METHODS IN MEDICAL RESEARCH

Volume 8 • H. D. BRUNER, *Editor-in-Chief*

METHODS IN

Medical Research

Volume 8

H. D. BRUNER, *Editor-in-Chief*

LIFE HISTORY OF THE ERYTHROCYTE, *Walter S. Root, Editor*

MEASUREMENT OF RESPONSES OF INVOLUNTARY MUSCLE,
A. M. Lands, Editor

PERIPHERAL BLOOD FLOW MEASUREMENT, *H. D. Bruner, Editor*

THE YEAR BOOK PUBLISHERS, INC.
200 EAST ILLINOIS STREET, CHICAGO

Library of Congress Catalog Card Number: 48-10150

PRINTED IN U.S A.

METHODS IN

MEDICAL RESEARCH

Volume 8

METHODS IN MEDICAL RESEARCH

VOLUME 1

VAN R. POTTER, *Editor-in-Chief*

ASSAY OF ANTIBIOTICS, *Henry Welch, Editor*
CIRCULATION—BLOOD FLOW MEASUREMENT, *Harold D. Green, Editor*
SELECTED METHODS IN GASTROENTEROLOGIC RESEARCH, *A. C. Ivy, Editor*
CELLULAR RESPIRATION, *Van R. Potter, Editor*

VOLUME 2

JULIUS H. COMROE, JR., *Editor-in-Chief*

METHODS OF STUDY OF BACTERIAL VIRUSES, *Mark H. Adams, Editor*
PULMONARY FUNCTION TESTS, *Julius H. Comroe, Jr., Editor*
ASSAY OF HORMONAL SECRETIONS, *Eleanor H. Venning, Editor*

VOLUME 3

RALPH W. GERARD, *Editor-in-Chief*

GENETICS OF MICRO-ORGANISMS, *S. E. Luria, Editor*
ASSAY OF NEUROHUMORS, *J. H. Gaddum, Editor*
SELECTED PSYCHOMOTOR MEASUREMENT METHODS, *Walter R. Miles, Editor*
METHODS FOR STUDY OF PEPTIDE STRUCTURE, *Choh Hao Li, Editor*

VOLUME 4

MAURICE B. VISSCHER, *Editor-in-Chief*

HISTOCHEMICAL STAINING METHODS, *George Gomori, Editor*
FLUID AND ELECTROLYTE DISTRIBUTION, *Louis B. Flexner, Editor*
STUDIES ON GASTROINTESTINAL PRESSURES, INNERVATION AND SECRETIONS, *J. P. Quigley, Editor*
TISSUE CULTURE METHODS, *C. M. Pomerat, Editor*

(continued on next page)

METHODS IN MEDICAL RESEARCH

VOLUME 5

A. C. CORCORAN, *Editor-in-Chief*

METHODS FOR SEPARATION OF COMPLEX MIXTURES AND HIGHER MOLECULAR WEIGHT SUBSTANCES, *Lyman C. Craig, Editor*
METHODS OF RENAL STUDY, *A. C. Corcoran, Editor*
IMMUNOCHEMICAL METHODS OF DETERMINING HOMOGENEITY OF PROTEINS AND POLYSACCHARIDES, *Melvin Cohn, Editor*

VOLUME 6

J. MURRAY STEELE, *Editor-in-Chief*

SOME METHODS OF STUDYING HUMAN GENETICS, *Antonio Ciocco, Editor*
METHODS IN ENVIRONMENTAL RESEARCH, *Ray G. Daggs, Editor*
STATISTICS IN MEDICAL RESEARCH, *Donald Mainland, Editor*
DESIGN AND CONSTRUCTION OF METABOLISM CAGES, *Arnold Lazarow, Editor*

VOLUME 7

J. V. WARREN, *Editor-in-Chief*

CHEMICAL INVESTIGATION OF MUSCULAR TISSUES, *W. F. H. M. Mommaerts, Editor*
HEMODYNAMIC METHODS—HEART AND LUNGS, *Richard Gorlin* and *James V. Warren, Editors*
METHODS FOR THE STUDY OF HUMAN LEUKOCYTES, *Samuel P. Martin, Editor*
METHODS FOR STUDY OF THE HISTOLOGY AND CYTOLOGY OF THE RETINA, *E. N. Willmer, Editor*

