebanon, March 30, 1951.

Pineal.

Silver Diaminohydroxide after Sensitizing with Sodium Sulfite for Neuroglia.

R. E. STOWELL, Department of Oncology, University of Kansas Medical Center, Kansas City 2, Kansas, January 19, 1951.

Photoelectric Microphotometer.

CHARLOTTE M. STREET, Department of Anatomy, Cornell University Medical College, New York, New York, May 21, 1951.

Papanicolaou Techniques in Exfoliative Cytology.

LLOYD E. THOMAS, Department of Biochemistry, University of Missouri School of Medicine, Columbia, Missouri, July 8, 1951.

Arginine Reaction.

MILDRED TROTTER and O. H. DUGGINS, Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri, May 8, 1951.

Hairs.

L. F. WICKS, Veterans Administration Hospital, Jefferson Barracks, Missouri, February 1, 1951.

Hydrogen Ion Indicators.

Piccolyte Resins.

G. B. WISLOCKI, Department o Anatomy Harvard Medical School, Boston 15, Massachusetts, March 8, 1951.

Placenta.

R. C. WILLIAMS, Department of Biochemistry, University of California, Berkeley, California, June 9, 1950.

Shadow-Casting (with W. T. Dempster)

PREFACE TO THE FIRST EDITION

What appeared altogether impossible twenty-five years ago has in several cases been attained by improvements in technique. Who would have believed at that time that ultramicroscopes would now be manufactured in quantity, built without any optical lenses, and capable of revealing a world of structures quite beyond their ken? Who would have thought that a whole series of different atoms could be tagged and their distribution to the several tissues, when introduced into the body, accurately measured? Who would have anticipated the significant and unexpected new developments which have been made in polarization optical methods? Had we been told twenty-five years ago that the cell itself can be broken up into parts several of which can be collected in quantity and chemically analyzed, we would have been incredulous. All this and more has been achieved as a result of team work between the biological and physical sciences. And we may believe that more surprises are in store.

Yet some of us individually are still extraordinarily conservative in the methods we use. The possibilities of improving old techniques, of replacing some of them by new ones and of relying more upon microchemical and physical procedures are not explored as they should be. The purpose of this book is to extend the horizon by exposing in an introductory way a few of the many opportunities awaiting workers in biology and medicine interested in the minute structure of living things. Success will depend upon ability to anticipate and meet the needs of those likely to consult it. Definite information about specific matters is likely to be more in demand than general statements. The latter are limited to a few pages and deal with "choice of methods" and "organization of laboratory."

Some may turn to the names of the structures in which they happen to be most interested at the moment—**Nissl Bodies, Nerve Fibers, Capillaries** and so forth—on the off chance of finding some useful hints as to methods better adapted for their microscopic study, the most likely experimental errors and so on. Because the range of cells, parts of cells, tissues, organs and systems is obviously so immense, mention is only possible of a small proportion of them so that much depends on the selection made.

Others may seek information under the headings of elements such as **Iron, Potassium** and **Calcium,** of enzymes like **Pepsin** and **Phosphatase** and of many other components of living material. It is difficult to draw the line but most of those that can be localized microscopically are mentioned, likewise techniques for the determination of permeability, viscosity, pH and other properties of tissues.

It often happens, however, that data are required about a particular technique, which the workers are using or expect to use, and which is known to them by the names of those who discovered it, as for example the methods of **Giemsa** and

Mallory. Consequently information also must be supplied under various names though this is usually less satisfactory than under subjects. A very annoying handicap is the host of synonyms for dyes. Being ignorant of chemistry, I have with confidence listed those given by Dr. H. J. Conn. Many more will be found in The Colour Index of the Society of Dyers and Colourists.

Since all are busy people, time is a factor and they will wish to dig out what they want as directly and quickly as possible. It is for this reason that everything is listed alphabetically. Obviously this book can be nothing more than a brief entré to microscopic technique. Therefore, numerous references to the literature are supplied for follow up. Again to save time, these are given each in its appropriate place, thus avoiding the necessity of turning the pages and locating them in a large bibliography. But no attempt is made to trace the techniques to their original exponents and to apportion credit for numerous modifications. Often the most recent and accessible reference is provided relying on the author to state history fairly. Evidently, in order to keep up to date as to methods, the reader must repeatedly consult the latest issues of many journals. Stain Techn.; J. Lab. & Clin. Med.; J. Tech. Meth.; Bull. d'Hist. Appl.; and Zeit. f. mikr. Tech. are particularly valuable.

Finally I wish to thank my colleagues for their help, particularly Drs. L. R. Boling, C. Carruthers, William Cramer, Morris Moore, J. L. O'Leary, W. L. Simpson, R. E. Stowell, Lester Wicks and Dr. H. J. Conn, Chairman of the Biological Stain Commission, who very kindly read the manuscript and made several useful suggestions.

