INTERNATIONAL ACADEMY OF PATHOLOGY MONOGRAPH

THE PLATELET

by 40 authors

EDITED BY **K. M. BRINKHOUS, M.D.**

Alumni Distinguished Professor and Chairman of Pathology, University of North Carolina, Chapel Hill

R. W. SHERMER, M.D.

Assistant Professor of Pathology, University of North Carolina, Chapel Hill

AND **F. K. MOSTOFI, M.D.**

Chief, Genitourinary Pathology Branch, Armed Forces Institute of Pathology, and Veterans Administration Special Laboratory for Pathology at the Armed Forces Institute of Pathology, Washington, D. C.

THE WILLIAMS & WILKINS COMPANY

Baltimore • 1971

INTERNATIONAL ACADEMY OF PATHOLOGY MONOGRAPH

THE PLATELET

by 40 authors

EDITED BY **K. M. BRINKHOUS, M.D.**

Alumni Distinguished Professor and Chairman of Pathology, University of North Carolina, Chapel Hill

R. W. SHERMER, M.D.

Assistant Professor of Pathology, University of North Carolina, Chapel Hill

AND **F. K. MOSTOFI, M.D.**

Chief, Genitourinary Pathology Branch, Armed Forces Institute of Pathology, and Veterans Administration Special Laboratory for Pathology at the Armed Forces Institute of Pathology, Washington, D. C.

THE WILLIAMS & WILKINS COMPANY

Baltimore • 1971

Made in the United States of America

Library of Congress Catalog Card Number 76-150978
SBN 683-01055-7

COMPOSED AND PRINTED AT
WAVERLY PRESS, INC.
Mt. Royal and Guilford Aves.
Baltimore, Md. 21202, U.S.A.

Contributing Authors

PAUL M. AGGELER, M.D.*
Professor of Medicine, Chief, Hematology Section, University of California, San Francisco General Hospital, San Francisco

RICHARD H. ASTER, M.D.
Executive Director Milwaukee Blood Center and Professor of Medicine, Medical College of Wisconsin, Milwaukee

E. J. WALTER BOWIE, M.A., B.M., F.A.C.P.
Assistant Professor of Medicine, Mayo Graduate School, University of Minnesota, Consultant, Department of Clinical Pathology, Mayo Clinic, Rochester

CAROLE B. BOYD, M.D.
Chief Resident in Pathology, Henry Ford Hospital, Detroit

GEORGE BRECHER, M.D.
Professor and Chairman, Division of Clinical Pathology and Laboratory Medicine, University of California, San Francisco

K. M. BRINKHOUS, M.D.
Alumni Distinguished Professor and Chairman of Pathology, University of North Carolina, Chapel Hill

JOHN R. CARTER, M.D.
Director of the Department of Pathology, Case Western Reserve University, Cleveland

A. B. CHANDLER, M.D.
Professor of Pathology, Medical College of Georgia, Eugene Talmage Memorial Hospital, Augusta

WILLIAM H. CROSBY, M.D.
Director of Hematology and Professor of Medicine, Tufts-New England Medical Center, Boston

PAUL DIDISHEIM, M.D.
Assistant Professor of Experimental Pathology, Mayo Graduate School, University of Minnesota, and Consultant in Clinical Pathology, Mayo Clinic, Rochester

SHIRLEY EBBE, M.D.
Associate Director of Research, St. Elizabeth's Hospital, and Associate Professor of Medicine, Tufts University School of Medicine, Boston

