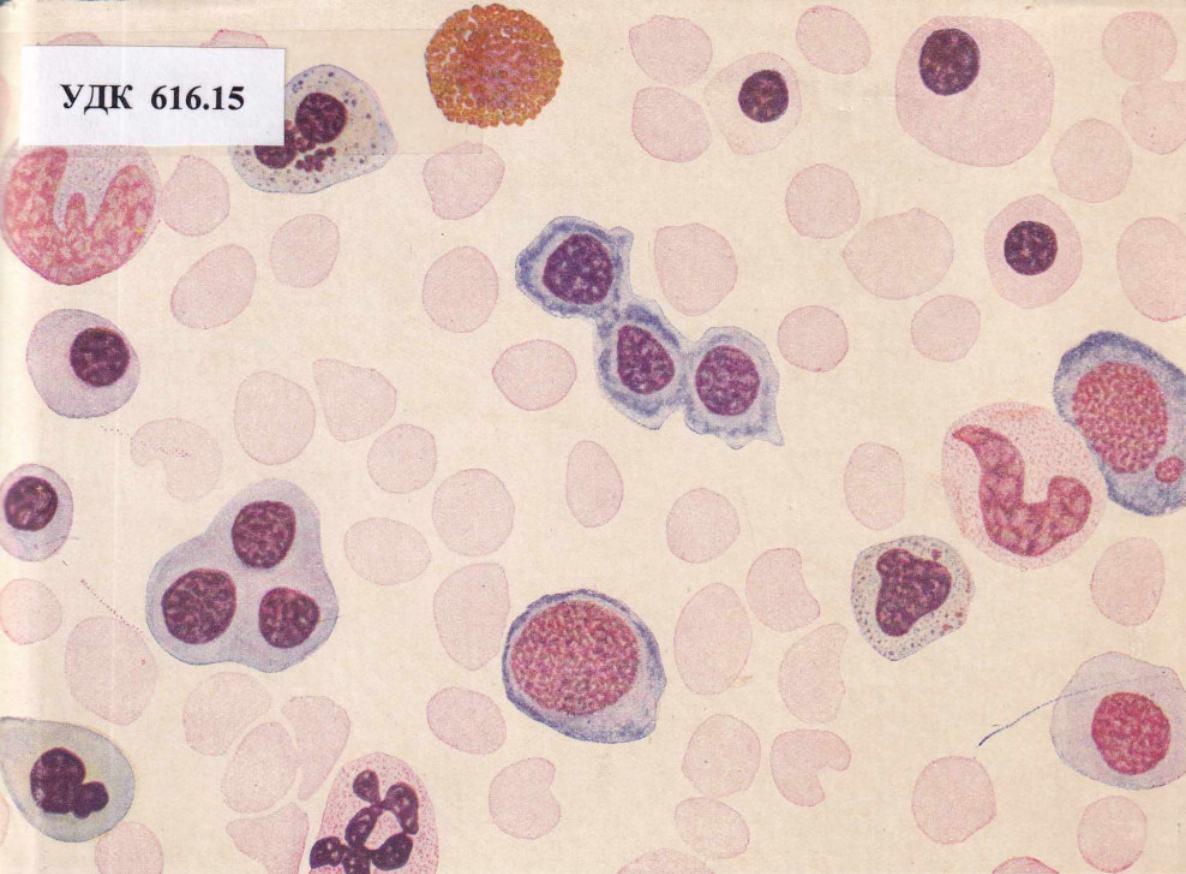


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W. ŁAWKOWICZ AND I. KRZEMIŃSKA-ŁAWKOWICZ

ATLAS  
*of*  
HAEMATOLOGY



POLISH STATE MEDICAL PUBLISHERS · WARSAW

ATLAS OF  
HAEMATOLOGY  
and  
THE PRINCIPLES  
OF DIAGNOSIS OF  
BLOOD DISEASES

by

W. LAWKOWICZ

and

I. KRZEMIŃSKA-LAWKOWICZ

1957, pp. xii + 288, pl. 103

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The pictorial part of the

Atlas of Haematology  
comprises 103 coloured  
plates. Each plate is ac-  
companied by a commen-  
tary and a concise de-  
scription of the disease.  
The authors describe, in  
addition to the common-  
ly known blood diseases,  
a number of rare and only  
recently identified clin-  
ical entities. A large  
quantity of clinical data  
is included.

The didactic value of this  
Atlas, together with high  
artistic value of the plates,  
should make it extremely  
helpful not only in clin-  
ical and laboratory work  
but also in teaching hae-  
matology.

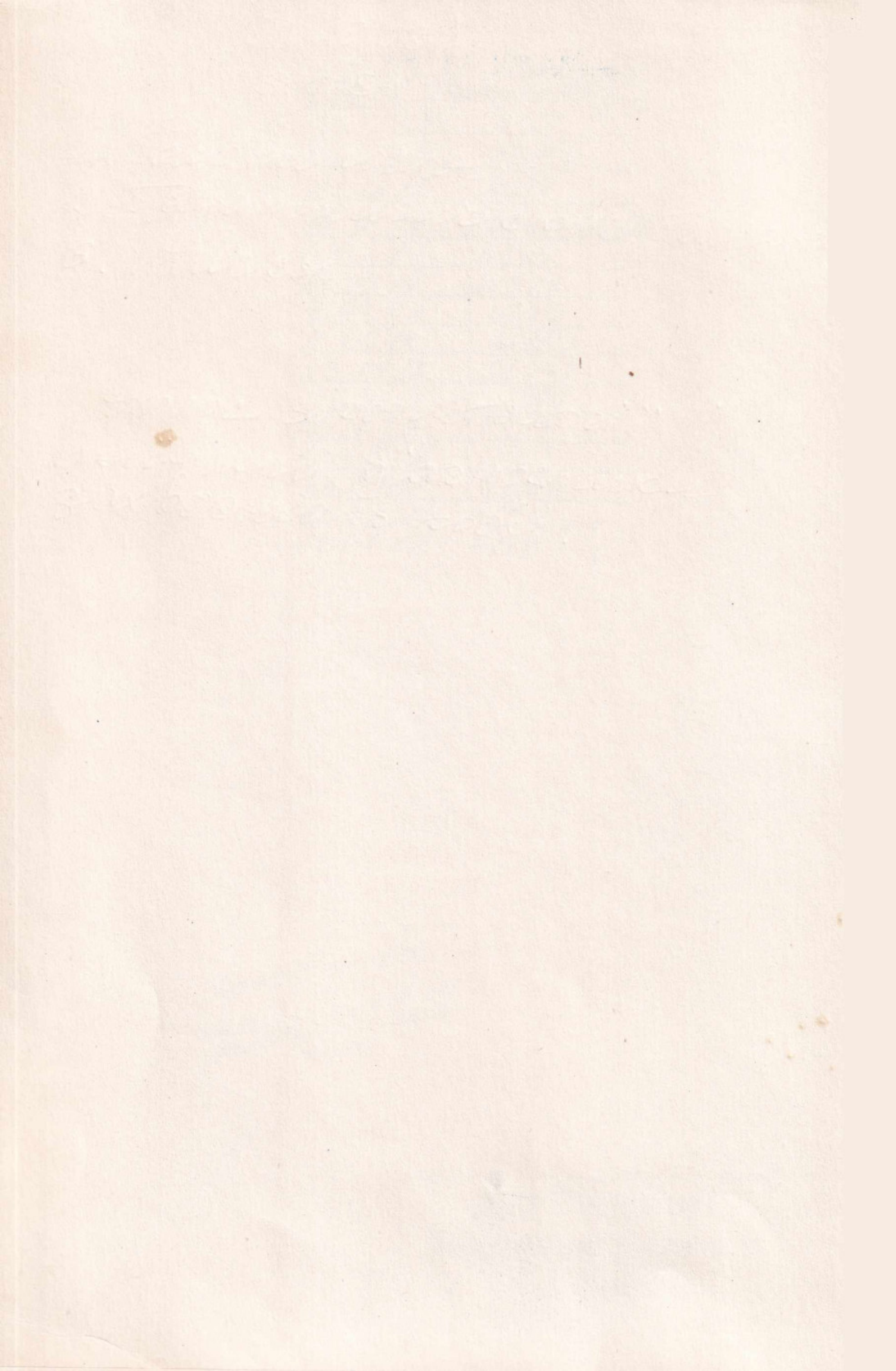
The English translation,  
is prepared from the se-  
cond Polish edition is-  
sued in 1956. The Russian  
and German translations  
are in preparation.

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M E D I C A L  
P U B L I S H E R S

Дар Окружної  
лікарської палати в  
м. Варшаві



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ATLAS  
*of*  
HAEMATOTOLOGY  
*and*  
THE PRINCIPLES OF DIAGNOSIS  
OF BLOOD DISEASES

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POLISH STATE MEDICAL PUBLISHERS

WARSAW

1957

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1. Гематология
2. Гематологические болезни
3. Атласы

Атлас з гематології і  
принципи діагностики  
захворювань крові.



## FOREWORD TO THE I-st EDITION

This work was undertaken by us on the incentive of our Teacher, the Head of the II Medicinal Clinic of the Medical Academy in Gdańsk, Professor M. Semerau-Siemianowski, who being aware of the difficulties of learning haematology without adequate illustrative material, spurned us on to prepare the first Polish atlas of the cells of the blood and bone marrow.

This work took four years. Often we met with difficulties — lack of adequate material for the preparation of drawings, lack of time for the time-consuming execution of tables, the stress of the everyday duties of hard-working physicians. In all such moments Professor Semerau-Siemianowski was ready to help and to encourage us.

Therefore we are happy to express here our deep gratitude to Him, for He enabled us to bring our task to end.

The tables, with the exception of a few, represent the smears from our personal cases, which have been followed by us in the Medicinal Clinic of the Medical Academy in Gdańsk and afterwards in the II Medicinal Clinic of the Medical Academy in Warsaw. Several blood smears as well as bone-marrow smears from the cases of tropical diseases or of diseases not occurring in Poland were obtained by us during a scholarship journey abroad, in the year 1948. All originals of colour-tables were executed by associate professor I. Krzemińska-Ławkowicz with watercolours.

Our work does not represent the whole scope of haematology, it is rather an attempt to depict the basic morphological features of the blood and bone-marrow cells. We omitted splenograms, because these as well as a detailed description of tumour cells will be subjects of a separate work. On the other hand, in several cases the lymph-node biopsy was taken into account.

The present work represents rather an introduction to morphologic haematology and it may be useful in teaching students or in routine work in haematological laboratories.

It could be said that the scope of the text is broader than it is usual in an atlas. In giving, however, such extensive interpretations of the tables we were moved by following considerations:

- 1) there are only few textbooks of haematology;

2) the descriptions of the drawings could be made more interesting and more readily understandable when supplemented by physiopathologic remarks;

3) the usefulness of such a work to the clinician, by supplementing additionally other diagnostic points besides the morphology of blood or bone-marrow cells;

4) we endeavoured also to inform the reader of some new acquisitions in diagnostic methods.

During the execution of this work we were moved also by an additional aim, which is readily understood by anybody, who would see haematology developing and progressing. This aim is the awakening of interest for haematology in a greater number of workers and an increase of the number of haematologists. The importance of haematology becomes greater every day, it surpasses mere laboratory considerations, it reaches into the field of prophylactics. Haematological ambulatories and periodic examinations of blood morphology of the populace are of greater and greater importance. As an example the rôle of haematological examinations in early diagnosis cancer may be cited, when discrete signs of anaemia lead the clinician to the diagnosis of the causative disease.

There follows the importance of clinical haematology, which grows because of the growing frequency of disorders of the blood. The training of haematologists is among the pressing exigencies of the present moment, because their number in Poland is far too scarce in proportion to the needs.

We should consider our aim fulfilled, if this work could at least partially induce a wider interest in haematology.

*THE AUTHORS*



## FOREWORD TO THE II-nd EDITION

The first edition of the "Atlas of Haematology" was out of press in 1952, in the following year — the Czech translation. The first issue was sold out in a short time. The friendly reception it received and many letters from various countries induced us to the preparation of a second edition.

An additional incentive to the revision and extending of the text was the proposal to translate this book into Russian, English and German with a view of facilitating the study of haematology to students and young physicians in their own language.

During the preparation of the second edition the work was equal to that of the preparation of a new book. In printing the first edition and the Czech translation in Prague the delicate original watercolours became so used up, that all tables were painted anew from the preparations remaining in our collection.

We began this time-consuming task with pleasure. Now our work was given to Polish polygraphist and Polish printers. Their work was so excellent that we had difficulties in finding differences between the copies and the originals of our drawings.

We obtained also new technical aids — in the Institute of Haematology in Warsaw we were able, due to excellent modern microscopes, to draw the cells in a more detailed and true manner.

As in the first edition, all tables were painted in watercolours by associate prof. I. Krzemińska-Ławkowicz. In view of the progress made by haematology the text was thoroughly revised. We endeavoured to take into account new acquisitions as well as our personal experience gained during the time elapsed. The second edition contains moreover selected references to the literature. It was necessary to omit many basic publications from our literature as well as from the world's literature. To atone for this, a special division of literature has been adopted and works with an extensive bibliography (textbooks, atlases, monographs etc.) have been grouped separately. The remaining references are the works cited in the chapters of the "Atlas of Haematology", these are in the first place new publications not cited elsewhere.

In spite of many additions to the text the present work remains an attempt to represent only basic traits of haematological morphology and does not



cover the ever growing field of this science. The comparatively extensive text (in comparison to other atlases) should serve the clinician, as it contains the basic diagnostic points.

A continuation of the present work will be instituted by the "Textbook of Haematological Diagnostics" which is now in preparation. This textbook will contain chapters on haematological cytology, cytochemistry, blood coagulation, immunohaematology and related subjects.

In presenting to the reader the second, revised edition of the "Atlas of Haematology" we should like to express our sincere thanks to the Polish State Medical Publishers for technical help and careful edition of this book as well as to all those, whose nameless work enabled the second edition and the translations into English, Russian and German to appear.

We are also indebted to dr. Przemysław Czerski for his aid in the tedious task of selecting and searching under the microscope for cells, which served as models for the drawings, for his help in the preparation of the list of references, as well as for his meticulous English translation.

*THE AUTHORS*

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*Note.* All drawings were made from original preparations using objective 90 x. imm. and ocular 15 x. Under such magnification all details are readily discerned, using lower magnifications and less perfect optical equipment the cellular structures may be blurred, the images obtained being less distinct.





## INTRODUCTION

Recent years have brought many advances in haematology, both as regards diagnostic procedures and such special laboratory techniques as cytochemistry, biochemistry, electron- and phasemicroscopy. Among the numerous new microscopic techniques, fluorescence microscopy has lately been successfully applied in haematologic investigations. These advances, have made possible a better understanding of vital processes of normal and pathological blood cells, and have contributed towards explaining a number of intricate and obscure phenomena in cell metabolism.

It might be thought that such progress would reduce the importance of the time-honoured morphological methods of blood examination, hitherto considered essential in the diagnosis of blood disorders. Morphologic haematology is, however, far from being in decline. On the contrary, the introduction of cytochemical methods is causing it to gain new ground. This is reflected in the constantly increasing number of haematological publications devoted to morphologic diagnostic methods, nor is this trend limited to haematology only; a new branch of medical sciences — clinical cytology — is acquiring greater and greater importance in the diagnosis of various diseases.

The times are not remote, in which the diagnosis of blood diseases was based entirely on peripheral blood examination. Joint biopsies of several organs has enabled us today to acquire direct insight into the blood-forming system.

Although blood examination is essential in diagnosis, nevertheless the supplementary information derived from direct examination of the blood-forming tissue — the bone marrow — is of great value; even if it does not contribute any new positive diagnostic findings indicating a definite disease, it can be helpful in ruling out certain syndromes.

When examining a bone marrow or lymph node aspiration smear pictures strictly specific for a definite blood disorder should not be expected. Rather should we endeavour to evaluate the functional efficiency of the bone marrow on the basis of the morphological pattern.

There are but few haematological syndromes, which are accompanied by a pathognomonic bone-marrow picture, making it possible to identify the



disorder at first glance. Among these are Addison-Biermer's disease and allied megaloblastic anaemias, multiple myeloma, acute aleukaemic myeloses, Gaucher's disease, kala-azar and certain parasitic diseases.

This explains why increasing attention is being devoted to joint biopsies, which, supplemented if necessary, by lymph node, spleen, liver or tumour puncture, enable us to correlate the findings in peripheral blood and bone marrow. Although the clinician intrudes in such cases upon the alien domain of histopathology, he is compensated by numerous advantages, the smears of bioptic specimens bringing out such details of cell structure as become obscured or totally lost in histological sections of fixed tissue.