CHOICE OF METHODS

The selections will depend on several considerations. The first is what one wants to discover. Many investigators in biology and medicine wish to obtain more information about structural components of the body whether gross, microscopic or submicroscopic in size. They desire to obtain further data on the physical and chemical properties of these components whether solid or fluid in different physiological stages of activity and in disease in both man and in animals. They are interested in subjects, not personalities, so that in this alphabetically arranged presentation the names of individuals are seldom listed. Emphasis is given to subjects. After all the problems continue while the names of generation after generation of individuals fade out.

Those who perchance may consult this book will need help at two levels. It may be a simple question of the nature of some dye, or the composition of some solution, or the making of a well known test, or it may be the selection of a technique to be employed in a comprehensive series of experiments. In the latter case it involves a major decision. It is necessary not only to choose the technique most likely to lead to the answer but also give some thought to the equipment required and the training demanded for its proper use.

A few leading references to the most recent utilization of the technique in question may be helpful. But to discuss the history of its development and to assess priority is not attempted. A complete discussion of the literature may indeed constitute a handicap by providing an excuse for doing nothing. To be stuffed with information may give a feeling of frustration.

Some chemical and physical procedures are well within the reach of people who are neither chemists nor physicists while others will simply lead them beyond their depth into futility. It is equally true that well trained chemists and physicists are likely to fail to realize the complexity of vital processes and to make little progress through ignorance of physiology and pathology. Consequently one should never hesitate to seek advice from friends in other departments.

1. To Examine Directly *in Vivo*

The ideal arrangement is to look into the body and to study its parts as they function without causing any disturbance. With protozoa and certain small transparent invertebrates this is relatively simple. The web of a frog's foot is thin and can easily be looked through without seriously interfering with the frog. Some other parts of the bodies of various aquatic lower forms lend themselves to direct examination *in vivo*; but there are definite limitations in such a study of what is going on in the human body. It is possible to peer into the various apertures but to get close enough to the living tissues to use high magnifications is not feasible. The cornea and lens of the eye are transparent and

much valuable information can be secured by direct examination of the retinal blood vessels. Even here their distance from the surface is considerable and magnification is therefore limited. As far as we know at present the best that can be done is to take advantage of a discovery, made by Lombard (W. P., Am. J. Physiol., 1911–12, **29,** 335–362) that the epidermis can be rendered transparent by the addition of a little highly refractile oil without noticeably injuring it or disturbing the underlying tissues. By this means the blood vessels of the dermal papillae in the fold of skin over the nail bed, which are very near to the surface, can be studied directly at fairly high magnification and over long periods of time thus permitting the making of excellent pictures. See review of literature by Wright, I. S. and Duryee, A. W., Arch. Int. Med., 1933, **52,** 545–575.

That the lymphatics in the human skin can be made visible *in vivo* by the injection of small amounts of Patent Blue V has been demonstrated by Hudack, S. S. and McMaster, P. D., J. Exp. Med., 1933, **57,** 751–774. The vessels in the ears of living mice can readily be seen without any surgical procedure. It is even possible to directly watch the dye, Chicago blue, after intravenous injection elsewhere in the body, leak out into the tissues especially through the walls of the venules (Smith, F. and Rous, P., J. Exp. Med., 1931, **54,** 499–514). Ideas as to the relative hydrogen ion concentrations of some of the tissues visible from without can be secured by the injection of **Hydrogen Ion Indicators** (Rous, P., J. Exp. Med., 1925, **41,** 739–759). The opportunities are many especially in animal experimentation.

Another way to examine structure *in vivo* is to record the structure by x-ray photographs and to magnify the photographs, see **Microradiographic** examination.

2. To Examine through Windows *in Vivo*

The construction of windows in the skin or body wall through which the tissues can be examined *in vivo* is a less ideal technique because it involves surgical interference with the body. In the most used of these techniques a hole is made through a rabbit's ear from one surface to the other. A glass chamber is then sewed into the hole in such a way that a blood vessel is included between a thin layer of glass (serving as a cover glass) and a thicker one serving as a slide. After a time the epidermis adheres to the edges of the chamber and blood vessels, nerves and other tissues grow into it where they can be studied under oil immersion objectives. This technique was first reported by Sandison (J. C., Anat. Rec., 1924, **28,** 281) working under Dr. E. R. Clark at the University of Pennsylvania. It has since been very greatly improved (Clark, E. R., et al., Anat. Rec., 1930, **47,** 187–211 and Abell, R. G., and Clark, E. R., Anat. Rec., 1932, **53,** 121–140) by the introduction of "round table" and "moat" chambers.

To place a window in the wall of the skull and to observe what is going on within has been done with more or less success on several occasions. The technique devised by Forbes (H. S., Arch. Neurol. and Psych., 1928, **19,** 75) permits direct observation at low magnification of the blood vessels over the cerebral

convolutions with so little injury that their behaviour in various experimental conditions can be investigated (see also Clark, E. R., and Wentsler, N. E., Proc. Assoc. Res. Nerv. and Ment. Dis., 1937, **18**, 218–228). Through a window in the thoracic wall Wearn and his associates (Wearn, J. T. et al., Am. J. Physiol., 1934, **109**, 236–256) have similarly studied the pulmonary arterioles and capillaries. They employed a fused quartz cone to conduct light to the tissue.