* Deceased

FRANK H. GARDNER, M.D.
Professor of Medicine, Director, Hematology Research Laboratory, Presbyterian-University of Pennsylvania Medical Center, Philadelphia
IRA M. GOLDSTEIN, M.D.
Instructor of Medicine, New York University Medical Center, New York
LAURENCE A. HARKER, M.D.
Assistant Professor of Medicine, Division of Hematology, Washington University School of Medicine, Seattle
WILLIAM J. HARRINGTON, M.D.
Professor and Chairman of Medicine, University of Miami School of Medicine, Miami
ROBERT C. HARTMANN, M.D.
Professor of Medicine, Director, Division of Hematology, Vanderbilt University School of Medicine, Nashville
EDWARD S. HENDERSON, M.D.
Head, Leukemia Service, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda
SHIRLEY A. JOHNSON, PH.D.*
Associate Professor of Physiology, Georgetown University School of Medicine, and Research Physiologist, Research Service, Veterans Administration Hospital, Washington
FREDERICK G. KRONCKE, M.D.
Departments of Pathology and Medicine, University of North Carolina, Chapel Hill
E. F. LÜSCHER, PH.D.
Professor of Biochemistry, Theodor Kocher Institute, University of Berne, Berne, Switzerland
DONALD G. MCKAY, M.D.
Professor of Pathology, University of California, San Francisco General Hospital, San Francisco
ARON J. MARCUS, M.D., F.A.C.P.
Chief, Hematology Section, New York Veterans Administration Hospital, and Associate Professor of Medicine, Cornell University Medical College; Associate Attending Physician, New York Hospital; Associate Attending Physician, Bellevue Hospital, New York
WILLIAM MARGARETTEN, M.D.
Associate Professor of Pathology, University of California, San Francisco General Hospital, San Francisco
R. G. MASON, M.D., PH.D.
Associate Professor of Pathology, University of North Carolina, Chapel Hill
RAYMOND W. MONTO, M.D.
Chief, Division of Hematology, Department of Medicine, Henry Ford Hospital, Detroit

* Deceased

SCOTT MURPHY, M.D.
Associate Director of the Hematology Research Laboratory, Presbyterian-University of Pennsylvania Medical Center, Philadelphia
J. F. MUSTARD, M.D., PH.D.
Professor of Pathology, McMaster University, Hamilton, Ontario, Canada
RALPH L. NACHMAN, M.D.
Chief, Division of Hematology, The New York Hospital, and Associate Professor of Medicine, Cornell University Medical College, New York
CHARLES A. OWEN, JR., M.D., PH.D.
Professor of Medicine (Medical Research), Mayo Graduate School, University of Minnesota, and Chairman of Clinical Pathology, Mayo Clinic, Rochester
M. A. PACKHAM, PH.D.
Assistant Professor of Biochemistry, University of Toronto, Toronto, Ontario, Canada
JOHN W. REBUCK, M.D., PH.D.
Chief, Division of Hematology, Department of Pathology, Henry Ford Hospital, Detroit
HAROLD R. ROBERTS, M.D.
Professor of Medicine and Pathology, University of North Carolina, Chapel Hill
A. JEAN ROBINSON, PH.D.
Assistant Research Physiologist, Coagulation Research Laboratory, University of California, San Francisco General Hospital, San Francisco
NATHANIEL F. RODMAN, M.D.
Department of Pathology, University of Iowa, College of Medicine, Iowa City
LENORE B. SAFIER
Research Associate, Hematology Research Laboratory, New York Veterans Administration Hospital, New York
MERVYN A. SAHUD, M.D.
Research Associate in Medicine, Special Hematology Laboratory, University of California, San Francisco General Hospital, San Francisco
EDWIN W. SALZMAN, M.D.
Associate Professor of Surgery, Beth Israel Hospital, and Associate Professor of Surgery, Harvard Medical School, Boston
R. W. SHERMER, M.D.
Assistant Professor of Pathology, University of North Carolina, Chapel Hill
JOHN H. THOMPSON, JR., PH.D.
Associate Professor of Clinical Pathology, Mayo Graduate School, University of Minnesota, and Consultant in Clinical Pathology, Mayo Clinic, Rochester
JAMES G. WHITE, M.D.
Professor of Pediatrics, University of Minnesota School of Medicine, Minneapolis

Foreword

"*The Platelet*" is the eleventh of a series of monographs in pathology initiated by the International Academy of Pathology. Its various chapters are expansions of the lectures given at the 58th annual meeting held in San Francisco where the principal course, entitled "Pathologic Physiology and Anatomy of the Platelet", was organized by Dr. Kenneth M. Brinkhous, Alumni Distinguished Professor and Chairman of Pathology, University of North Carolina, Chapel Hill.