Therefore oligobiopsy (puncture procedures) should be regarded as an independent diagnostic method. Smears prepared from aspirated material or imprints from excision biopsy specimens are to be seen as a valuable addition to classical histopathological techniques. What are needed, are further investigations by both these techniques (confronted by microphotography) with a view to forming a scientific basis for haematological cytodiagnosics.

This should be extended as regards oncological research. Such a work is planned by the present authors, in view of the keen interest of haematologists in the problems of neoplastic proliferation.

Bone-marrow biopsy has, since Arinkin introduced sternal puncture in 1929, been adopted as a routine clinical examination. This technique has been generally accepted and is in common use throughout the world.

Nowadays, bone-marrow examination is performed daily by the clinical haematologist in all cases of blood diseases. The interpretation of the morphology of bone-marrow cells is not always easy. Not only is profound knowledge of characteristic cell types necessary, but also long experience and constant study of atypical cells. It may be said that the clinician's personal haematological knowledge depends on the number of cases followed. There are, however, cases in which even an eminent haematologist of long experience cannot recognise an individual cell or diagnose a syndrome. One of the gravest difficulties in precise diagnosis, is the frequent occurrence of intermediate conditions between individual haematological syndromes. A single examination may be compared to a snapshot of an object in motion — that is the findings pertain only to the given moment. The morphological picture represents the state of the blood-forming system at the moment of aspiration of the specimen. It may change in the near future, and it is often impossible to predict, with any degree of certainty, in what direction. Generally known are the difficulties encountered in differential diagnosis of granulocytopenia or bone-marrow aplasia, which may correspond to an initial period of acute

myelosis. Typical "textbook" bone marrow, lymph node or spleen aspiration smear pictures are, unfortunately, encountered somewhat infrequently in clinical practice. Conclusions from the examination of haematological preparations must be drawn with the utmost prudence, and confronted with the entire clinical picture. Diagnosis based solely upon microscopical findings often proves incorrect. A hasty diagnosis may lead to serious errors and cause loss of the patient's confidence. The cytological diagnosis should be rather descriptive, the diagnosis of a syndrome only tentative. For example, the presence of megaloblasts does not always indicate Addison-Biermer's disease or some related syndrome of the "acastlose" group; it may be caused by other, hitherto incompletely understood pathogenetic mechanisms.

Only when the cytological picture is confronted with the remaining symptoms, and often after a prolonged period of clinical observation can diagnosis be established. It is these reservations which have caused us to supplement with short clinical notes the plates representing morphological pictures.

The present work has been divided into chapters according to the generally adopted classification of cellular elements of the blood-forming system. Disorders characterised by hyperplasia of the leucocytic and reticulo-endothelial systems, parasitic diseases and tumours are discussed in separate chapters. Such an arrangement of the contents was felt to be best suited to the aim of this book, which is to provide basic haematologic information in an orderly and systematic form. On the other hand, this division was imposed upon the authors by the limited scope of their work as well as by the necessity to cover not only the pathology but also normal haematologic morphology and the development of the cellular elements of the blood.

The authors are conscious of the shortcomings of the morphologic criteria upon which haematologic diagnosis has hitherto been based. It may well be that advances in cytochemistry and cytoimmunology will cast on the chemical structure and antigenic properties of the cell such new light as to bring out the individual nature of the elements of the blood-forming system.

Although research work of this kind has made great progress, nevertheless it has not as yet achieved application in routine practice. It may be presumed that when, in future, new methods are included in routine clinical examinations, classical morphology will acquire a new and wider meaning.

It is not so long ago that haematology was a discipline cultivated only by a small group of initiated investigators. In our day, it has entered the ranks of the most universal sciences covering almost all fields of modern



clinical and experimental medicine. The blood, being an ubiquitous tissue instituting the "internal environment" of the human body, reflects the life processes of all the organs and systems. Biology interests itself in the rôle and interrelationship of the various systems in the functions of the organism considered as a whole. It is evident, therefore, that the phenomena occurring in the blood and blood-forming tissues also cannot be regarded as a strictly individual separate problem, not connected with the remaining organs and tissues.

The discovery of the nervous regulation of the course of haematopoiesis opens up a vast field for haematologic investigations. Pavlov's theory concerning the rôle of the nervous system, his concept of the integrity of the organism, the idea of nervism and of the evolution of functions, has prepared the way for new ideas about the onset, development and the final outcome of diseases.

New opportunities thus arise for the elaboration of modern methods of prevention, diagnosis and treatment of blood diseases.

A better understanding of the laws which govern haematopoiesis will clarify the mechanism of the responses and diseases of blood-forming organs, and will probably enable us to influence their course.

## OUTLINES OF BONE MARROW EXAMINATION TECHNIQUE

Aspiration biopsy has been found, among the various methods proposed for obtaining bone-marrow samples for examination, to be the most convenient procedure. In the case of adults, sternal puncture according to Arinkin's technique is generally performed. It should, however, be borne in mind that other bones may be punctured in cases, where the presence of localised lesions (*e.g. tumor metastases*) or of a generalised disease of the blood-forming system are suspected. In addition to the sternum, the iliac crest, the ribs or spinal processes of lumbar vertebrae are commonly selected for diagnostic puncture. There are instances in which the sternal pulp remains normal in the course of a blood disorder, while the biopsy of another bone (*e.g. of the iliac crest*) yields pathological findings. A "dry" sternal marrow also constitutes an indication of the desirability of effecting puncture of another bone.

Sternal puncture is in children under three years hazardous, since the needle may easily penetrate the posterior lamina and enter the mediastinum. The large vessels are in such cases usually injured with fatal consequences. In children under three years, therefore, the tibia is the bone of choice, the upper third of the diaphysis, medially from the tuberosity, being selected as the site for puncture. This procedure is known to be quite safe and easy.

The needle for bone puncture should be of hard steel, with a sharp bevelled point and stylet. On the needle should be placed a movable guard to prevent unnecessary (and dangerous) deep penetration. Different types of needles are advocated by various authors. The needle devised by M. Kubiczek is among the more convenient instruments. The present authors used successfully for a long time Strauss' vene puncture needles, for sternal puncture. Lumbar or pleural puncture needles may be also adapted for this purpose and particularly for bone puncture in children.

In adults, the manubrium of the sternum is selected as the site for puncture, at a point approximately 2 cm. below the sternal incisure. A thin needle is inserted and the skin and subcutaneous tissue, as well as the periosteum are infiltrated with 2% sterile solution of novocaine. An amount



equal to 2 ml. of the solution is usually sufficient to spare the patient all pain.<sup>1</sup> Following anaesthesia, the puncture needle, held vertically, is inserted. The bone cortex is penetrated with slight pressure and a gentle, rotary motion, to and fro twist. When the needle enters the bone-marrow cavity, a sudden "give" is usually felt. The stylet is then withdrawn, a syringe promptly attached and a small amount of bone marrow aspirated. Usually from 0.25 to 0.3 ml. of the bone marrow is sufficient for all purposes (intravital staining, phase-contrast microscopy, tissue culture, etc.). For the preparation of smears for routine staining, the contents of the lumen of the needle are more than ample.

The aspirate is placed on a watch glass, liquid blood being poured off. Bone-marrow particles are selected, using a small forceps or bacteriological loop, posed upon an object or cover glass for the execution of smears. In addition to May-Grünwald-Giemsa stain, supravital stains (brilliant-cresil blue or Nile-blue sulphate) should be used.

The iliac crest is punctured approximately 5 cm. posterior to the spina iliaca anterior. The patient lies on his back, or, in cases of ascites, on his side. Following local anaesthesia the needle is inserted at right angles to the frontal plane. The skin and subcutaneous tissue are penetrated, the needle being at an angle of 45 degrees to the iliac crest. When the outer cortex is reached, the needle is posed at right angles to the iliac crest and bored into the bone by rotary motion, its tip pointing in the direction of the lower extremities. On entering the marrow cavity, a distinct "give" is felt. A 20 ml. syringe is best suited for aspiration. This procedure is completely safe. A word of warning must, however, be given against entering the bone from the lateral aspect in the direction of the abdominal cavity.

If a spinal process of the lumbar vertebra is to be punctured, the patient takes a sitting position, inclined forward. Broad processes, such as those of the third or fourth lumbar vertebra, are selected. Adipose subjects are not suited for this procedure. Following local anaesthesia, the puncture needle is inserted at right angles to the surface of the spinal process. Penetration to a depth of from 1.5 to 2 cm. is usually quite sufficient. The puncture is usually almost painless and more tolerable for sensitive patients than sternal puncture. The ribs are best punctured at a point several centimeters laterally to the vertebral column.

The technique of biopsy of the remaining organs lies beyond the scope of the present work. Detailed descriptions are to be found in standard textbooks of haematology or in specialised monographs.

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<sup>1</sup> Rigid sterile precautions must, of course, be ensured throughout the entire procedure, the skin of patient and the operators' hands sterilised with antiseptic solutions etc.

## CHAPTER I

### THE RED-CELL SERIES

The course of human erythropoietic activity may be divided into two different periods: the foetal (megaloblastic) generation and the normoblastic generation. Each of them is characterised by the presence of morphologically distinct red-cell types — the megaloblast and the normoblast.

In physiological conditions, the megaloblastic phase is of short duration, comprising only the first two months of foetal life. As development proceeds, the temporary oxygen carriers — megalocytes — are replaced by normal red cells — normocytes — and the normoblastic trend of erythropoiesis prevails.

Although both red-cell types have similar functions in common, they are morphologically different. Precise knowledge of their characteristic traits is of fundamental importance in the identification of pathological states of the haematopoietic system.

It should be emphasised, that the megalocytes are a red-cell species possessing full functional efficiency, and that they participate in the physiological development of the human foetus and its haematopoietic system during intrauterine life. The appearance of megaloblasts during extrauterine life is considered as an abnormal condition suggesting a pathological phenomenon.

### NORMOBLASTIC ERYTHROPOIESIS

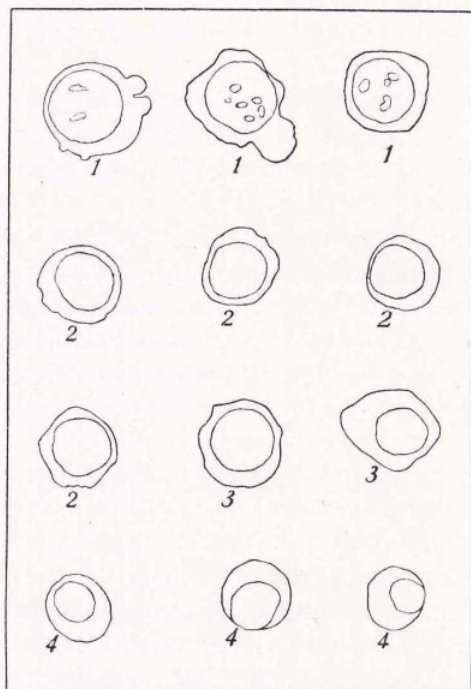
The stem-cell of the normocytic red-cell series is termed proerythroblast. It is a cell measuring 14—20  $\mu$  in diameter. This cell possesses a large oval or round nucleus. Its chromatin is arranged in fine interwoven strands and filaments. Parachromatin (oxychromatin) is, as in most immature cells, abundant. It is generally held, that with progressing differentiation, parachromatin is converted into basochromatin, which intensively takes basic stains. Several faint nucleoli may often be observed. These are usually darker than the rest of the nuclear structure. This trait is helpful in differentiation between proerythroblasts and myeloblasts. The nucleoli



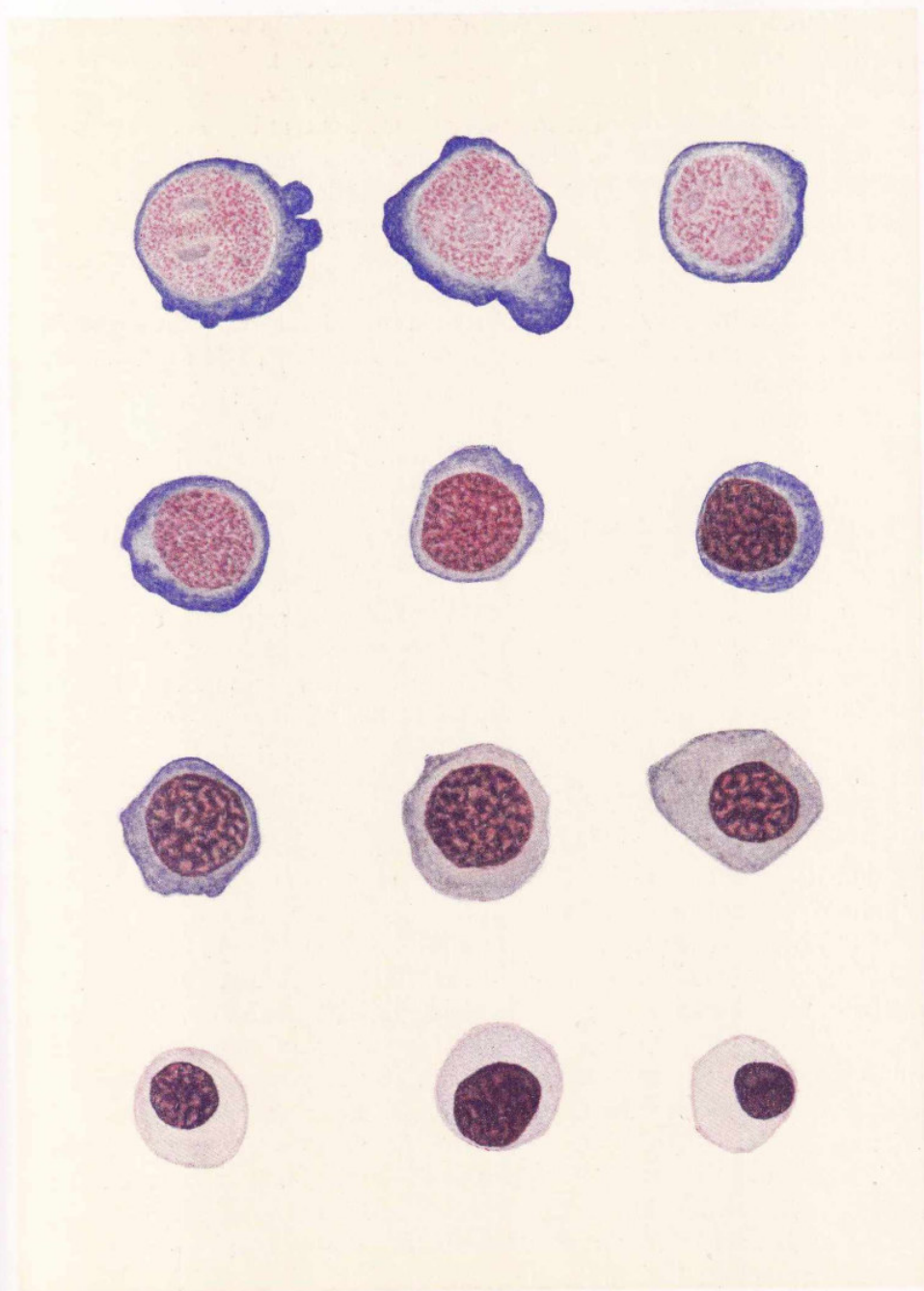
of the latter are larger, vesicular and stain greyish-blue. The nucleus fills almost the whole cell, and is surrounded by a narrow rim of deeply basophilic agranular cytoplasm. In the perinuclear region, a chromophobic whitish-clear border is visible. Sometimes the cytoplasm forms one or several bud-like pseudopodia.

The next developmental stage of the red cell is the basophilic erythroblast. It is of variable dimensions ranging in diameter from 10 to 19  $\mu$ . The outlines of this cell are usually round, occasionally oval, the cytoplasm is distinctly basophilic. The oval nucleus loses its fine reticular structure, the nucleoli are no longer demonstrable. The chromatin differentiates into deeply dark-stained basochromatin, forming the main nuclear contents, and parachromatin (oxychromatin) becomes arranged into whitish stripes, which give to the nucleus its wheel-spoke appearance.

The presence of nucleoproteins, in particular of ribonucleic acid, is responsible for the basophilia of the cytoplasm in immature cells. At this stage, the cells multiply intensively and their capacity for synthesising proteins and nucleic acids develops to a high degree.



*Plate 1.* — The developmental cycle of the red cells (normoblastic erythropoiesis): 1, Proerythroblast. 2, Basophilic erythroblast. 3, Polychromatic erythroblast. 4, Orthochromatic erythroblast. A close inspection of proerythroblasts reveals the presence of faintly visible nucleoli.



*Plate 1.* — The developmental cycle of the red cells  
(normoblastic erythropoiesis).