Other investigators have availed themselves of the natural window, the cornea, through which what goes on immediately within it in the anterior chamber of the eye can be observed. Several tissues have been successfully transplanted into this chamber. Perhaps the most dramatic is the behavior of transplanted uterine mucosa in the rhesus monkey. In it the menstrual changes can be seen in detail and the influence of hormones noted (Markee, J. E., Contrib. to Embryol., Carnegie Inst. of Washington, 1940, **28**, 219–308). For some kinds of work the fact that the tissue fluid (aqueous humor) in this chamber differs from others in the same animal by the absence of certain species specific growth inhibiting factors is a priceless asset. Thus Greene (H. S. N., Science, 1938, **88**, 357–358) was able to grow pieces of human cancers, which ordinarily quickly die in other species, in the anterior chambers of the eyes of some mammals. The existence of a barrier protecting this fluid against the entry of antibodies from blood plasma and thus making possible the growth of tumor transplants, while all other tissues are resistant to their growth, has recently been emphasized (Saphir, O., Appel, M. and Strauss, H. A., Cancer Res., 1941, **1**, 545–547).

In order to view the less accessible living tissues, techniques have been devised that include opening the body and partly withdrawing the organ so that it can be placed on the stage of a microscope but with circulation and nerve supply intact and adequate regulation of temperature and humidity. Particularly fruitful has been the direct observation through oil immersion objectives of secretion by acinous cells of the **Pancreas** by Covell (W. P., Anat. Rec., 1928, **40**, 213–223) and of islet cells by O'Leary, (J. L., Anat. Rec., 1930, **45**, 27–58). Thus the influence of drugs on the secretory process can now be followed in minute detail.

Knisely (M. H., Anat. Rec., 1936, **64**, 499–523; **65**, 23–50) has perfected a technique for the study of the living **Spleen** at somewhat lower magnification. The essential features are slight displacement of the spleen so that it can be transilluminated by light delivered through a quartz rod. This allows for the first time direct examination of the behavior of the venous sinuses. Undoubtedly the **Quartz Rod** technique will be of great service in providing light for similar examination of other organs.

3. To Study the Arrangement of Parts in the Body

Since the body is structurally so very complex it is often illuminating to view parts of it in their normal shape and size but unobscured by all the neighboring components. There are several ways by which this can be accomplished.

The first method of **Reconstruction** from serial sections is well known. Briefly stated the particular tissue, organ or system is outlined, as it appears in section after section, at the desired magnification on sheets of material of uniform and carefully selected thickness. The outlined areas are then cut out and when superimposed they constitute a reconstruction of the original structure. This technique is tedious but it may reveal topographical relations that can be discovered by no other means.

The second kind of technique is to make casts of vascular, respiratory and other lumina. Woods' metal, formerly used for this purpose, has now been almost displaced by **Celloidin** and other substances. The surrounding tissue is freed from the cast by digestion in concentrated hydrochloric acid and gentle brushing away in a stream of water. Very beautiful **Corrosion** preparations of the lungs and kidneys have been obtained by this method which should be more widely employed.

The third is by **Maceration** to soak the organs, without previous preparation, in fluids that either digest away the tissues which it is desired to eliminate or loosen their connections with those under investigation, which, latter, can then be individually examined. Techniques of this sort are the only available means for the isolation of individual seminiferous and renal tubules. Oliver's researches on the kidney illustrate the value of reconstruction and maceration in pathology. Only three other examples will be submitted. **Thyroid follicles** can be isolated by maceration (Jackson, J. L., Anat. Rec., 1931, 48, 219–239). Their study as individuals provides data as to size and shape only obtainable otherwise by the tedious examination of serial sections. The **Epidermis** is so tightly bound to the underlying dermis that separation is extremely difficult; but, after treatment of skin with dilute acetic acid, the attachment is loosened and the epidermis can readily be removed as a complete sheet of tissue which can be stained, made transparent and examined as a whole mount. Opportunities are thus afforded for the detection of regional differences which might not be located even by painstaking study of sections and the making of mitotic counts is greatly facilitated. By macerating in the same fashion the nasal mucous membrane covering the septum can also be removed for study. Perhaps still other epithelial sheets can be similarly isolated. However such sheets are of little value for chemical analysis because of the action of the acetic acid. Fortunately it has been found that the epidermis may also be quickly loosened by simply heating the skin to 50°C. when it can be peeled off like the covering of a scalded tomato (Baumberger, J. P., Suntzeff, V. and Cowdry, E. V., J. Nat. Cancer Inst., 1942, 2, 413–423).

There is still another alternative. Instead of simply omitting the unwanted material by reconstructing only the structures chosen for demonstration, or of removing the material by corrosion or maceration, it can be left in and rendered transparent so that it does not obstruct the view. After marking the particular structures by vital dyes or other means the whole tissue is cleared by the method of **Spalteholz** or **Schultze.** These techniques give admirable results in the study