The thoughtful planning of the course and the symposium added immeasurably to the depth and breadth of the coverage of the subject of the platelet. In addition to their careful editing of the monograph, Dr. Brinkhous and Dr. Shermer have contributed significantly to the volume by the excellent closing chapter which has summarized the present concepts of the Platelet.

The Council of the International Academy of Pathology has sponsored the publication of this and previous monographs to provide a permanent reference source for the subjects especially presented at the meetings of the Academy. The goals are to assemble material of the finest scientific standards and educational value relative to the normal and abnormal structure and function of the platelet and to present the information in a manner that will be of continuing benefit to the entire scientific community, as well as to those who attended the meeting.

F. K. Mostofi, M.D.
Series Editor

Washington, D. C.

Preface

This volume consists of a series of lectures developed in connection with the long course of the International Academy of Pathology on the subject of the Pathologic Physiology and Anatomy of Platelets. After remaining relatively dormant for many years, the platelet field has been the subject of intensive study by newer methods since the late 1950's. In the past 15 years the field has grown vigorously, largely by the convergence method. Investigators from many disciplines have applied new technologies, including those of electron and scanning electron microscopy, enzymology, and cellular and subcellular biology with its emphasis on lysosomes, nucleotide metabolism, membranes, and cell contractility. This work by many investigators has increased tremendously the scope of understanding of the role of the platelet in physiology and pathology. This volume presents many of the newer aspects of the platelet, and the reader will obtain an up-to-date background of the subject from many of the individuals who have contributed significantly to the field over the recent past. The bibliography for some chapters is very comprehensive and could well serve as a base for those starting serious work in the field. The final chapter provides a brief annotated summary of the whole volume. Also included here is a list of recent reviews and monographs on the subject.

The understanding of the pathogenesis of many platelet diseases has improved greatly in the recent past, but it is evident from the reading of this monograph that many gaps in knowledge persist. With the knowledge of the subject as presented here, and with the new methodologies available, the reader should be well prepared for advances which will undoubtedly occur in the future.

A sad note developed between the time of the lectures and the publication of this volume. This sadness was caused by the death of three outstanding American hematologists who contributed to the program. Dr. Paul Aggeler died on September 1, 1969. His lecture, developed with his co-workers, appears in this volume as Chapter 11. Dr. William Dameshek, whose lecture was entitled "Drug Toxicity: Mechanisms of Thrombocytopenia," died on October 6, 1969. His manuscript had not been completed for publication. Dr. Shirley Johnson died on September 12, 1970. Her lecture appears as Chapter 13.

Special thanks are given the United States Atomic Energy Commission for its support of the Conference through a grant to Dr. N. F. Rodman, University of North Carolina (Contract #AT (49-7)-3034), co-chairman of the Conference. Dr. K. Mostofi, series editor of the International Academy of Pathology Monographs, gave much valued advice throughout. To all of these individuals and organizations grateful appreciation is expressed.

K. M. Brinkhous, M.D.
R. W. Shermer, M.D.
Monograph Editors

Chapel Hill, N.C.

Contents

Platelets and Disease States

Clinical Evaluation of Platelet Function

Summary

Chapter 1

The Megakaryocyte: Maturation and Self-Renewal*

SHIRLEY EBBE

In the past, it was believed that the compartment of recognizable megakaryocytes was capable of cell proliferation at the megakaryoblast level. Megakaryocytes were recognized as polyploid cells, but they were thought to undergo endomitotic nuclear replication simultaneously with cytoplasmic maturation. Recent observations have made these notions with regard to cellular and nuclear proliferation untenable, but have also failed to yield a completely clear picture of these processes. In this paper, some of these observations will be summarized. An attempt will be made to correlate them into a model for megakaryocytopoiesis and to indicate some of the unsolved problems with regard to this interesting cell system.