As the content of ribonucleic acid decreases, the cytoplasm of erythroblasts loses its affinity for basic dyes and begins to take acid stains. This change in the tinctorial properties of the cytoplasm is brought about by the gradual accumulation of heme and globin. Cells at this stage are called polychromatic erythroblasts. They are usually smaller than the basophilic erythroblasts. Their cytoplasm may either be reddish-violet or show alternating acidophilic and basophilic zones. The nucleus is smaller, pycnotic and often assumes the wheel-spoke appearance. Nucleoli are not demonstrable.

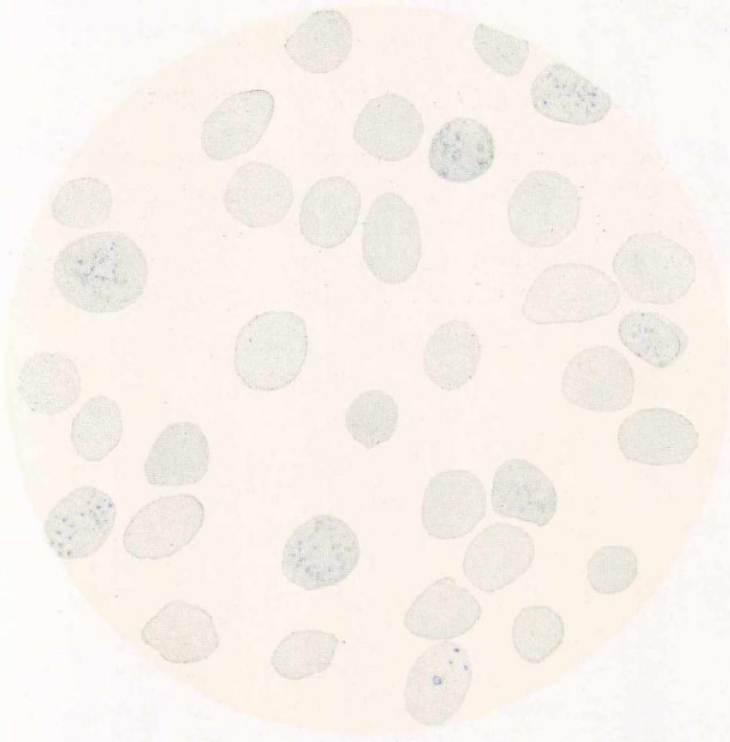
The next step in the course of differentiation is initiated by the orthochromatic or acidophilic erythroblast. In normal conditions, this cell is smaller, its fully haemoglobinated cytoplasm reveals no difference in colour from that of mature red cells. The pycnotic nucleus is uniformly stained dark violet, although its wheel-spoke arrangement occasionally persists.

### RETICULOCYTES

By using supravital stains (for instance brilliant-cresil blue, Nile blue, methylene blue), fine blue strands and granula may be demonstrated in the cytoplasm of some nucleated and mature red cells. Their distribution around the nucleus resembles a wreath. In nonnucleated red cells, these filaments and granula are located centrally, forming a loose skein or a reticulum. Sometimes only a few solitary filaments or granula may be seen. This *substantia reticulo-filamentosa* is not a nuclear derivative, but it is formed in the cytoplasm of transitory stages between the nucleated erythroblast and the mature red cell. These cells, called reticulocytes (skein cells), institute a developmental phase preceding the definitive maturity of the red cell. It has been established that the polychromatophilic erythrocytes, occasionally encountered in blood smears stained by Pappenheim's method, contain this basophilic substance. The precise chemical nature of this substance is still unknown. Some more recent investigations indicate that its presence may be associated with a higher content of free protoporphyrin, which phenomenon accompanies incomplete haemoglobin synthesis. Dustin claims, that ribonucleic acid may be one of the constituents of the "substantia reticulo-filamentosa". Reticulocytes should be then regarded as young red cells. Their count in peripheral blood or in bone marrow may be used for the evaluation of the regenerative activity of the erythropoietic tissue.

In the peripheral blood of normal humans 3 to 15%<sub>00</sub> reticulocytes are present.

Eilers has recently described the presence of what she calls achromoreticulocytes. These are larger in diameter than normal reticulocytes, and



*Plate 2.* — Reticulocytes.

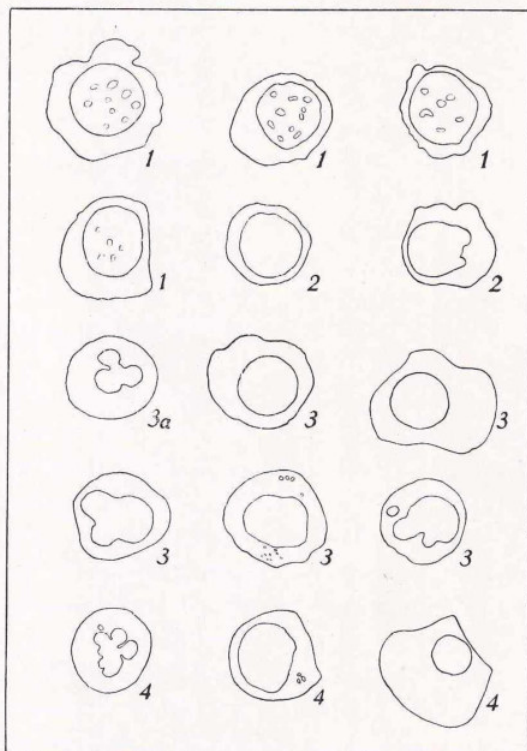


are to some extent chromophobic. These cells may be stained by means of Eilers' technique — Giemsa's stain 20-times diluted for 12 hours at 4°C. Clinical observations indicate (Haenel, personal observations) that classic techniques make it impossible to evaluate fully the regenerative activity of the erythropoietic system.

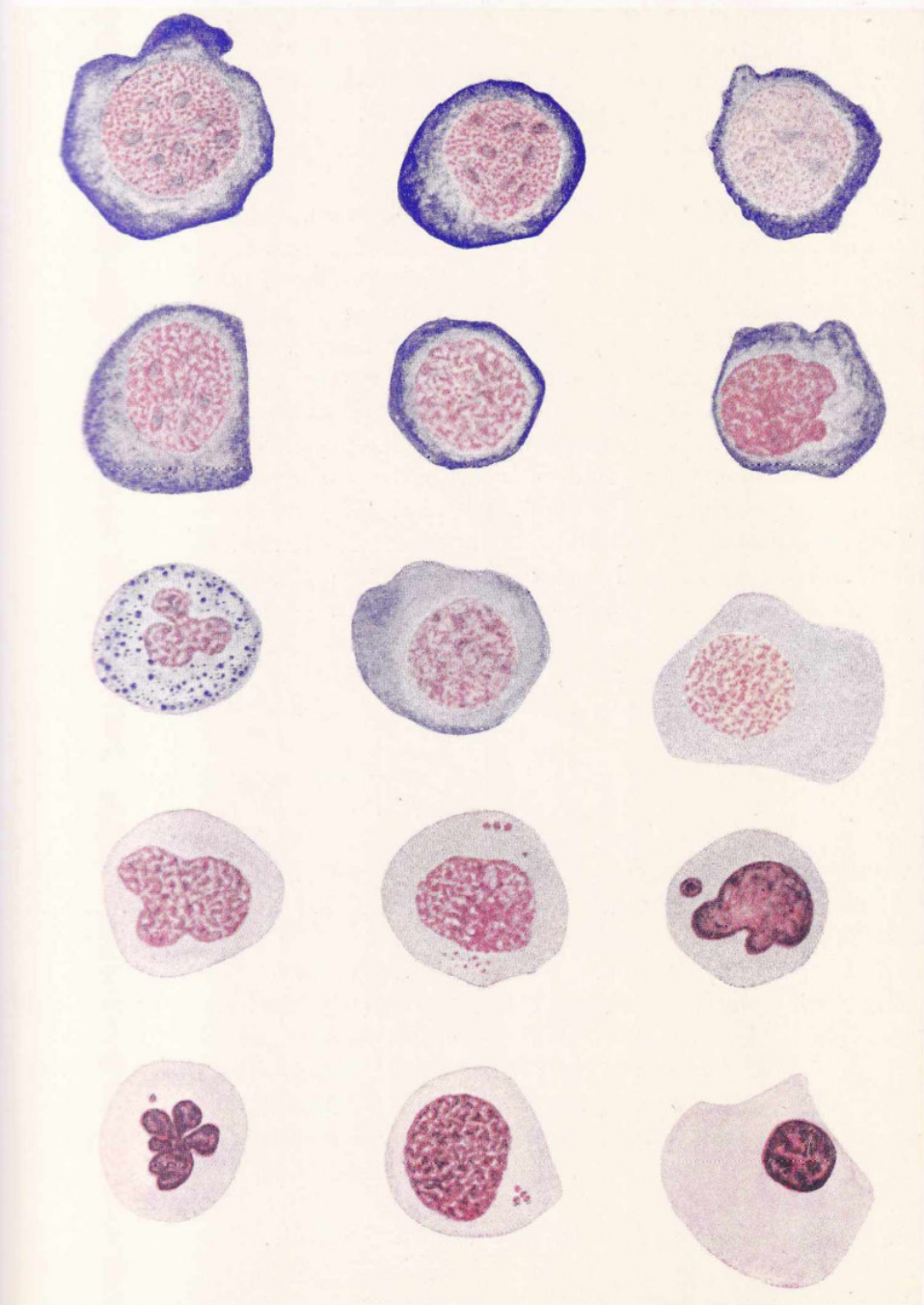
As soon as the basophilic reticulum disappears, the erythrocyte attains full maturity.

### MEGALOBLASTIC ERYTHROPOIESIS

States of deficiency, which influence the haematopoietic activity of the bone marrow, may lead to the development of various types of anaemia. Iron deficiency causes anaemia, which manifests itself morphologically by changes in the cytoplasm of the erythrocyte associated with disturbed haemoglobin synthesis. Another variety of anaemia caused



*Plate 3.* — The developmental cycle of the red cells (megaloblastic erythropoiesis): 1. Pro-megaloblast. 2. Basophilic megaloblast. 3. Polychromatic megaloblast. 3a. — polychromatic megaloblast presenting atypical stippling in the cytoplasm. 4. Orthochromatic megaloblast. cf plate 1 (the intensity of nuclear staining) and the description in text.



*Plate 3.* — The developmental cycle of the red cells (megaloblastic erythropoiesis).



by deficiency in maturation factors manifests itself by profound disturbances in erythropoiesis, expressed by dysplastic alterations in regenerative activity, which then takes a course resembling the foetal period. This course of erythropoiesis, called megaloblastic is morphologically stigmatised by changes in the cell nucleus expressed by delayed or abnormal maturation.

The diagnosis of megaloblastic erythropoiesis is of fundamental importance and should be based on the examination of bone-marrow smears. The finding of large oval mature red cells in peripheral blood smears, and even the presence of single nucleated red cells with fine chromatin stroma, is not always sufficient to justify diagnosis of erythropoiesis of the foetal type. In peripheral blood, macrocytosis may be produced by other factors, for instance, high reticulocytosis or a macronormoblastic reaction of the bone marrow. Apart from these considerations, it is the examination of the bone marrow which establishes the diagnosis of megaloblastic type of regeneration of the red-cell series.

The following stages, similar to those found in normoblastic erythropoiesis, are distinguished in the development of the megaloblast:

promegaloblast, basophilic —, polychromatic —, and orthochromatic megaloblast.

It should be borne in mind, that the size of the cell is not decisive in recognition of a megaloblast. The dimensions of these cells vary within wide limits.

The stem-cell of red cells of the foetal type — the promegaloblast — may, by comparison with a proerythroblast, reveal no points of difference. The appropriate term for such cells of moderate size is rather proerythroblast. According to Tempka, a typical promegaloblast may attain a diameter of 27  $\mu$ . The nucleus of this cell is round and has an extremely fine reticular structure and usually contains from 8 to 10 nucleoli. Parachromatin (oxychromatin) dominates over basochromatin.

Prominently large promegaloblasts (up to 40  $\mu$  in diameter) are called gigantoblasts. The nuclei of such cells are often indented and abnormal in shape. The cytoplasm of the promegaloblast stains deep blue and shows a clear zone in the perinuclear region. The identification of red cells of the foetal type is easier in later developmental stages.

In evaluating these cells attention should be focused on:

1. Nuclear structure,
2. The nucleo-cytoplasmatic ratio and the breadth of the cytoplasmatic rim,
3. The shape and size of the cell.

(1) In basophilic, polychromatic and occasionally even in orthochromatic megaloblasts, the nucleus has a loose, spongy structure, caused

by the preponderance of parachromatin. The basochromatin shows little tendency to form agglomerates. In later stages, basochromatin may condense in clumps, irregularly distributed, which gives the nucleus a motley appearance. During the stage of haemoglobin formation, the leptochromatic character of the nucleus persists, as evidence of its delayed maturation in comparison to the cytoplasm.

During mitosis, deep disturbances occur. Multipolar mitoses and atypical chromosomes are found. More mature cells in mitosis are characterised by a wide rim of abundant cytoplasm, which facilitates the recognition of their megaloblastic character.

The nucleus is often atypical, lobulated or of bizarre shape (teratological forms). Binucleated megaloblasts are not infrequently encountered. They often contain many nuclear splinters in the cytoplasm. The nucleus of these cells splits into many fragments (*karyorrhexis*). On rare occasions karyolytic mechanisms are operative in the denucleation process.

(2) Abnormal nucleo-cytoplasmatic ratio shifted in favour of the cytoplasm institutes another mean of identification of megaloblasts. The cytoplasm, contrarily as in erythroblasts, forms a broad zone around the nucleus.

(3) Megaloblasts are usually larger than erythroblasts. The abundance of cytoplasm suggests the hypothesis that in these cells the coordination between the process of growth and division is disturbed.

## PATHOLOGICAL ERYTHROBLASTS (PARAERYTHROBLASTS)

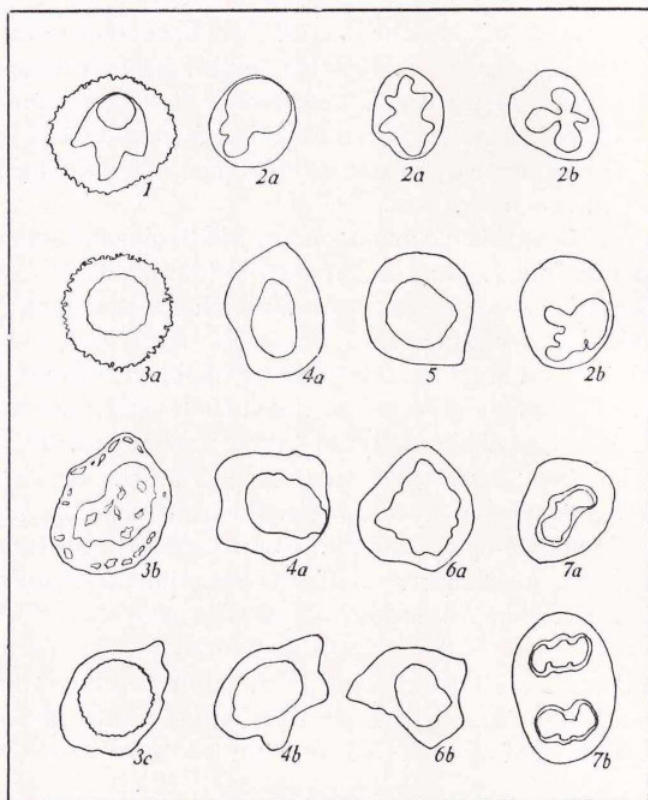
In some pathological states there may occur numerous deviations from the normal type of erythroblast caused by the damage of the cell by various factors. Erythroblasts of this type called paraerythroblasts, may be so changed that the classification of them as cells belonging to the red-cell series is possible only when less damaged, nucleated red cells are present, instituting identifiable transitory forms.

Pathological changes may concern the nucleus and the cytoplasm equally and may be present in cells at various developmental stages.

Plate 4 represents paraerythroblasts found in bone-marrow smears in cases of aplastic anaemia, acute myeloblastic leukaemia, severe toxæmias and in states following X-ray injury.