GOVERNING BOARD PREFACE

NOTHING could seem more conventional, indeed more old-fashioned, than the list of topics discussed in Volume 8 of METHODS IN MEDICAL RESEARCH. The life history of erythrocytes, the response of involuntary muscles, the measurement of blood flow—all these subjects have long been in the minds of investigators and teachers. But in contrast to the traditional character of the list of topics, what could be more modern than the outline of research technics described in this volume? Their very names, and the scientific concepts on which they are based, would have been meaningless to the physiologist of one or two generations ago. Moreover, the variety of these technics also would have bewildered the traditional physiologist. For example, he could hardly have imagined that radioactive elements as well as serologic methods would be used in his laboratory to determine the life span of erythrocytes.

Looking at the table of contents of this volume, one gets the impression that our predecessors had clearly seen and formulated most of the great problems of biology and medicine, but had left to us the task of working out quantitative technics for the analysis and solution of these problems. Hence the importance, and practical value, of the present series on METHODS IN MEDICAL RESEARCH. We feel confident that, like the preceding numbers of the series, Volume 8 will help the laboratory investigator to develop the tools necessary to convert the questions of yesterday into the knowledge of tomorrow.

IRVINE H. PAGE
RENÉ J. DUBOS
C. N. H. LONG
CARL F. SCHMIDT
DAVID L. THOMSON

EDITOR'S PREFACE

THE EDITORS of the preceding volumes have so thoroughly stated the reasons for the existence of this series that there is little for me to add. Each editor, in his own way, has pointed out that these volumes are a natural component of every biomedical scientist's library and in one way or another reaffirmed Karl Ludwig's dictum: "Die Methode ist alles." Ralph W. Gerard in a few clear sentences in the preface to Volume 3 has stated the case for the primacy of methodology most effectively.

Nevertheless, on a day when everything seemed to be going right, I was encouraged to dash off a brief essay to demonstrate that Ludwig's simple statement is too indefinite, too sweeping, too naive to apply to modern experimental planning. But he made the mistake of trying these ideas out on his colleagues and now, more than 3 months later, he must admit failure. He should have reserved his assertions for a captive audience since he succeeded in proving to some of his friends only that *alles* may be too absolute a word—or perhaps it gains too much in the translation.

It is self evident that a research can be no more significant than is permitted by the limits of reliability of the data gathering/processing procedures, but still there is a distressingly large amount of meaningless data obtained by perfectly valid methods; and there are other objections to methodology for the sake of methodology. Most scientists will readily concede the first point and some will agree to other exceptions, but then immediately insist that "die Methode" is the most important *single* component of research. At this point, logic vanishes and philosophy prevails.

Our teachers may take pride in the thoroughness with which they have indoctrinated us; there seems no escape for this generation from this catchy slogan. Still, even though it may be heresy within the covers of a volume such as this, I submit that "die Methode" is a part of the whole in the same sense that the thorax or the brain is a part of the body.

In any case, planning and gathering the papers for this volume has been a most pleasant and instructive experience, although it required about five times more effort than was estimated.

It is greatly to the credit of the contributors to this volume that they were willing to turn aside from the flow of their current studies to prepare manuscripts detailing the nuts and bolts of the proce-

TABLE OF CONTENTS

SECTION I. Life History of the Erythrocyte

ASSOCIATE EDITOR, *Walter S. Root*

SECTION II. Measurement of Responses of Involuntary Muscle

ASSOCIATE EDITOR, *A. M. Lands*

SECTION III. Peripheral Blood Flow Measurement

ASSOCIATE EDITOR, *H. D. Bruner*

SECTION I

Life History of the Erythrocyte

ASSOCIATE EDITOR—*Walter S. Root*

INTRODUCTION

SINCE the latter part of the nineteenth century the red bone marrow of the normal adult has been known to produce red blood cells. For this and other reasons, it has been the subject of countless investigations, and its routine examination is of importance to the clinical hematologist. More recently, studies of the culture of bone marrow cells have excited great interest.