With currently available data, the cell system which leads to production of blood platelets has been proposed to have the characteristics shown schematically in Figure 1.1.[11, 12] Recognizable megakaryocytes appear to be end-stage, highly specialized cells which do not reproduce themselves. The important cell pools which are responsible for renewal of the cell line and cellular multiplication have not been morphologically identified. Therefore, their characteristics can only be surmised from the manner in which their behavior is reflected by the recognizable megakaryocytes and platelets.

Recognizable megakaryocytes can be divided into three different types, based on morphological appearance: megakaryoblast (stage I), promegakaryocyte (stage II), and granular megakaryocyte (stage III). All of these are larger than myeloid or erythroid cells, and they represent successive stages of maturation. The megakaryoblast has a relatively small amount of non-granular basophilic cytoplasm, the granular megakaryocyte has abundant cytoplasm which is completely filled with azurophilic granules of the type seen in platelets, and the promegakaryocyte is an intermediate cell with basophilic cytoplasm which either has no visible granules or is incompletely granulated. The stage III megakaryocytes lose their basophilia. Nuclear changes with maturation are not characteristic, but, generally, the nucleus tends to show increasing degrees of lobulation or segmentation with maturation from stage I to III.

* Supported in part by Grant AM-08263 and Research Career Development Award 1-K3-AM8634 from the National Institute of Arthritis and Metabolic Diseases and by a research grant from The American Cancer Society (Massachusetts Division), Inc.

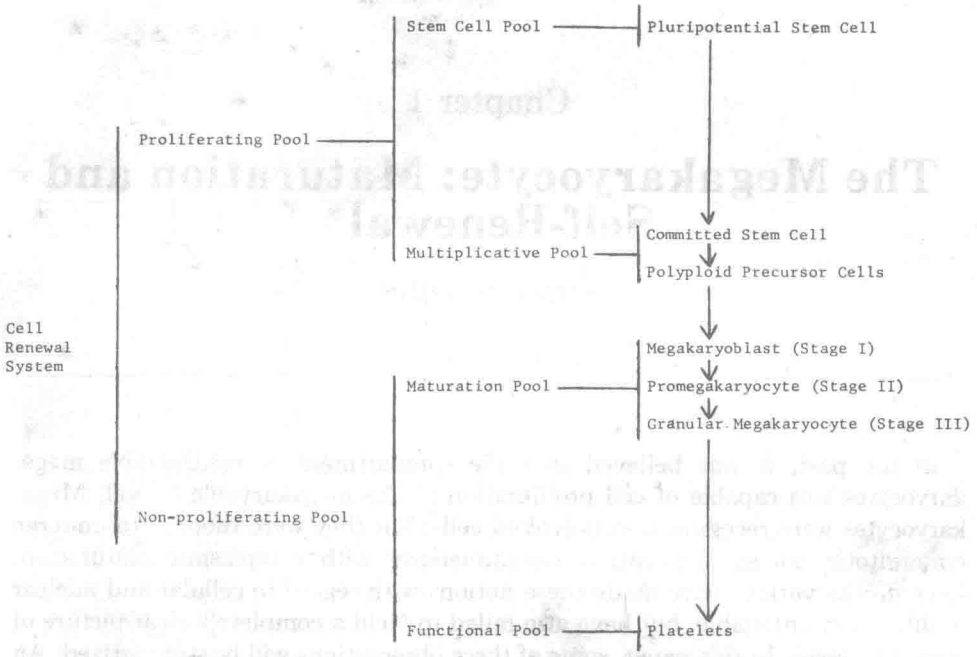

FIG. 1.1. Proposed cell renewal system for megakaryocytes. From reference 11.