The nucleus of pathologically changed cells loses its normal structure, its design becomes blurred, the chromatin homogenised. Sometimes, the basochromatin aggregates on the periphery of the nucleus, forming a ring-like border around a central field of lightly stained parachromatin. In other instances, the nucleus possesses stellar processes, poorly delineated protruding far into the cytoplasm. In this case, the basochromatin





*Plate 4.* — Paraerythroblasts: 1. The chromatin is dense, pycnotic, as in late erythroblasts; strands of an azurophilic and simultaneously acidophilic substance, surrounding the main chromatin aggregate, reach far into the cytoplasm; the cytoplasm is granular. 2. The abnormality consists in atypical bizarre shape of the nucleus, 2a. — the cytoplasm is faintly basophilic, demonstrating an affinity for basic stains uncharacteristic of erythroblasts, moreover one of these cells is stippled; 2b. — the cytoplasm is normal. 3. Erythroblasts with a marked basophilia of the cytoplasm, such as encountered at early developmental stages (in proerythroblasts or in early basophilic erythroblasts), 3a. — the nuclear structure corresponds to that of late polychromatic or orthochromatic erythroblasts, the cytoplasm is granular; 3b. — the nuclear outline is irregular, the structure is blurred, the nucleus and the cytoplasm vacuolated; 3c. — note the granular structure of the nucleus. 4. Polychromatic erythroblasts with atypical nuclear structure, the oval outlines of the cell, as well as of the nucleus, constitute another abnormality, 4a. — late polychromatic erythroblasts, the cytoplasm of one of these cells is vacuolated; 4b. — early polychromatic erythroblast, the cytoplasm is vacuolated. 5. Erythroblast with an atypical "lymphocytoidal" nucleus and abnormally stained cytoplasm. 6. Orthochromatic erythroblasts with atypical stippling of the cytoplasm, 6a. — the shape and structure of the nucleus are markedly abnormal, it is difficult to decide if this appearance is the result of an abnormal division or of abnormal karyolysis; 6b. — the cellular outline is abnormal, the nucleus demonstrates a "lymphocytoidal" structure. 7. Atypical cell divisions, 7a. — metaphasis, the chromosomes are clumped together, of blurred outlines; the basophilic chromatin is embedded in an acidophilic substance; the cytoplasm stains abnormal and demonstrates stippling; 7b. — anaphasis, abnormalities as above.

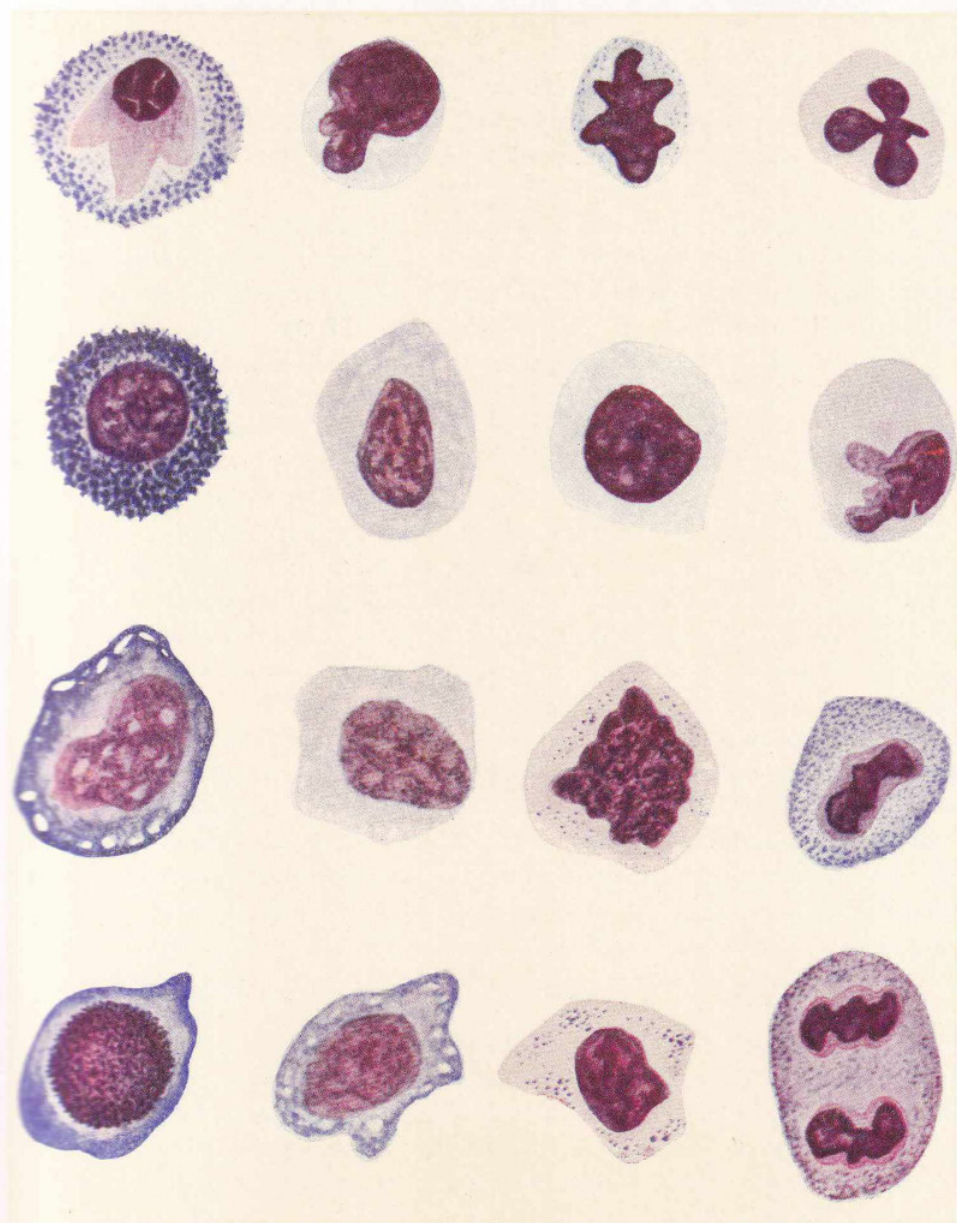


Plate 4. — Paraerythroblasts.



may accumulate on one pole of the nucleus presenting a darkly stained mass. The nucleus may also be of abnormal "fantastic" shape or may disintegrate into several fragments.

The cytoplasm is granular or foamy, which indicates serious disturbances in the maturation and haemoglobin formation. Some paraerythroblasts are morphologically similar to finely granular reticulum cells, but they differ from the latter by the presence of haemoglobin. The outlines of abnormal cells are often frayed and the cytoplasm is vacuolated. Vacuolation of the nucleus is rarely demonstrable. As a result of severe injury to the cell, disturbances in coordination of the maturation of the nucleus and the cytoplasm are present. Unlike the megaloblastic series, the nucleus matures in advance of the cytoplasm.

Mitotic figures are also evidence of deep lesions in the red-cell system. The chromosomes become thick, fuse together and are occasionally fragmented. In many cases chromosomes stain inhomogeneously.

Paraerythroblasts, a characteristic feature of di Guglielmos's acute erythraemia, attain extreme abnormality of form in a neoplastic variety of this disease described in one of the subsequent chapters.

Björkman has recently demonstrated that even immature and atypical erythroblasts agglutinate, in an analogical way, as mature red cells in the presence of influenza viruses (virus haemagglutination). This test should be considered as strictly specific. Stem-cells of the other series (myeloblasts and lymphoblasts) show no agglutination. The authors are of the opinion that this test may, in clinical practice, prove to be useful as a means of diagnosis of erythromyelosis. This disease often escapes attention and has hitherto usually been diagnosed as acute myelo- or haemocytoblastic leukaemia.

## ERYTHROCYTES IN PERIPHERAL BLOOD SMEARS

In the peripheral blood smears of healthy individuals there are found normal red cells (normocytes), almost constant in size, and reticulocytes.

Red cells from 6.686 to 7.718  $\mu$  in diameter are considered as normocytes. Smaller red cells are called microcytes, larger ones — macrocytes.

The dimensions of reticulocytes are usually greater, their mean diameter is from 8.06 to 8.28  $\mu$  (Netoušek).

Closer inspection of a smear of normal peripheral blood usually reveals minute differences in size and shape of individual red cells, considered as physiological anisocytosis and poikilocytosis.

The differences in size may be graphically expressed by means of the Price-Jones curve, established on the diameter measurements of at least 200 red cells.

## ANISOCYTOSIS AND POIKILOCYTOSIS

In pathological states the most frequent red-cell change is a marked inequality of size (anisocytosis) and differences of shape (poikilocytosis).

Poikilocytes are erythrocytes of abnormal shape, elongated, pear-shaped, wedge-shaped, dumb-bell-shaped or of frankly bizarre form.

They probably originate in peripheral circulation from red cells of increased mechanical fragility. Poikilocytosis is often accompanied by the presence of schizocytes, that is, minute fragments of erythrocytes resembling dots or commas.

Poikilocytes should not be confused with crenated erythrocytes. These forms arise under the influence of hypertonicity of the blood serum during slow drying of the smear.

## POLYCHROMASIA OF ERYTHROCYTES (POLYCHROMATOPHILIA)

Single greyish-blue erythrocytes may be encountered in smears of normal peripheral blood. They possess a simultaneous affinity to basic stains and acid stains. This symptom becomes marked during intensive erythropoiesis, as for example in posthaemorrhagic anaemia, pernicious anaemia (especially during remission following liver-extract treatment), in haemolytic disease, neoplastic metastases to the bone marrow and many other conditions.

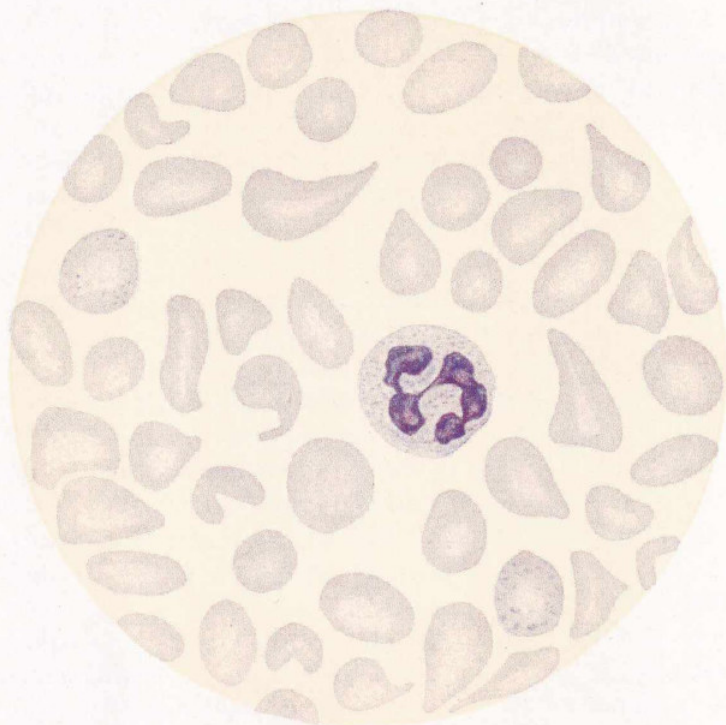
As evidence of increased erythropoiesis, polychromatic erythrocytes may occur not only in anaemic states, but also in polycythaemia. It follows that the presence of these cells must be interpreted in accordance with the clinical picture of the disease. Hasty conclusions as to the efficiency of regenerative activity of the bone marrow and optimistic prognosis may be misleading.

Polychromatic erythrocytes are generally considered as young elements, with inadequate haemoglobin content and possessing remnants of basophilic stroma spongioplasm. Histochemical investigations show that the basophilic substance is not of nuclear origin. The basophilia of these cells is associated with the presence of cytoplasmatic RNA (Plate 6).

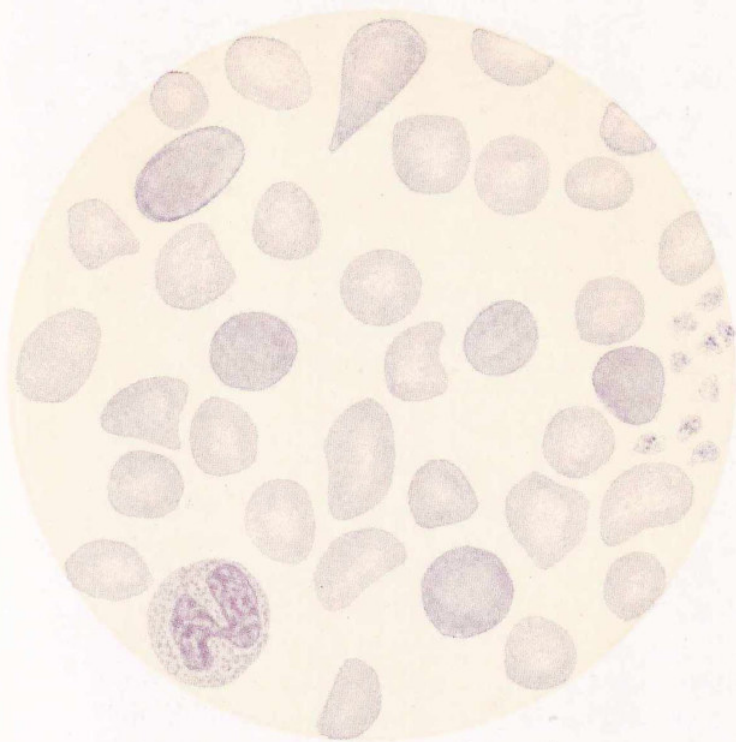
In smears stained with Nilc blue, polychromasia is more readily discerned. Polychromatic erythrocytes are, in such preparations, blue or dark blue, whilst normal red cells are green.

Netoušek asserts that polychromasia is not only characteristic of young elements, but may be observed also in erythrocytes undergoing destruction, as for instance in haematomas.





*Plate 5.* — Anisocytosis and poikilocytosis.



*Plate 6.* — Polychromasia of erythrocytes.



The number of polychromatic cells is usually in proportion to reticulocytosis. In many of the polychromatic erythrocytes, "substantia reticulo-filamentosa" may be demonstrated. Both these red-cell types should not, however, be confused.

### PUNCTUATE BASOPHILIA OF RED CELLS (BASOPHILIC STIPPLING)

This phenomenon may be observed in mature denucleated red cells, as well as in erythroblasts with an intact nucleus. The basophilic substance contains, as is shown by negative Feulgen's reaction, no desoxyribonucleic acid (basophilic nuclear constituent).

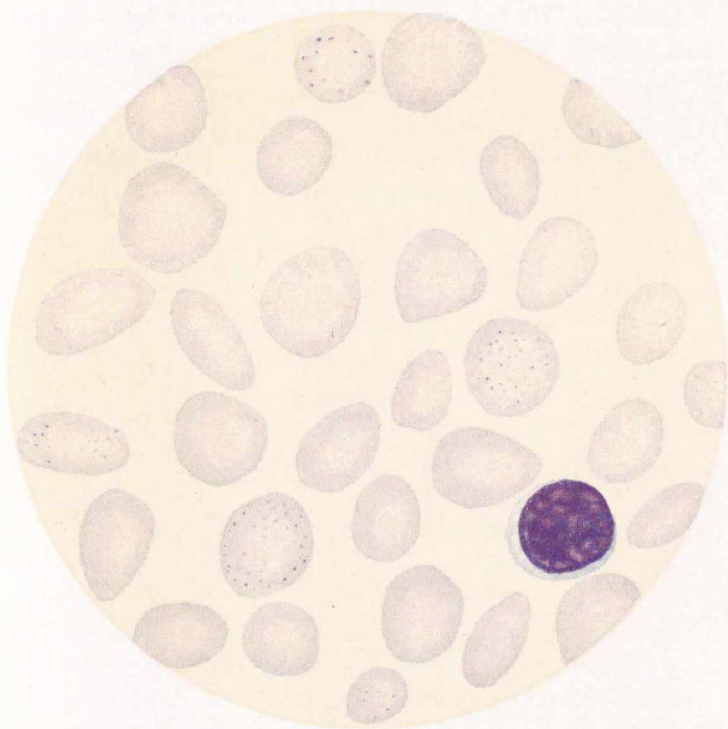
Stippled erythrocytes are young elements. Punctuate basophilia probably arises from flocculation phenomena. A basophilic substance normally present in a diffuse state, possibly identical with the rest of spongio-plasm, precipitates. According to this view, basophilic stippling, polychromasia and "substantia reticulo-filamentosa" are but varieties of the same cellular substance.

There are very few stippled cells present in normal peripheral blood. These may be encountered especially in women. The count of stippled cells is increased in foetal blood or in certain anaemias. Punctuate basophilia is also present in blood in cases of poisoning by heavy metals such as lead, bismuth, zinc, and mercury. The presence of stippled erythrocytes may be taken as a sign of abnormal erythropoiesis.

Special importance is attached to the appearance of punctuate basophilia in lead poisoning. This symptom, taken with the rest of the clinical picture makes it possible to evaluate the scope of the toxic influence exerted by lead. The increase in the number of stippled and polychromatic erythrocytes during a period of lead poisoning is explained by a regenerative response of the organism to the injury caused by the metal to the red cells. This symptom, however, lacks specificity and therefore is of doubtful value. Personal observations suggest that it is not always present in cases of lead poisoning.

The best method of demonstrating punctuate basophilia consists in fixation of a thoroughly dried smear (2—3 hrs. on air) with methanol, and staining with methylene blue.