Although changes in the amount of red bone marrow are known to occur, the size of the marrow is not an index of its ability to produce red blood cells (2). For many years, the appearance of reticulocytes in increasing numbers in the circulating blood has been regarded as an indication of marrow activity. However, the only quantitative measurements of red cell formation are those based upon the determination of the amount of iron incorporated during the formation of red cells, or on the volume of red cells which must be withdrawn to maintain the red cell volume constant, although reduced below the normal value.

The red cell, like other living organisms, exists for a finite time. Of the various methods used to study the life span of the erythrocyte those in which the cell is tagged by one or another means have proved most satisfactory. Determination of the life span of red cells has given useful information concerning red cell dynamics (3).

Disintegration of the red blood cell is followed by the breakdown of its hemoglobin. Many of the products of hemoglobin catabolism have been identified, and so quantitative measurements of the excretion of these substances have been used as an index of red cell destruction.

In a discussion of the history of hematology, Dameshek (1) calls attention to the increasing use of the physiologic approach in studies made since 1925. His thesis seems to be strikingly confirmed by the nature of the technics described below. Although recent advances in chemistry and physics have led to the development of procedures which have aided greatly in understanding the life history of the erythrocyte, many uncertainties exist and much is still unknown.

WALTER S. ROOT

REFERENCES

1. Dameshek, W.: The ten "greats" in hematology—1900–1950, Blood 5: 388, 1950.
2. Grant, W. C., and Root, W. S.: Fundamental stimulus for erythropoiesis, Physiol. Rev. 32: 449, 1952.
3. London, I. M., *et al.:* Heme synthesis and red blood cell dynamics in normal humans and in subjects with polycythemia vera, sickle cell anemia and pernicious anemia, J. Biol. Chem. 179: 463, 1949.

I. THE BONE MARROW

EXAMINATION OF BONE MARROW

RALPH L. ENGLE, JR., *Cornell University Medical College*

ALTHOUGH bone marrow examinations are a routine procedure, there is great variation in the ways in which the marrow is aspirated and handled. Bone marrow is relatively simple to obtain, even serially, in living patients and in animals, and it offers excellent opportunities for cytochemical and histochemical studies. Since it is impossible to cover all of the methods in use, the discussion will be limited to the more routine procedures carried out in most hematology laboratories.

OBTAINING BONE MARROW

The sites most commonly used for aspirating bone marrow in patients are the sternum (13), the iliac crest (22, 23), the spinous process of a vertebra (11, 16) and, in children, the head of the tibia (7). Recently, Bierman and Kelly (4) have recommended that marrow be obtained from the posterior ilium.

Hematologists prefer the sternum, despite slight risk of serious accident by perforation through the dorsal surface of the bone (2). They believe that consistently better specimens can be obtained from this location than from the others. The site for puncturing the sternum is in the midline opposite the second or third costal interspace. The portion of the sternum opposite a rib insertion should be avoided, since cartilage is frequently present there. The needle is pointed directly into the bone, making about a 90° angle with the skin.

The iliac crest, although less hazardous, presents certain difficulties, particularly in individuals with considerable subcutaneous fat or large muscles and in the occasional patient with unusually hard bone. In such patients, a strong needle must be used, lest it bend or break during the procedure. Many patients prefer this approach because they feel that it is at a distance from vital organs. Some clinics now use iliac crest aspiration instead of sternal aspiration because of the negligible risk involved.

Technic. The patient lies supine in bed and the hip on the side of the procedure may be raised on one pillow. The needle is inserted into the ilium in a medial direction 2 cm below the anterior superior spine. Or the needle is directed caudad into the top of the ilium $^1/_4$ of the distance from the anterior superior spine to the posterior superior spine. If the posterior ilium is used, the patient is placed in the prone or lateral position and the needle is inserted into the region of the posterior superior spine of the ilium where the bone is 2–4 inches thick.