The proliferative capacity and maturation rate of megakaryocytes have been determined from their pattern of labeling *in vivo* after a single injection of tritiated thymidine (^{3}HTdR). Several investigators[13, 19, 32] have reported similar results in the rat, and, over a period of years, the findings have been quite reproducible, as shown in Figure 1.2. Several points are noteworthy. First, only stage I cells are regularly labeled 30 minutes after injection of ^{3}HTdR. This finding indicates that DNA synthesis occurs only in this sub-population of megakaryocytes and invalidates the older concept that nuclear replication and cytoplasmic maturation were synchronous events. Second, labeled cells appear in stages II and III after delays of about 8 and 24 hours, respectively, confirming the impression that these morphological types are successive stages of maturation. As labeled cells mature into these compartments the intensity of the nuclear label, as determined by the mean grain count (MGC), is as high as it had been when the cells were in stage I (Fig. 1.3). Hence, cellular division does not follow DNA synthesis in stage I even though occasional mitotic figures are seen. Third, the labeling index of stage I progressively increases during the first 24 hours to over 90%. In the absence of cell division, this increase can be due only to differentiation of precursors from a compartment which is morphologically unrecognizable, but which is actively synthesizing DNA.[13, 19]

In a smaller group of mice, the pattern of labeling of megakaryocytes by ^{3}HTdR is almost identical to that of rats (Fig. 1.4). The number of observations is insufficient to make precise determinations of MGC, but, as in the rats, labeling intensity of stages II and III at 8 and 24 hours, respectively, was equal to or slightly greater than that of stage I at 30 minutes. In a single human being in whom megakaryocyte labeling by ^{3}HTdR was reported,[9] labeling of morpholog

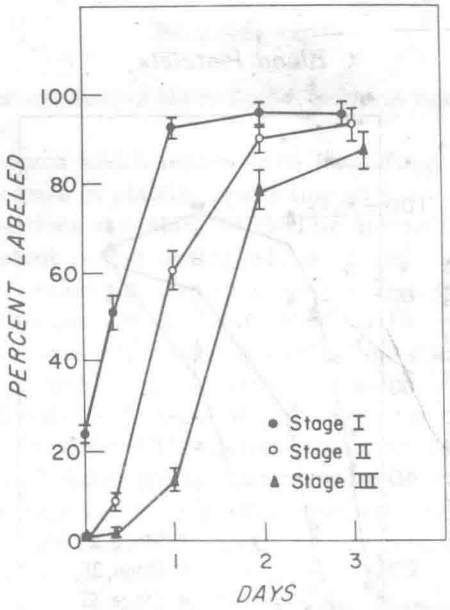

FIG. 1.2. Labeling indexes for rat megakaryocytes after a single injection of ^{3}HTdR. Methods described in reference 13. Stages I, II, and III refer to megakaryoblast, promegakaryocyte, and granular megakaryocyte, respectively. For each time interval, data from 20 experiments over a 5 year period were pooled. Each point shows the average for 20–30 rats +2 S.E.M. In each rat, at least 250 megakaryocytes were evaluated.

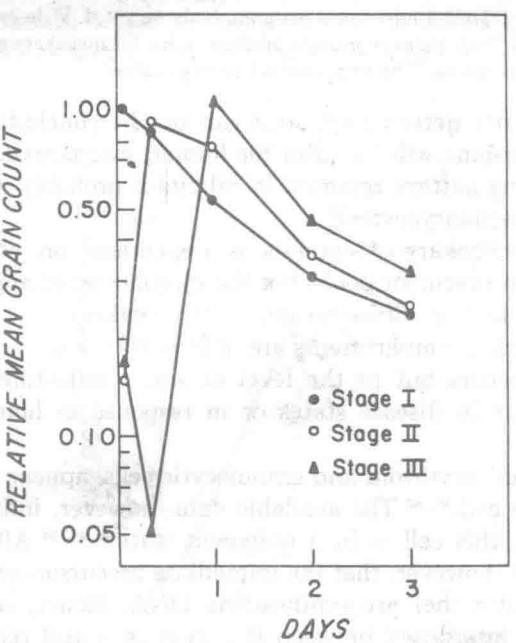

FIG. 1.3. Relative mean grain counts of labeled rat megakaryocytes after a single injection of ^{3}HTdR. Numbers of animals and experiments as in Figure 1.2. To eliminate technical variations in absolute grain counts among experiments, the average MGC of labeled stage I megakaryocytes 30 minutes after injection in each experiment was called 1.00, and all other values for that experiment were expressed as relative values.