According to Teisinger, one stippled cell in 5—10 fields of vision (that is ca 300 per 1,000,000 erythrocytes) is suggestive of lead poisoning in an individual exposed to lead hazard. Other authors consider 600 or even 3,000 stippled erythrocytes per million as the upper limit in healthy individuals.



*Plate 7.* — Basophilic stippling of erythrocytes.



Cases where one stippled erythrocyte may be found in the blood per every field of vision, are designated by Schulten as +++; cases where are several stippled cells per every field are designated as ++++ (after Netoušek).

Whitby and Britton regard stippled cells and reticulocytes as identical elements. They hold that the punctuation arises from injury to "substantia reticulo-filamentosa" caused by lead or other poisons. It is therefore possible to conclude that the reticulocyte count may substitute stippled-cells enumeration as a simpler method.

Plate 7 represents a blood smear from a case of severe lead poisoning.

Anaemia is of a slightly hypochromic type. Stippled cells as immature elements are usually larger in size than the normal red cells.

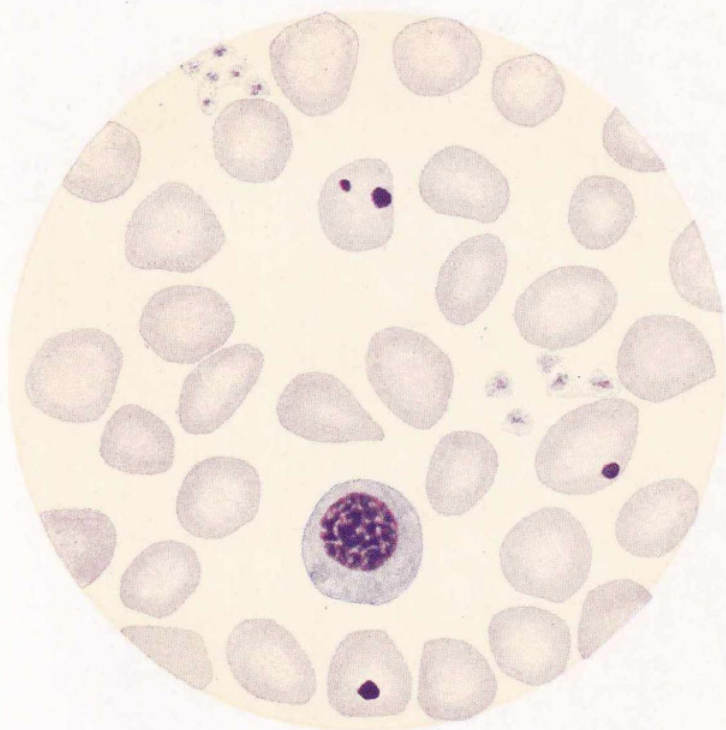
### HOWELL-JOLLY BODIES

The process of the maturation of the erythroblastic nucleus is slow, but when it attains the pycnotic phase it is rapidly destroyed. Although there are many hypotheses, the mechanism of denucleation of the red cells is unknown. Recent investigations on bone marrow cultivated in vitro, using phase-contrast cinematography, suggest that the nucleus may be extruded (Bessis and Bricka). Another view is that the nucleus is dissolved within the cell (*karyolysis*). It is held, that the enzymatic system concerned in the dissolution of the nucleus depends on the humoral regulation by the spleen. This hypothesis is based on the observation of patients' blood following splenectomy. In blood smears of such cases erythrocytes, containing minute round bodies from 0.5 to 1  $\mu$  in diameter taking nuclear stains, are nearly always present. These cell inclusions, called Howell-Jolly bodies, may remain in the blood of patients operated upon for many years after the surgical intervention (27 years in a case described by Naegeli).

It is now beyond discussion that Howell-Jolly bodies are nuclear remnants following its incomplete dissolution. This contention is strengthened by the fact that, as shown by positive Feulgen's reaction, they contain desoxyribonucleic acid.

Howell-Jolly bodies may be formed by fragmentation of the nucleus. They may also arise during mitosis from splitting off of chromosomes, which afterwards become round.

In most cases one, on rare occasions two, of these bodies may be encountered in a single erythrocyte. As dissolution progresses, they are transformed into almost invisible chromatin dust. It is not only after splenectomy that Howell-Jolly bodies may be found in the blood but also during disturbances in the function of the spleen, in severe anaemias,



*Plate 8.* — Howell-Jolly bodies.



haemolytic disease, pernicious anaemia, leukaemias and other diseases with abnormal haematopoiesis.

In normal conditions, Howell-Jolly bodies may be met in the blood of the newborn.

Plate 8 represents the peripheral blood of a child, with congenital haemolytic anaemia, during regenerative haematopoietic response. Howell-Jolly bodies are visible in several erythrocytes.

### CABOT'S RINGS

Cabot's rings are fine thread-like, elliptical or 8-shaped convolutions. In preparations stained by the May-Grünwald-Giemsa's process, they are of reddish hue.



*Plate 9.* — Cabot's rings. 1. Cabot's rings in erythrocytes. 2. Neutrophil segmented granulocyte. 3. Monocyte. 4. Platelets.

*Note:* marked anisocytosis and poikilocytosis of red cells.



*Plate 9. — Cabot's rings.*



Until recently it was held that these structures are remnants of the nuclear membrane, their presence being taken as a sign of disturbed karyolysis. However they were described in erythroblasts with an intact nucleus and Schleicher (1942) produced them artificially. He believes that their appearance is caused by injury to the lipid membrane of the red cell — for instance under the influence of haemolytic or toxic agents.

The appearance of Cabot's rings is not pathognomonic. They may be encountered in various anaemias, Addison-Biermer's disease, haemolytic syndromes, leukaemias and heavy metal poisoning.

Plate 9 illustrates Cabot's rings in a peripheral blood smear (Addison-Biermer's disease).

### HEINZ-EHRlich'S INTRACELLULAR BODIES

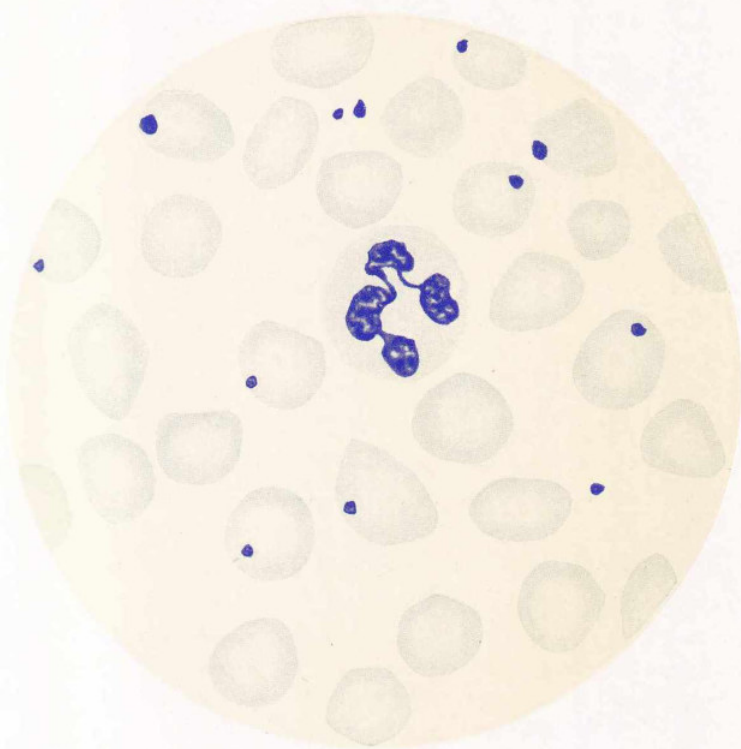
These inclusions are fine, round, of varying dimensions and stain dark blue with Nile blue in a 0.5% solution. They are located on the periphery of the erythrocyte; occasionally they may be found extracellularly.

The existence of such bodies is evidence of grave toxic lesions caused by the action of substances which oxidise haemoglobin. Such substances include phenylhydrazine, nitrobenzol, aniline, antifebrin, potassium chlorate, nitroglycerine, erythrolum tetranitricum, toluendiamine, pyridine and sulphonamides.

The view formerly held, that Heinz's bodies are identical with methaemoglobin, was recently criticised, because of the stability of the oxidation products of the blood pigment present in the erythrocytes. The chemical nature of Heinz's bodies is not known; it may be presumed, however, that they contain transformed proteins of the cell. Their presence is not always accompanied by methaemoglobinaemia or sulphhaemoglobinaemia. There may also be no anaemia. Severe poisoning with one of the compounds referred to may lead to toxic lesions of erythrocytes and haemolytic disease.

The general use of sulphonamides and the progress made by industrial medicine are giving new importance to these inclusions. The blood of patients treated with sulphonamides and of persons employed in chemical branches of industry should be closely investigated for the presence of these bodies.

Plate 10 illustrates a blood smear (stained with Nile blue) from a case of sulphapyridine poisoning.



*Plate 10.* — Heinz-Ehrlich's bodies.



## OVALOCYTOSIS, ELLIPTOCYTOSIS

The oval red cell is philogenetically older than the round form. Ovalocytosis is a characteristic trait of erythrocytes in lower vertebrates; in mammals it appears in *Camelidae*.

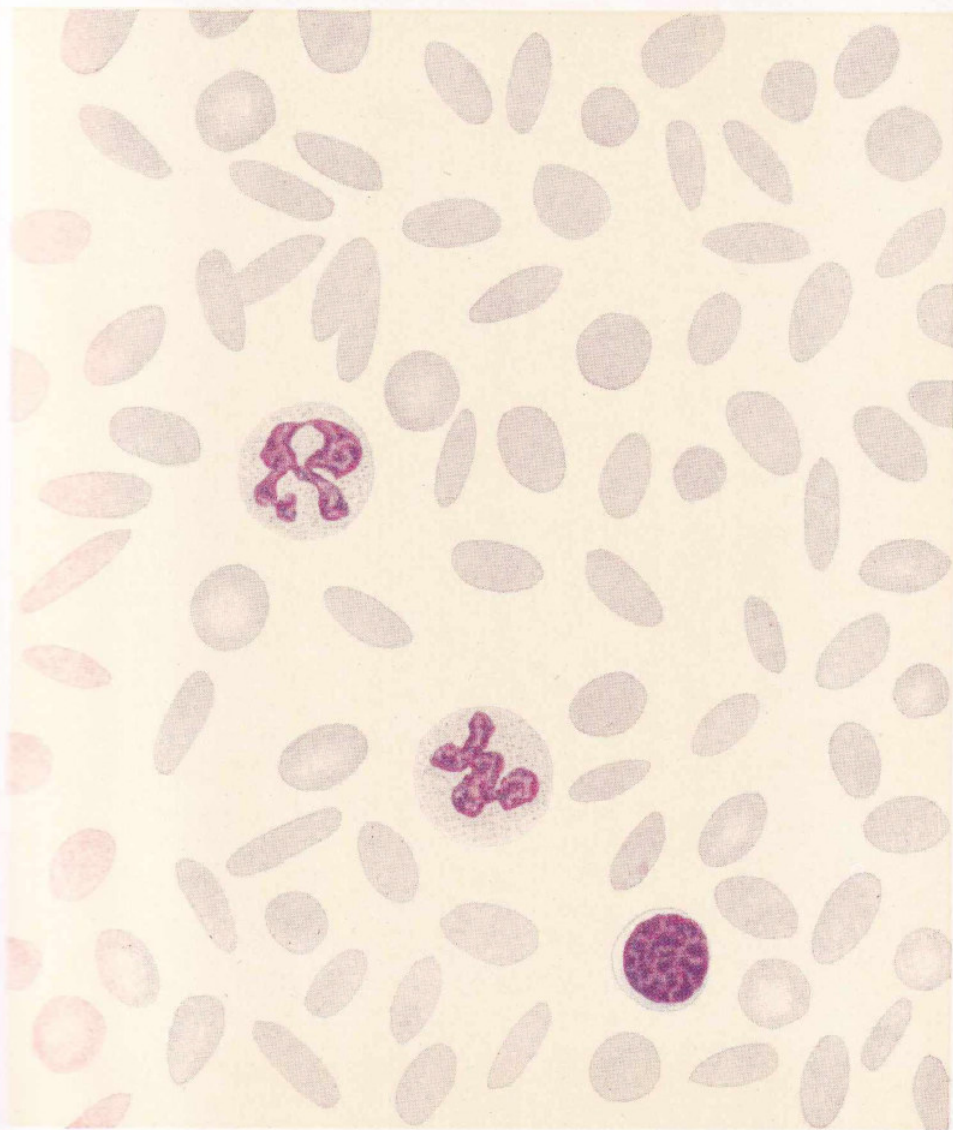
In normal human blood, slight deviations occur in the round shape, and precise measurements have indicated the presence of an average of 11 % ovalocytes.

When the number of ovalocytes reaches 80 to 90 % of the red-cell population, ovalocytosis or elliptocytosis is diagnosed.

This trait has been observed in individuals of various nationalities as a congenital anomaly. In most bearers of this trait, there is no anaemia or evidence of increased blood destruction. Ovalocytes show normal osmotic fragility. However there are conditions in which these cells are more easily destroyed than normal erythrocytes and they may be considered as less resistant. In such cases, comprising about 12 per cent of individuals bearing the ovalocytic trait, symptoms of increased haemolysis are present. Anaemia does not usually become marked, because of the regenerative response of the marrow evidenced by high reticulocytosis. Erythroblasts, as well as most of the reticulocytes, are round. It therefore seems that the erythrocyte becomes an ovalocyte or an elliptocyte after attaining full maturity.

Plate 11 shows peripheral blood from a case of congenital elliptocytosis.

A healthy 18 years old girl and her family (two brothers, a sister and mother) revealed the trait of ovalocytosis. The sister (17 years old) had a palpable spleen. Both sisters showed dental anomalies. One of the healthy brothers (12 years old) had the so-called "gothic palate", the other presented a very discrete subicterus. The mother (41 years old) revealed no abnormal findings (W. Łaukowicz and N. Włodarski — unpublished observations).



*Plate 11.* — Ovalocytosis.





## CHAPTER II

# DISORDERS OF THE ERYTHROPOIETIC SYSTEM

## ACUTE POSTHAEMORRHAGIC ANAEMIA

The clinical symptoms of acute posthaemorrhagic anaemia depend on the cause, the rapidity and the extent of the blood loss. The peripheral blood picture, during the initial period, does not show any conspicuous changes. It is a "monotonous" blood picture. The red cells are of normal dimensions, shape and colour (normochromic). Anisocytosis and poikilocytosis remain within normal limits. The mean red-cell volume is normal. Thrombocytosis and neutrophilic leukocytosis may appear as the result of a haemorrhage.

The succeeding changes in peripheral blood depend on the regenerative capacity of the bone marrow. If the blood-forming activity is stimulated to a certain degree, anisocytosis and poikilocytosis develop in the course of a few days. This phenomenon reflects the increased reticulocyte count in the peripheral blood. These cells possess a greater diameter. Thus when reticulocytes are sufficiently numerous, the blood picture may be characterised by macrocytosis. This is usually of brief duration and disappears, together with increased reticulocytosis. In some cases, sporadic erythroblasts may be found. No hypochromia of the red cells is generally observed. For this to occur, the blood loss must be repeated and other factors causing iron deficiency in the organism, as for example a faulty diet or deficient iron absorption by the gastro-intestinal tract, must be present. The finding of hypochromic erythrocytes in peripheral blood smears suggests chronic blood loss.

The bone-marrow findings after an acute haemorrhage vary according to the underlying illness, the age of the patient, the regenerative activity of the blood-forming system and the eventual presence of additional complications — for example a chronic infection.

If the bone marrow is capable of an active response to loss of blood, signs of stimulated haematopoiesis will be found. Erythropoiesis is of the normoblastic type. The number of erythroblasts is greatly increased (it



may exceed 50% of all nucleated elements of the bone marrow) and all stages of maturity are represented. Symptoms of maturation arrest (in contradistinction to the picture of iron-deficiency anaemias) are absent, as also no disturbances of haemoglobin formation are observed. Numerous erythroblasts as well as cells of the granulopoietic series in mitosis are found.

During later periods following a haemorrhage, the peak of the maturation curve of erythroblasts passes to the orthochromatic stage.

Some haematologists (Piechl) ascribe great importance to the appearance of eosinophilia, accompanying blood regeneration. This symptom is taken to be a sensitive indicator of an active erythropoietic response.

The haematologic findings, described above, may be taken as evidence of coordinated regenerative activity of the three main blood-forming series of the bone marrow.