The spinous processes of the lower thoracic and lumbar vertebrae are often used. It is difficult to get more than a few drops of marrow from this location and it is sometimes necessary to withdraw and reposition the needle in order to obtain the specimen. However, many patients whose spines are not sensitive prefer this method because they cannot see the procedure. The patient may be positioned prone in bed, on the side as for a spinal tap, or sitting up and leaning slightly forward. The needle is inserted either straight into the midpoint of the spine at about a 90° angle with the skin, or through the side of the spine directed medially and anteriorly.

In children up to the age of 3 years, many hematologists prefer the iliac crest; this approach has been used successfully even in premature infants. Others use the head of the tibia, inserting the needle just below the tubercle and medial to the tibial tuberosity. In children over the age of 3, the sites used, in order of preference, are the iliac crest, the spinous process of a vertebra, and sterum (22, 25, 31).

Several types of needles have been recommended for marrow aspirations. For sternal aspirations, where the bone usually is not too hard, satisfactory needles can be made by shortening 18 gage spinal puncture needles with their stilets to about 1 inch (1). The bevel should make a 140°–160° angle with the shaft of the needle. This is enough bevel to give a point and at the same time be short enough to stay within the marrow cavity. A similar but somewhat sturdier needle is that described by Osgood (19).*

For aspiration from the spinous process of a vertebra and from the crest of the ilium, where the bone is often quite firm, the University of Illinois needle† is recommended (15). This is a very large needle and stilet with an adjustable combination guard and finger rest. One can manipulate this needle close to its point, thereby obtaining maximum pressure and maximum control; in our experience, however, this has been a rather frightening needle to use for sternal aspirations. In some instances in

* Becton, Dickinson and Company, Rutherford, N. J.
† V. Mueller & Co., Chicago, Ill.

which it is desirable to have small pieces of marrow, the Turkel needle should be used (33). The stilet is removed after the needle has been inserted down to the bone and replaced by a cutting needle which cuts through the bone when it is rotated. A piece of the bone and marrow lodges in the lumen of the cutting needle and can be removed for study. This particular needle, when used for sternal aspiration, is inserted at an angle of 45° with the point of the needle directed posteriorly and cephalad.

Apprehensive patients should be given a sedative about $^1/_2$ hr before the bone marrow aspiration. Prepare the site of aspiration and an area about 10 inches around it by shaving, if necessary, and an antiseptic such as pHysoHex* or alcohol. Drape sterile towels around the site. Complete asepsis, including the use of sterile rubber gloves, is essential. With a short 25 gage needle and 2 ml syringe, make a skin bleb with 1% procaine at the puncture spot. With the same needle, or, if necessary, with a larger 20 gage needle, infiltrate the tissues beneath with procaine down to the bone and beneath the periosteum. The density of the bone can be determined by gentle probing with this needle. After a 2 min wait, insert the aspiration needle in the exact path of the procaine needle down to the bone, then with a twisting, pushing motion advance the needle through the outer table of bone; at this point there is usually a distinct loss of resistance against the needle. When the spinous process of a vertebra, and occasionally other sites, are used, this loss of resistance may not be felt; one should then determine whether the marrow has been reached by attaching the syringe and attempting to withdraw a sample. After the marrow cavity has been entered, remove the stilet and attach a clean, dry, 10 ml syringe, preferably without a Luer lock, to the needle, and withdraw 0.1–0.3 ml marrow by gently but firmly drawing back on the plunger. This first small amount is usually highly cellular. If additional marrow is required, attach another syringe and withdraw the required amount; this is usually mixed with considerable blood. The patient almost always complains of moderately severe pain during withdrawal of the marrow. This is said to be due to the pressure on the endosteum. After the withdrawal, insert the stilet, quickly remove the needle, and apply a dry sterile bandage. Occasionally, in patients with purpura, pressure must be applied on the bandage to stop the bleeding.

Hemophilia is the only contraindication to bone marrow aspiration (35). Bleeding in patients with thrombocytopenia can be stopped with a pressure bandage. In the 13 deaths that have been

---

* Winthrop Laboratories, New York, N. Y.