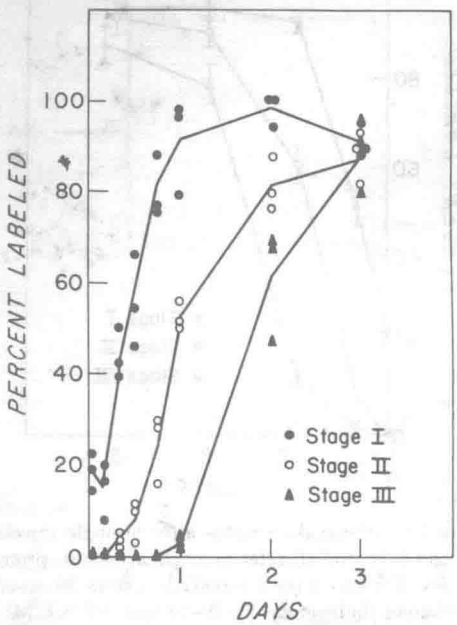

FIG. 1.4. Labeling indexes of megakaryocytes of 12 week old, female CF_1 mice (Carworth Farms) after a single injection of ^{3}HTdR, 1 microcurie per gram body weight, I. V. In each mouse, 250 megakaryocytes were evaluated; each point represents labeling index for megakaryocytes of a given morphological group in a single mouse. The lines connect average values.

ical sub-groups was not determined, so it can not be concluded with certainty that the same conclusions will be valid for human megakaryocytes. The somewhat different labeling pattern reported in rabbits is probably due to differences in classification of megakaryocytes.[7]

Maintenance of megakaryocytopoiesis is dependent on continuous differentiation of cells from precursor pools, but the morphological appearance of these precursor cells is not known. Since recognizable megakaryocytes are nonproliferating cells, the precursor compartments are of importance not only as the sites at which self-renewal occurs but as the level at which adjustments in thrombocytopoiesis may occur in disease states or in response to homeostatic mechanisms.

Megakaryocytes, like erythroid and granulocytic cells, appear to originate from a pluripotential stem cell.[49, 52] The available data, however, indicate that, in the normal steady state, this cell is in a quiescent state.[4, 20, 43] After labeling with ^{3}HTdR it is apparent, however, that the immediate precursors of megakaryocytes are not quiescent but rather are synthesizing DNA. Hence, intermediate precursor cells must be interposed between the stem cells and recognizable megakaryocytes. The data leading to the proposal that nuclear replication, in addition to cellular proliferation, occurs in this intermediate compartment have recently been reviewed.[11, 12] If there is, in fact, a pool of precursor cells which undergoes nuclear replication without cell division, they might be expected to have characteristics more of a maturing cell population than a proliferating cell population.

Observations of post-irradiation thrombocytopenia in mice and rats suggest that this may be the case.

The thrombocytopenia which results from irradiation is due to bone marrow suppression and decrease in platelet production. It is of interest that the level of circulating platelets does not start to decline immediately after irradiation; rather, there is a latent period during which normal platelet counts are maintained. This latent period appears to correspond to the length of time that normal thrombocytopoiesis can be maintained by the nonproliferating pool of megakaryocytes which was unaffected by irradiation. An estimate of total maturation time for recognizable megakaryocytes can be derived from the rate at which the unlabeled cells of all stages of maturation are replaced by labeled cells after an injection of ^{3}HTdR.[9, 13] This determination is predicated on the assumption that all cells which enter the compartment are labeled. Since this assumption is probably not completely valid, this method tends to overestimate true maturation time. In rats (Fig. 1.5), about 40% of megakaryocytes are replaced daily during the first two days and in mice, about 35% (Fig. 1.6). The respective times for the entire population to be replaced are, therefore, 60 and 68 hours. In the single human being studied with ^{3}HTdR, megakaryocyte turnover time was thus estimated to be 10 days or less.[9] Odell and Kniseley[35] summarized the observations in human beings after irradiation and concluded that there is a 10 day post-irradiation period during which megakaryocytes continue to function.