### CHRONIC POSTHAEMORRHAGIC ANAEMIA

Repeated haemorrhages or prolonged occult bleedings cause exhaustion of the iron reserves in the organism and wearing out of the regenerative activity of the bone marrow. In consequence, there develops a different type of anaemia, than the one described in the preceding chapter. This syndrome is characterised by a disproportion between the marked fall of the haemoglobin level and a relatively mild erythrocytopenia. The mean corpuscular haemoglobin concentration is low, which constitutes evidence of the hypochromia of the red cells. The mean corpuscular volume is also usually decreased, in rare instances it may be normal. The cardinal haematologic feature of this syndrome is instituted by the hypochromia of the erythrocytes. The more severe the anaemia, the more marked the hypochromia of the red cells. They resemble pale discs or ovals with a narrow pink border ("ring-forms" or annulocytes). In the course of severe forms of anaemia of this type, a high degree of anisocytosis and poikilocytosis is found in blood smears and schizocytes appear also. Microcytosis, however, prevails. The varying number of macrocytes and polychromatic cells reflects the regenerative activity of the blood-forming tissues. Single normochromic erythrocytes are, however, usually present.

By contrast with the haematologic picture of the acute posthaemorrhagic anaemia, no symptoms of increased leuco- or thrombocytopoietic activity are usually observed. The number of leukocytes is normal or, in some instances, leukopenia with relative lymphocytosis and thrombocytopenia may be found.

The response of the bone marrow in the course of chronic blood loss depends on the state of exhaustion of this organ. The evaluation of the

responsive capacity is of great importance in establishing the prognosis. The symptoms of erythropoietic activity range from hypoplastic to hyperplastic reactions. In some cases, the number of erythroblasts is greatly increased. The preponderance of basophilic erythroblasts and stem-cells (proerythroblasts) suggests the presence of arrested maturation. In cases of an increased number of polychromatic and orthochromatic erythroblasts the prognosis is more favourable.

If the bone marrow is exhausted, the number of erythroblasts may be greatly diminished. The granulocytic and megakaryocytic series participate in bone-marrow response in a smaller degree than in the course of acute posthaemorrhagic anaemia.

Anaemia resulting from chronic blood loss is closely related to the large group of iron-deficiency anaemias. Hypochromic anaemia is a very widespread disorder and, unfortunately, oftentimes overlooked. In spite of its frequent occurrence, the importance of this syndrome is underestimated. On the basis of clinical observations, it has been proved that a relatively slight decrease of haemoglobin below the critical level exerts a marked influence on the general condition of the organism. The patient feels ill, his physical fitness and intellectual faculties are impaired. From these facts there arises the social and economic importance of an early diagnosis of this ailment and of the investigation and removal of its causes.

Stress is laid on these points because an indifferent attitude on the part of the physician to even a mild hypochromic anaemia should be considered a grave error. The causes should be thoroughly investigated and all possible causative factors considered. The most commonly encountered, are the following:

1. Chronic blood loss.
2. Inflammatory lesions of the gastro-intestinal tract (anatomical changes of the mucosa), which may lead in consequence to faulty absorption; dysentery; sprue, steatorrhoea idiopathica; gastritis, ileitis, jejunitis etc.
3. Hypo- or avitaminoses, such as pellagra.
4. Anatomical changes of the gastro-intestinal tract following surgical interventions.
5. Such anatomical changes, as diaphragmatic hernia, megacolon, dolichosigma or so-called coeliakia.
6. Hypoacidity or anacidity of the gastric juice.
7. Iron deficient diet or, in general, faulty, insufficient alimentation.
8. An increased demand for iron in a growing organism or during pregnancy.
9. Disturbances in iron utilisation or in haemoglobin synthesis, the diet and iron absorption being normal.
10. Chronic infections.



11. Intestinal infestation.
12. Idiopathic hypochromic anaemia.
13. Chlorosis; this ailment, formerly frequently diagnosed in girls at puberty, is now extremely rare.

The most common cause, from the clinical point of view, is chronic blood loss. There is no doubt that an early location of the bleeding will often be helpful in detecting and obviating grave organic disorders. The importance of haematological examinations should be stressed, especially in view of the widespread campaign against cancer.

The diagnosis of the underlying causative disorder must be based upon meticulously collected historical data (bleedings, abortions, deliveries), clinical, laboratory and specialist examinations. The peripheral blood findings indicate only to presence of "asiderosis", which is expressed by hypochromia and usually microcytosis. These haematological traits are common to all asiderotic syndromes irrespective of their cause.

### CHRONIC HYPOCHROMOCYTIC ANAEMIA (MOORE)

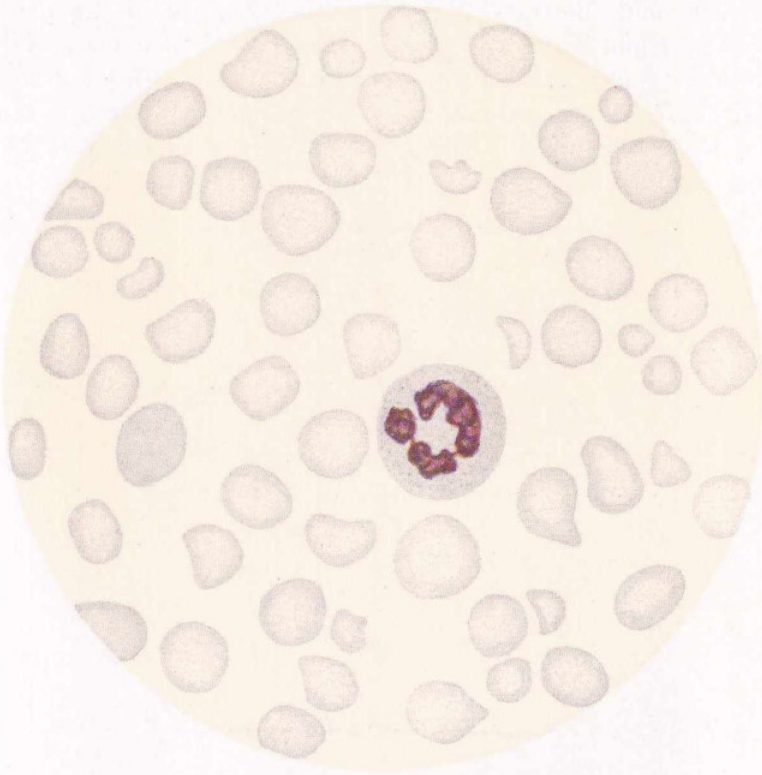
*Asiderosis idiopathica* (Tempka) — *Gastrogenная гипохромная анемия* (Kassirsky and Alekseev) — *Anaemia hypochromica essentialis* (Schulten) — *Idiopathic Hypochromic Anaemia*

The peripheral blood picture during this disorder is identical with the findings in cases of chronic loss of blood.

Plate 12 illustrates a peripheral blood smear from a case of idiopathic hypochromic anaemia.

The most remarkable trait consists in the marked hypochromia of red cells, which are stained only on the periphery (annulocytes). They are distinctly thinner than normal cells. In diameter they are usually below  $7.2 \mu$ , microcytosis prevails. Distinct anisocytosis, poikilocytosis and schizocytosis may be observed. Occasional polychromatic erythrocytes are present. The white-cell picture is characterised by leukopenia (to a lesser degree than in Addison-Biermer's anaemia) with a shift to the right in neutrophilic granulocytes and relative lymphocytosis.

Plate 13. Idiopathic hypochromic anaemia — not treated (bone marrow). The drawing represents a picture of stimulated haematopoiesis. This brings to mind the similarity with Biermer's syndrome, where the hyperactivity of the bone marrow stands out in contrast to the severe anaemia found in the peripheral blood. In this case, however, erythropoiesis is of the normoblastic type. Proerythroblasts and numerous basophilic erythroblasts, forming clusters, are observed in the preparation. In addition,



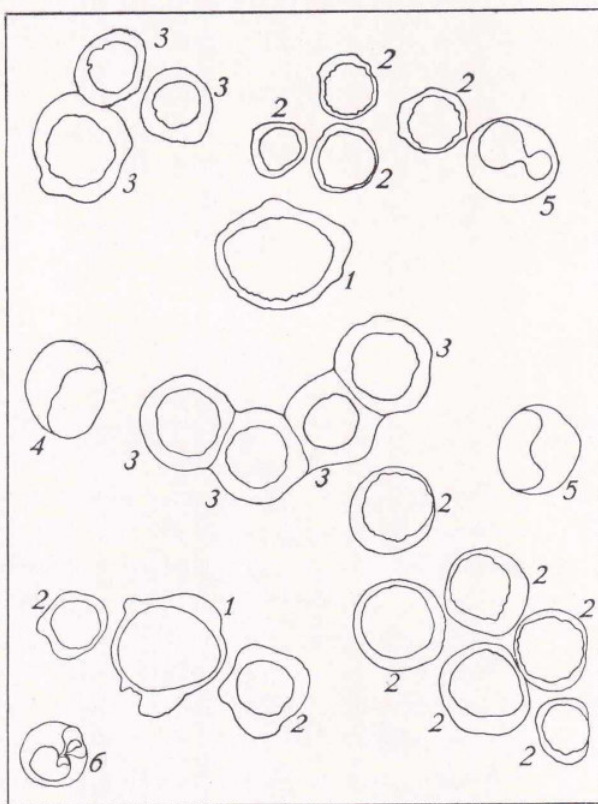
*Plate 12.* — Chronic hypochromic anaemia (peripheral blood).

*Note:* the presence of markedly hypochromic erythrocytes — annulocytes. A single polychromatic red cell is to be seen.



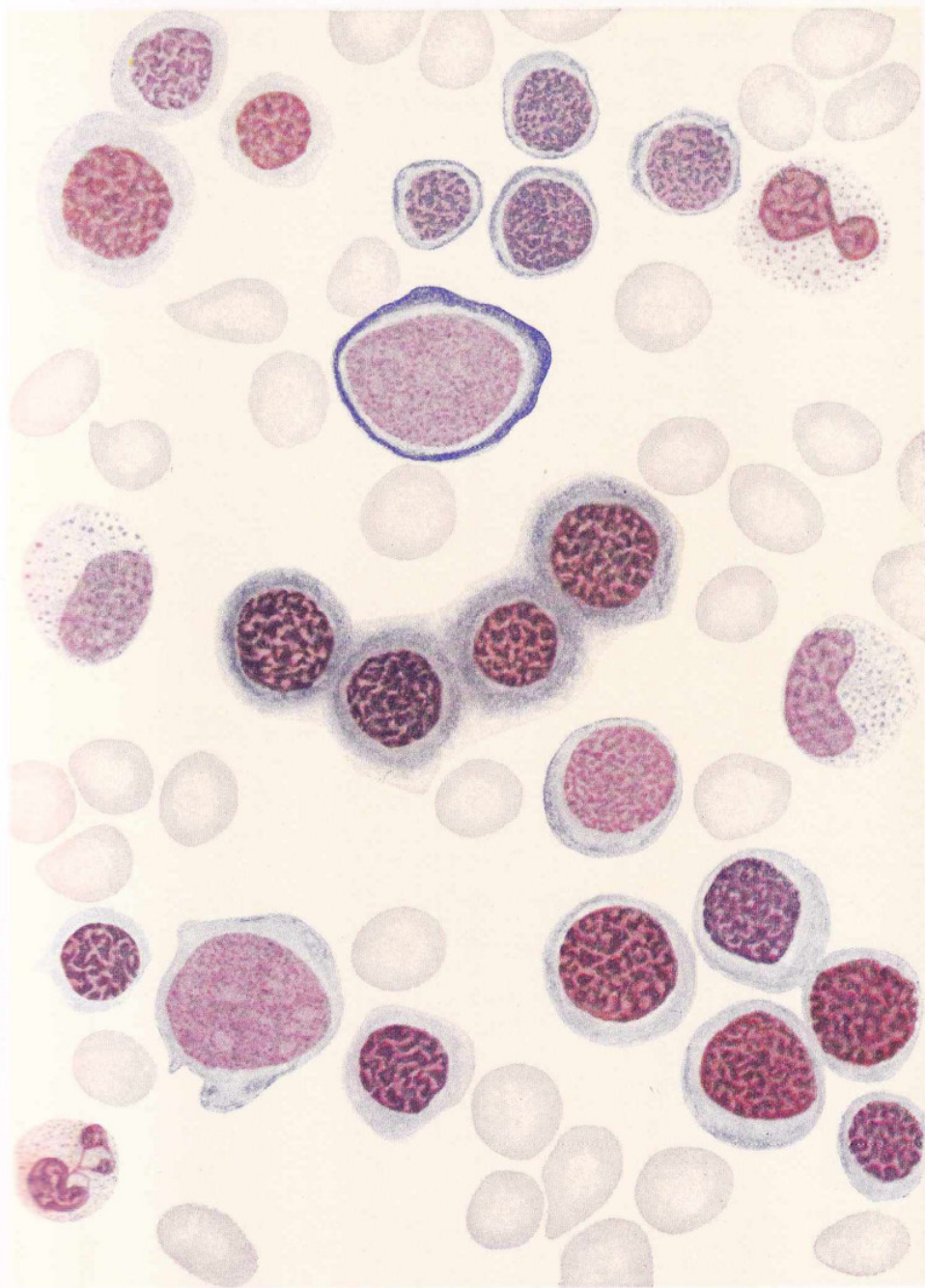
a number of polychromatic erythroblasts is present. The marked preponderance of immature forms (shift to the left in the erythrocytic series) indicates the presence of maturation arrest. This picture undergoes a change in the course of iron therapy. The maturation (haemoglobin formation) of the cytoplasm progresses rapidly and the number of orthochromatic erythroblasts increases.

By contrast with Biermer's anaemia, the changes of the granulocytic and megakaryocytic series are more discrete and not necessarily present. Some authors (Rohr) mention the sporadic occurrence of giant metamyelocytes and staff cells. In certain cases, the megakaryocytes may show an increased basophilia of the cytoplasm and an abnormally high number of nuclear segments.



*Plate 13.* — Chronic hypochromic anaemia (bone marrow): 1. Proerythroblast. 2. Basophilic erythroblast. 3. Polychromatic erythroblast (early and late). 4. Neutrophilic myelocyte. 5. Neutrophilic metamyelocyte. 6. Neutrophilic polymorphonuclear granulocyte.

*Note:* the marked erythropoietic response; the numerous basophilic erythroblasts and two proerythroblasts; more mature erythroblasts are sparse. Such a bone-marrow picture indicates the arrest of maturation in the erythropoietic series.



*Plate 13.* — Chronic hypochromic anaemia (bone marrow).



All known factors leading to iron deficiency must be ruled out, before diagnosis of idiopathic hypochromic anaemia is established. An exact history, thorough laboratory examinations and a long period of clinical observation are necessary. The causes of iron deficiency are manifold and are better known and understood with every passing day. Therefore, the instances in which idiopathic hypochromic anaemia may be diagnosed become increasingly rare. It seems more prudent, as proposed by Moore, to call this condition "chronic hypochromic anaemia".

The following points may be helpful in establishing the diagnosis of idiopathic hypochromic anaemia:

1. Haematological examinations: a low colour index, decrease of mean haemoglobin concentration in the red cells<sup>1</sup>.
2. Constitutional traits resembling those described in Addison-Biermer's disease: widely set eyes, light in colour and a tendency to premature greying of hair.
3. In a majority of cases (about 80 %), there is no free hydrochloric acid in the gastric juice; in about fifty per cent of cases, achlorhydria is histamine-resistant.
4. Idiopathic hypochromic anaemia occurs almost exclusively in women during the climax.
5. The patients present a tendency to diarrhoea, in some cases atrophic changes of the oral cavity mucosa.
6. Rossolimo-Bechterev's (1900—1901) or Plummer-Vinson's (1914—1920) syndrome may be present. This syndrome is, however, inconstant.
7. Trophic changes of the skin and skin-appendices; the skin is dry and inelastic; the finger nails become flattened or even concave (*koilonychia*), the hair is prematurely grey and brittle.
8. Neuralgic pains, numbness and tingling; the severe neurological symptoms characteristic of Addison-Biermer's anaemia are observed only in exceptional cases.
9. The serum is pale, the bilirubin level decreased.
10. The iron content of the serum is low.

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<sup>1</sup> The haematological examinations should, in addition to the red-cell count and the haemoglobin determination, comprise the calculation of the mean corpuscular volume and mean corpuscular haemoglobin concentration. These calculations are readily made with the haematocrit method.

## ADDISON-BIERMER'S DISEASE

*Pernicious anaemia*

Addison-Biermer's disease is the most important and most commonly encountered syndrome of the group of macrocytic anaemias in temperate zones.<sup>1</sup> The main haematological feature of this disorder is set up by macrocytosis and megalocytosis. Although in peripheral blood smears of typical and untreated cases, a high degree of anisocytosis is observed, the majority of erythrocytes are of larger dimensions than normal red cells. The haematocrit determinations show an increase in mean corpuscular volume. The normal values range from 74 to 94 cu.μ. During the course of Addison-Biermer's disease the mean corpuscular volume attains more than 100 cu.μ, and may even reach the values of 150—170 cu.μ. Poikilocytosis and macrocytosis may be also expressed by means of the Price-Jones curve. In pernicious anaemia, this curve is flattened; it has a broad base and the peak undergoes a shift to the right — in the direction of higher values.

The concept of the megalocyte comprises not only greater cell dimensions, but also normal haemoglobin concentration and even a certain "hyperchromia" together with a tendency to acquire an oval shape. A faultlessly executed smear is indispensable for the diagnosis of megalocytosis. Such diagnosis should be carefully made taking into account the possibilities of overstaining the preparation, the flattening of erythrocytes in peripheral parts of the smear and the phenomenon of macroplanocytosis (Aleksandrowicz). The detection of typical megaloblasts confirms the diagnosis of megalocytosis.

Plate 14 illustrates the peripheral blood of a nontreated case of severe pernicious anaemia.

In a thin blood smear attention is arrested by the decreased number of erythrocytes, by their anisocytosis, poikilocytosis, and polychromasia. Cells of greater dimensions and oval in shape, intensively stained (megalocytes) are clearly visible. Numerous poikilocytes, as well as schizocytes may be observed. It should be noted that nearly all the cells are deeply stained and show the lack of a thinner central area. Several stippled cells are present, reflecting the regenerative haematopoietic activity. A polychromatic megaloblast with a typical spongy nuclear structure and two megaloblasts with fragmented nuclei (*karyorrhexis*) are also included in the drawing. When only more mature stages with pyknotic nuclei are present, the recognition of megaloblasts may be rather difficult.

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<sup>1</sup> There exist several tropical blood disorders also characterised by macrocytosis and megalocytosis.



Leukopenia with relative lymphocytosis and a shift to the right in neutrophilic granulocytes is encountered in the great majority of cases of Addison-Biermer's disease. The shift to the right is represented by the occurrence of multisegmented granulocytes. Such a cell can be seen on the drawing. In addition to the shift to the right, sparse metamyelocytes and even myelocytes may be found in the blood smears. The shift to the right is almost constantly present in pernicious anaemia and may persist during remission. The absence of this symptom should give rise to doubts as to the correctness of the diagnosis.

During fully developed disease, the myelogram is characteristic and shows disturbances in erythropoiesis, granulopoiesis, as also in the megakaryocytic system.

The bone marrow is markedly cellular. The number of the cells of the erythropoietic series is greatly increased and often exceeds 50% of all nucleated elements. The most characteristic feature consists in the megaloblastic course of erythropoiesis; certain normoblastic cells may, however, be found. There are signs of arrested maturation — a preponderance of early stem-cells. The number of the stem-cells of the erythropoietic series is markedly increased. Numerous proerythroblasts and cells of greater dimensions — promegaloblasts — are present in the preparation. These cells often appear in clusters. The nuclei of some of the promegaloblasts possess a distinctly reticular structure and bluish nucleoli. Their cytoplasm stains less intensively blue with a greyish hue. It may be that these cells are genetically younger and originate from the multipotential reticulum cell.

In the later developmental stages of megaloblasts which are also present in considerable numbers in smears, attention is drawn to the disproportion between the degree of maturity attained by the nucleus and by the cytoplasm. The nuclear structure remains "young", spongy, while the cytoplasm contains haemoglobin. Megaloblasts with atypical nuclei or nuclei undergoing fragmentation (*karyorrhexis*) may be often encountered.

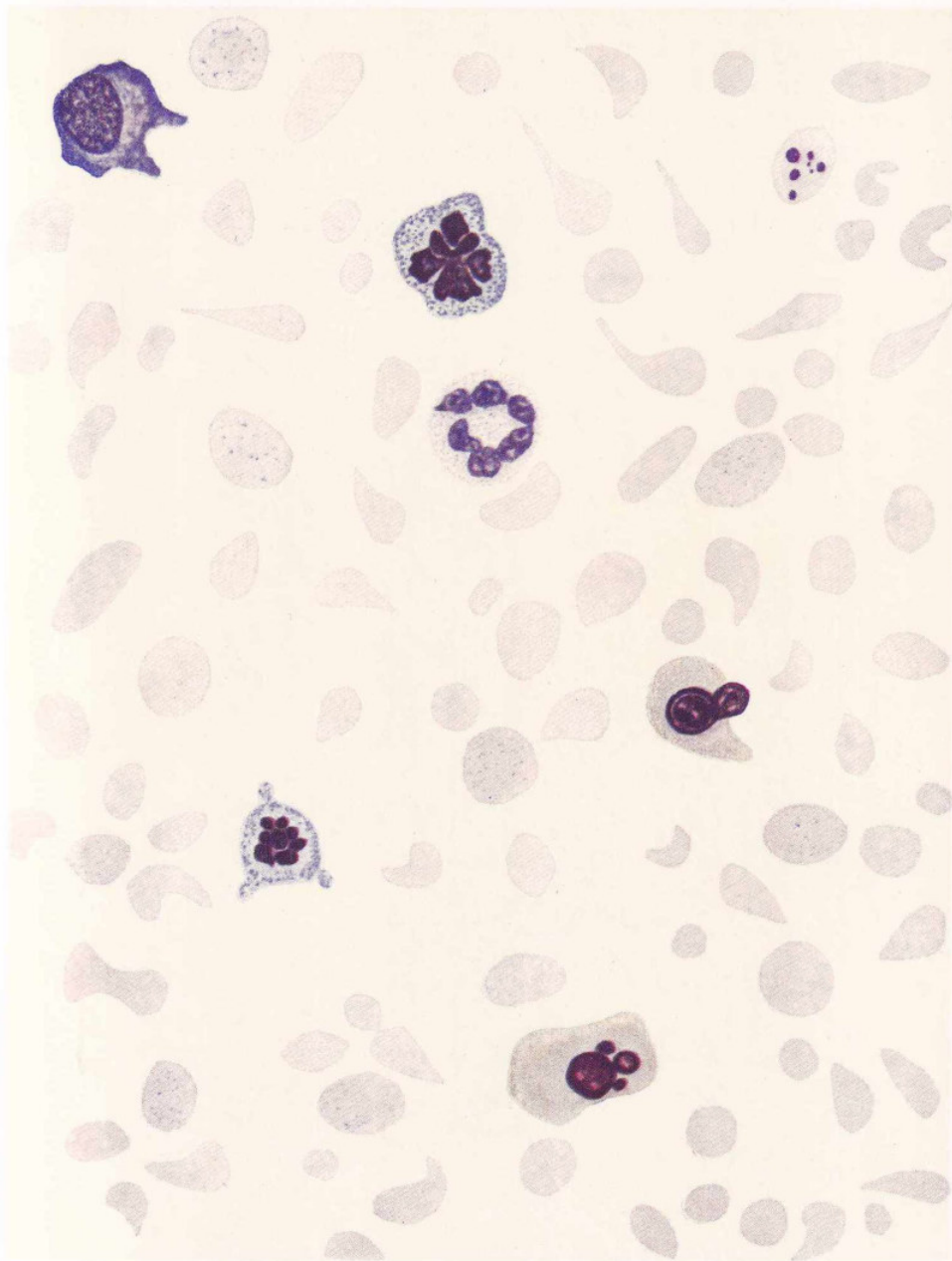
The percentage of the megaloblasts is in proportion to the degree of anaemia. It should be borne in mind, however, that the megaloblastic course of erythropoiesis is not pathognomonic for Addison-Biermer's disease, but may also be present in other "acastloses"<sup>1</sup>.

The simultaneous presence of normoblastic erythropoiesis expresses the tendency of the organism to replace the functionally less efficient megacytes by normocytes.

The mature red cells show the same abnormalities as were described above in the discussion of the peripheral blood changes. Megalocytes,

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<sup>1</sup> The term "acastlosis" was introduced by T. Tempka.

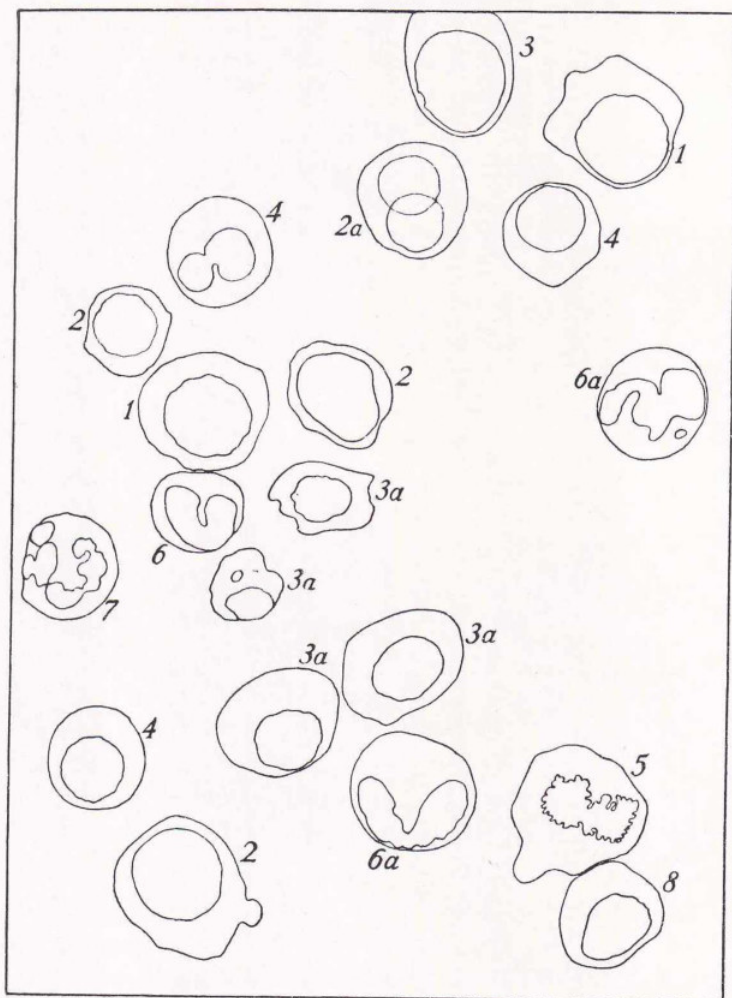


*Plate 14.* — Addison-Biermer's disease (peripheral blood).

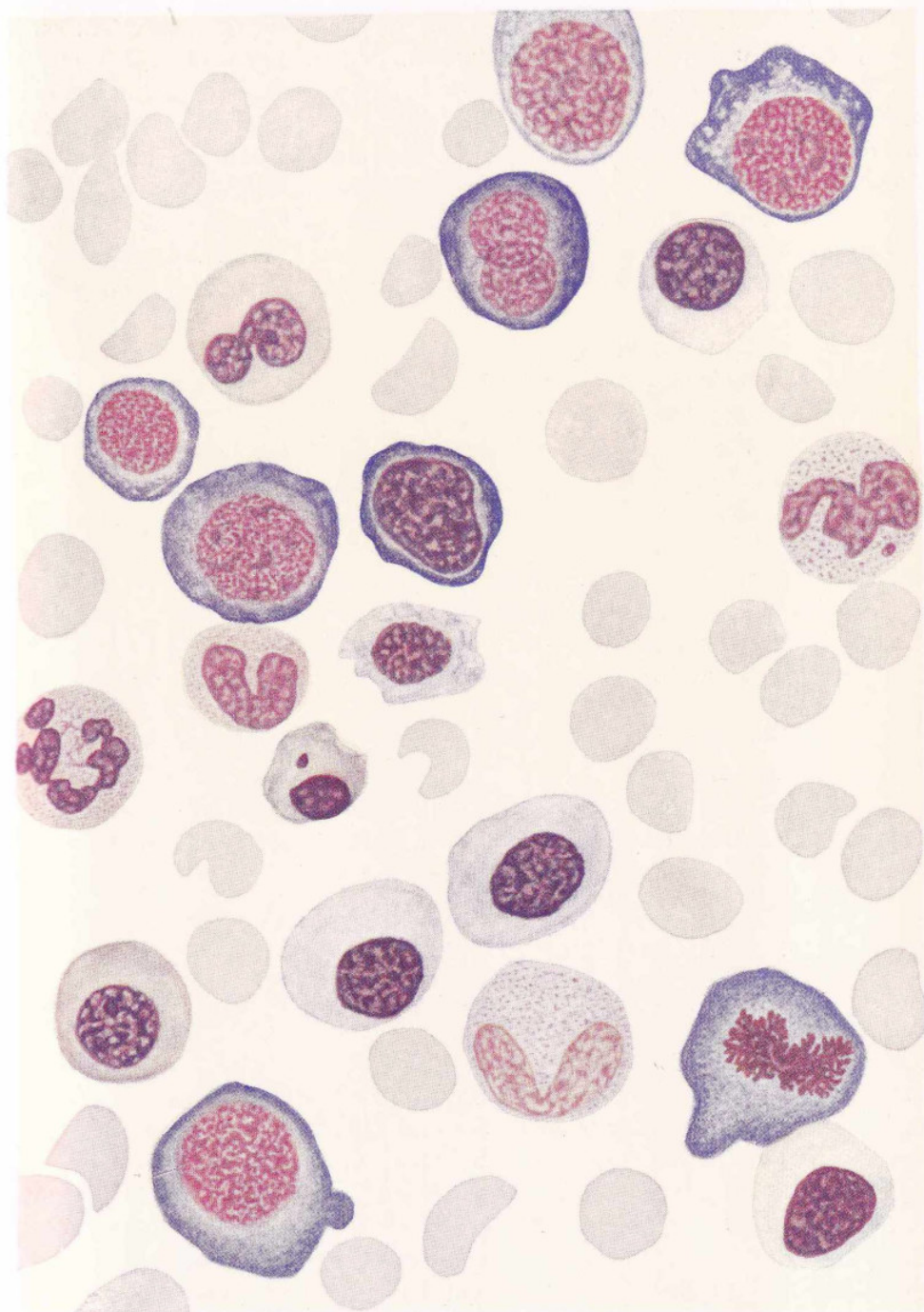


gigantocytes, red cells with nuclear remnants (Howell-Jolly bodies), basophilic stippling and Cabot's rings are encountered.

Far reaching qualitative changes may be demonstrated also in the granulopoietic series, a fact which indicates the presence of profound disturbances in the differentiation of these cells, together with degenerative changes. Nuclei mature in shape but immature in structure are often seen; abnormalities of segmentation, atypical or blurred nuclear structure may



*Plate 15.* — Addison-Biermer's disease (bone marrow): 1. Promegaloblast. 2. Basophilic megaloblast. 2a. — binucleated basophilic megaloblast. 3. Early polychromatic megaloblast. 3a. — late polychromatic megaloblast. 4. Orthochromatic megaloblast. 5. Megaloblast in mitosis. 6. Metamyelocyte. 6a. — giant metamyelocyte. 7. Multisegmented neutrophil. 8. Lymphoid cell.



*Plate 15.* — Addison-Biermer's disease (bone marrow).



be encountered. The presence of giant metamyelocytes and giant staff cells, first described by Tempka and Braun, are among the most characteristic deviations in the leukopoietic series. These cells may attain considerable dimensions and are distinguished by a gigantic, thick, loosely knit nucleus, which gives the cell a specific appearance. Both the nucleus and the cytoplasm of these cells are frequently vacuolated. In the cytoplasm of the remaining granulocytes, qualitative changes may also be observed. They may take the form of persistent basophilia of the cytoplasm, diffuse or circumscribed; it is not uncommon for vacuolation of the cytoplasm to occur.

In the megakaryocytic system, the number of megakaryocytes may diminish and their ability to produce platelets may be impaired.

Megakaryocytes with a multisegmented nucleus are often present. This phenomenon may be visible both in mature (megakaryocytes) and in immature stages (megakaryoblasts and promegakaryocytes).

These symptoms may be taken as evidence that the deficiency of maturation factors, in the light of recent researches concerning vitamin B<sub>12</sub> foremost, influences not only the course of erythropoiesis, but also the remaining cellular series of the blood-forming tissues. Clinical and pathological observations demonstrate the wide scope of metabolic disturbances occurring in the course of this disease (*e.g.* neurological and adrenal disorders).