Platelet counts of mice following sublethal irradiation are presented in Figure 1.7. In these animals, leukocyte and reticulocyte values were substantially decreased by the first or second day, but there was a latent period of four days post-

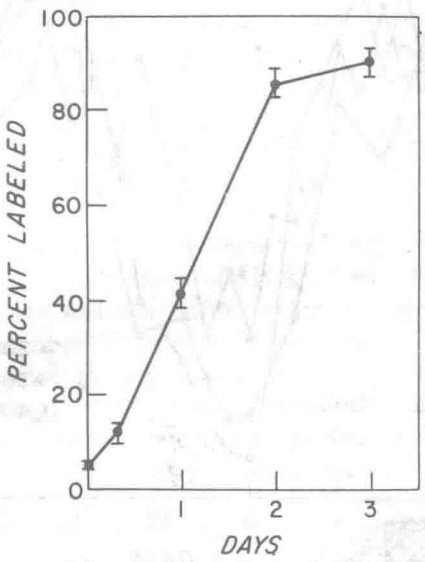

FIG. 1.5. Labeling indexes of the megakaryocyte population as a whole in rats after a single injection of ^{3}HTdR. Each point is the average ±2 S.E.M. for 20–30 rats. Increase of ~40%/24 hours indicates a total maturation time of ~60 hours.

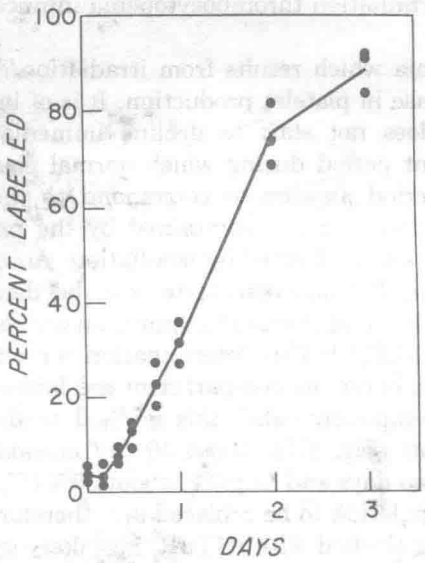

FIG. 1.6. Labeling indexes of the megakaryocyte population as a whole in CF_1 mice after a single injection of ^{3}HTdR. Each point represents one mouse; the line connects average values. Increase of ~35%/24 hours indicates a total maturation time of ~68 hours.

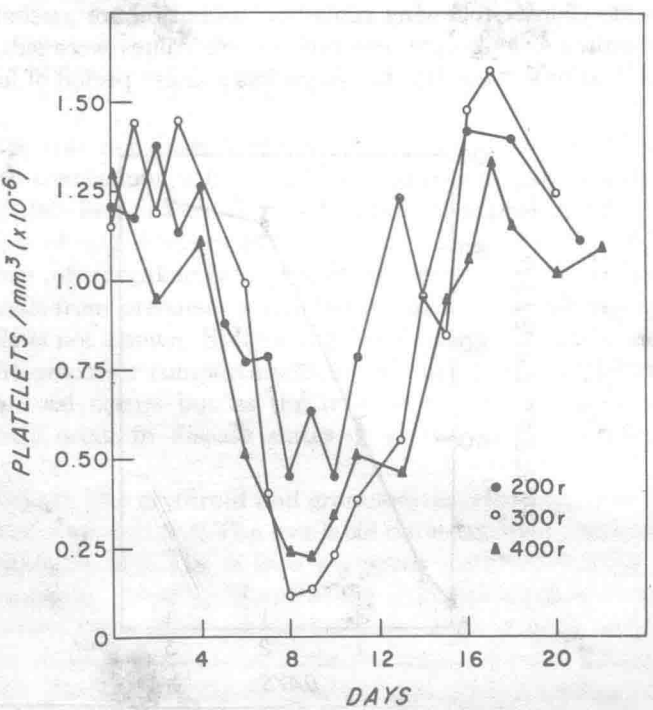

FIG. 1.7. Platelet counts of mice after sublethal, total body irradiation. Platelet counts were done on cardiac blood from serially sacrificed animals. For 200 r, each point is the average of 3–9 mice; for 300 r, 3 mice; for 400 r, 3–6 mice.

irradiation before the level of circulating platelets began to drop. The subsequent decline to minimum values on the eighth or ninth day was consistent with a platelet survival time of four or five days.[36] After irradiation of rats there was also a four day period before platelet production apparently ceased.[16] In both of these species, therefore, the numbers of mature, platelet-producing megakaryocytes were apparently maintained for about a day or a day and a half longer than the maturation time which had been estimated for recognizable megakaryocytes. Prolongation of platelet production beyond their maturation time lends support to the notion that the immediate precursors may also be non-proliferating cells.

The complete cell system for megakaryocytopoiesis appears to begin with the pluripotential stem cell. There is practically no data on the relationship between this cell and the later stages of megakaryocytes either in the normal steady state or in perturbed thrombocytopoiesis other than a recent report that the age of recipient irradiated mice may influence megakaryocytic differentiation of transplanted hematopoietic stem cells.[10] Stem cell differentiation is probably followed by a period of both cellular and nuclear replication before further differentiation into the compartment of recognizable megakaryocytes occurs.[11, 33] With the latter, a limited potential for DNA synthesis may persist,[13, 19, 32] but cellular proliferation ceases. Onset of cytoplasmic growth and specialization leading to platelet production is associated with loss of even this reproductive ability.

By light microscopy, the major manifestations of maturation of recognizable megakaryocytes are cellular enlargement and cytoplasmic differentiation. The increase in size appears to be due to cytoplasmic growth, as there is a progressive decrease in the nuclear:cytoplasmic ratio. Megakaryocytes are polyploid cells, and those of the rat have been shown to have quantities of DNA equivalent to 4N, 8N, 16N or 32N when compared to a population of normal diploid (2N) cells.[32, 33] The 4N cells are confined to the most immature class of megakaryocytes which, however, also contains cells of the higher ploidy values. More mature rat megakaryocytes are of the 8-32N types. Final cell size may be partially determined by DNA content, but the unimodal distribution curves of areas of cells in bone marrow smears (Fig. 1.8) indicate that, if this is true, there must be considerable overlap of sizes among cells of the different ploidy values. In cultures of human diploid/triploid cells, it has been found that individual diploid and triploid could not be distinguished on the basis of nuclear size even though there was a general correlation between DNA value and nuclear size.[29] Our results of megakaryocyte size contrast with those of Harker[24] who found that megakaryocytic diameter and volume and cytoplasmic volume, as determined from tissue sections, paralleled the number of nuclei in the cells. Japa[26] also reported that the amount of cytoplasm seemed to be proportional to the size of the nuclear body. One problem that needs to be clarified is the relationship of nuclear number and DNA content. Nuclei in human megakaryocytes were found to be present in numbers corresponding to 2^x.[26] DNA values, measured microspectrophotometrically in individual megakaryocytes of rats[33] and rabbits,[22] are ordered about 2^xN quantities. Both of these observations indicate that polyploidy is achieved by synchronous replication of all diploid chromatin groups of the cell. The question arises: is DNA replication necessarily followed by endomitosis