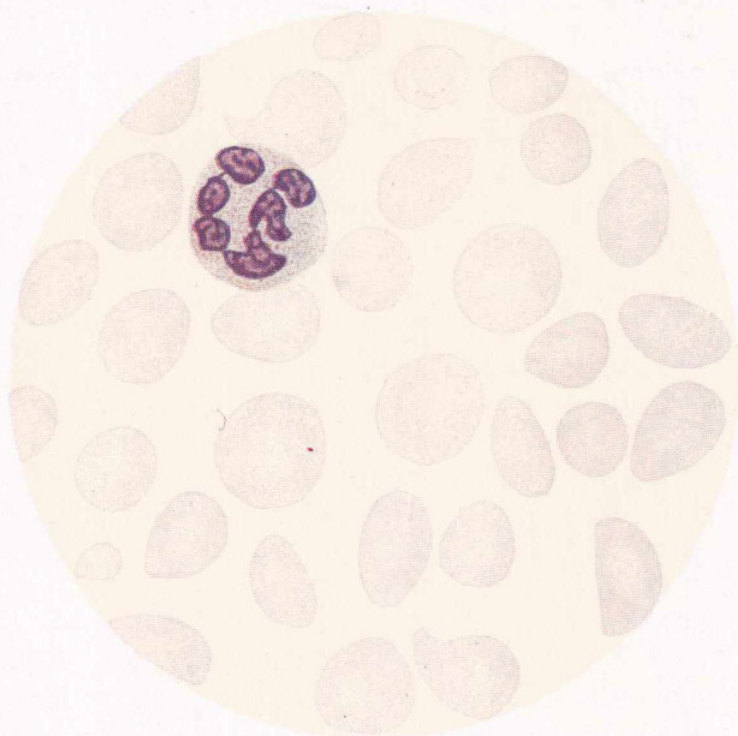
Plate 15 illustrates the bone marrow of a nontreated case of Addison-Biermer's anaemia.

Among less constant symptoms is an increase of reticulum cells, which show degenerative features, as well as a plasmocytic reaction.

#### THE BLOOD PICTURE IN ADDISON-BIERMER'S DISEASE IN THE COURSE OF VITAMIN B<sub>12</sub> or LIVER THERAPY

Plate 16 illustrates a peripheral blood smear in the eighth day of liver therapy.

Even during the first period of liver therapy, the blood picture becomes, because of the regression of anisocytosis and poikilocytosis, more uniform. In the first order, schizocytes and microcytes disappear. Distinct megalocytosis, however, persists. The mean corpuscular volume is high, and may even increase. This last phenomenon is explained by the maturation of megaloblasts and, in consequence, release of reticulocytes into the peripheral blood. In the smears are visible, first of all, large, often polychromatic erythrocytes. Megaloblasts and normoblasts disappear, usually at an early stage, from the peripheral blood. An increase in normal erythrocytes (normocytes) may be observed at the end of the second and



*Plate 16.* — Pernicious anaemia (peripheral blood) following 8-day treatment with liver extract.



during the third week of therapy. Afterwards, the blood picture gradually undergoes normalisation. In some instances, however, sporadic megalocytes may be found for long periods.

The presence of multisegmented neutrophilic granulocytes is one of the most persistent symptoms. Although this phenomenon is not specific, it may facilitate the recognition of treated cases of Addison-Biermer's disease.

During remission, leukocytosis with a shift to the left may be observed, as evidence of stimulated regenerative activity of the bone marrow. The number of platelets also increases.

High eosinophilia may frequently be induced by the use of liver preparations, particularly by those containing impurities. The authors observed eosinophilic reactions attaining 48 %. Eosinophilia was known to occur also in untreated cases of Addison-Biermer's disease.

#### RETICULOCYTOSIS IN PERIPHERAL BLOOD IN THE COURSE OF ADDISON-BIERMER'S DISEASE TREATED BY LIVER EXTRACT OR VITAMIN B<sub>12</sub>

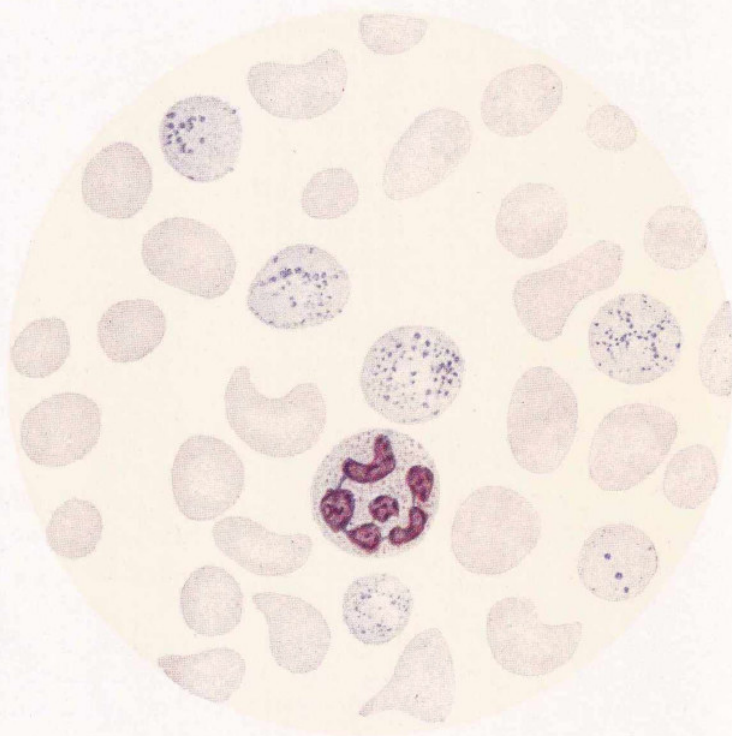
Plate 17 illustrates a peripheral blood smear from a case of Addison-Biermer's disease on the eighth day of liver-extract treatment.

Reticulocytosis is the earliest and, at the same time, the most sensitive symptom of a regenerative response of the bone marrow in the course of treatment of Addison-Biermer's disease. This symptom precedes increase in the number of red cells in peripheral blood and is useful in estimating the therapeutic value of the treatment chosen.

The promptness of the appearance of reticulocytosis depends on the doses of the liver extract, its concentration, the mode of administration and the degree of anaemia. After intravenous administration, reticulocytosis appears during the first 24 hours and reaches its maximum in the course of 4—5 days. After intramuscular administration, the curve of reticulocytosis attains its peak between the 5-th and the 9-th day and drops to normal values at the end of the third week of therapy.

Reticulocytosis and improvement of the blood picture may also be obtained by oral treatment with folic acid. It is now generally known that pteroylglutamic acid is of limited value in the management of Addison-Biermer's disease, and may even be contraindicated. Lately its use in the treatment of Addison's anaemia was abandoned.

The reticulocytic reaction after administration of vitamin B<sub>12</sub> also depends on the doses used. After large doses (160—200 gamma) the peak of the reaction occurs on the 6-th or 7-th day.



*Plate 17.* — Pernicious anaemia (reticulocytosis in the course of treatment with liver extract). Peripheral blood.



The increase in the number of erythrocytes is not always preceded by high reticulocytosis. Therefore, the evaluation of the improvement in the peripheral blood picture should be made also on the basis of the red-cell count.

The main changes in the bone marrow under the influence of liver extracts or other haematopoietic substances (such as vitamin B<sub>12</sub>) consist in the reversion of erythropoiesis from the megaloblastic to the normoblastic type. The course of erythropoiesis may undergo such a change very rapidly even during the first 24 hours. Usually, however, the therapeutic effects become marked in the myelogram after three days. The bone marrow is markedly cellular with extremely vivid erythropoietic activity. The total number of nucleated elements is greatly increased, and they are basically different in character. The number of promegaloblasts decreases. In their place there appear cells of smaller dimensions — proerythroblasts, which give rise to cells of the normocytic series. The majority of the nucleated red cells are set up by typical basophilic and polychromatic erythroblasts. As the treatment progresses, the number of orthochromatic erythroblasts increases. These transformations take place so rapidly that they may be more readily explained by an accelerated maturation of megaloblasts than by the proliferation of normoblasts.

During the first period of treatment, megaloblasts are found in the bone-marrow smears, particularly polychromatic and orthochromatic stages with signs of karyorrhexis. Nuclear remnants (Howell-Jolly bodies) are frequently encountered.

The granulocytic series also shows symptoms of marked proliferation, as well as of regression of degenerative changes. In the second week from the beginning of therapy, giant metamyelocytes and giant staff cells disappear.

The abnormal segmentation of the nuclei of promyelocytes and myelocytes also disappears. Eosinophilia may in many cases be present in the bone marrow.

The normalisation of the erythropoietic and granulopoietic series is accompanied by a regression of the changes in the megakaryocytic system. This, however, proceeds more slowly. The number of megakaryocytes increases, but their nuclei remain multisegmented for a long time. This trait does not interfere with normal platelet production.

Plate 18 illustrates the bone marrow from a case of Addison-Biermer's disease (eighth day of liver therapy).

Diagnosis of a fully developed Addison-Biermer's disease is usually simple. It should be made on the basis of haematological findings in peripheral blood and bone marrow, supplemented by the demonstration of gastric achlorhydria. Nevertheless, errors may occur. For instance, it

should be borne in mind that the megaloblastic syndrome may be present in other diseases, caused by disturbances in the functions of the hepato-gastric system or by deficiency states of extrinsic origin.

In this group of disorders, are included:

1. Disturbances associated with surgical procedures, such as gastrectomy, gastro-enterostomy, intestinal anastomoses and strictures; megaloblastic anaemia is in these conditions of rare occurrence; the appearance of hypochromic anaemia is more typical.

2. Carcinoma of the stomach.

3. Tropical sprue and idiopathic steatorrhoea, as well as the so-called coeliac disease; it is more common, however, for coeliac disease to be associated with hypochromic anaemia.

4. Cirrhosis and other diseases of the liver — it is conceivable, that disturbances in storage or in the metabolism of the antianaemic principle are present in those conditions; megaloblastic anaemia rarely occurs in association with them.

5. Nutritional megaloblastic anaemia; such cases occur chiefly in tropical zones.

6. Pregnancy or the puerperal period; megaloblastic anaemia rarely develops here.

7. Infestation with fish-tape worm (*Bothriocephalus latus*).

8. Megaloblastic anaemia of infancy.

9. Achrestic anaemia; this syndrome is very rarely encountered; its very existence is disputed; it is characterised by the presence of free hydrochloric acid in the gastric juice and the absence of neurologic complications; no signs of abnormal haemolysis were reported; this syndrome is refractory to liver therapy;

10. Macrocytic anaemia (without megaloblastic reaction in the bone marrow) with achlorhydria in hypothyroidism.

The findings of megalocytosis in the peripheral blood, indicates first of all the presence of Addison-Biermer's disease, because this "acastlosis" is most commonly encountered in the temperate climate.

On practical grounds the following criteria, which may be helpful in establishing the diagnosis of Addison-Biermer's disease are given:

1. Haematological examinations.

2. Positive Hijmans van den Bergh's test and the level of bilirubin in the serum. The patient's serum is usually greenish, in severe cases — olive. This symptom is usually absent in sprue, or in nutritional anaemia without haemolysis.

3. An increase of urobilinogen and urobiline in urine.

4. Increased saponin-fragility of red cells (Tempka and Braun).

5. Histamine-resistant achlorhydria.

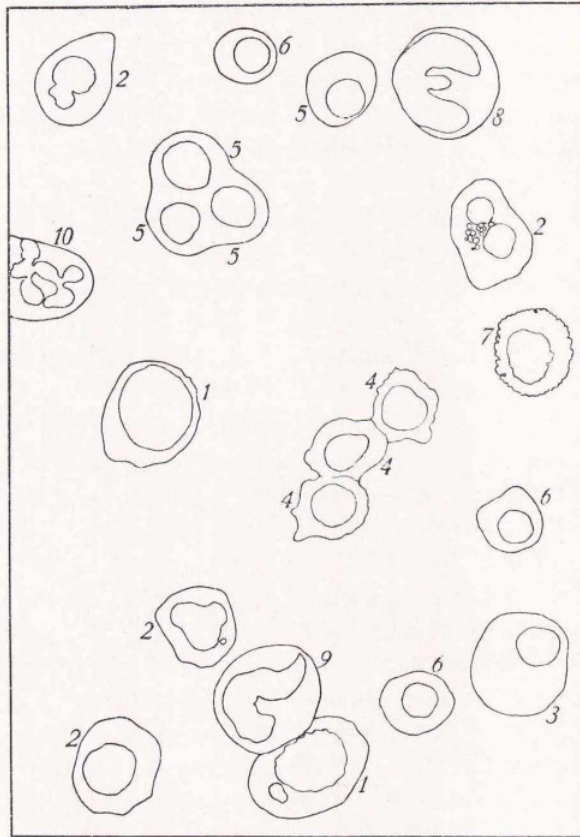


6. Roentgenologic examination of the gastro-intestinal tract with view to ruling out the presence of a carcinoma of the stomach.

7. The glucose-tolerance curve following the ingestion of a test dose (100 gm.) is in Addison-Biermer's disease usually normal. On the other hand, in sprue, the curve due to faulty absorption is flattened.

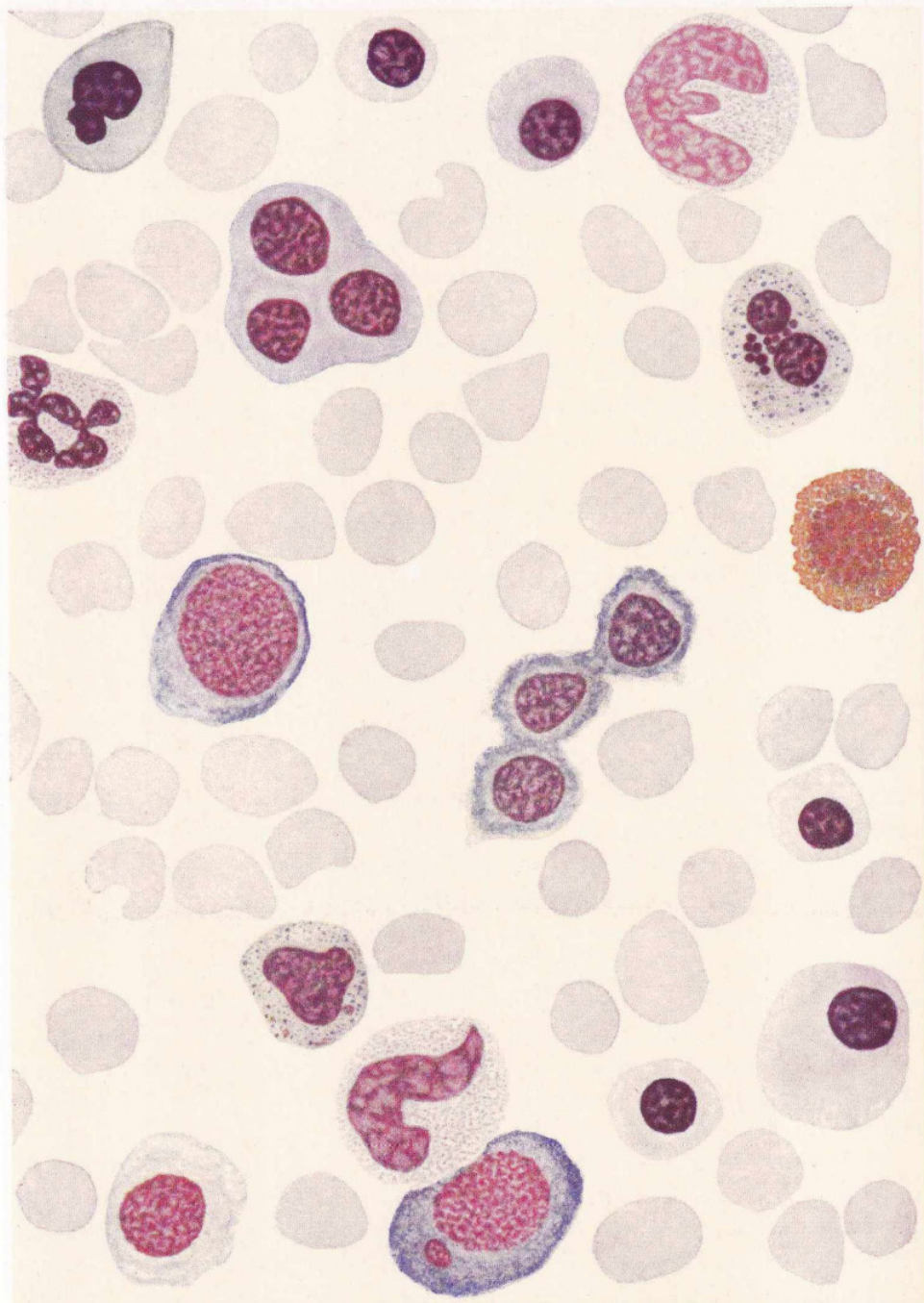
8. Low cholesterol level in the serum.

9. Determination of serum iron (normal values according to Donner are 115 to 135 gamma % in men and 70—95 gamma % in women). An untreated case of Biermer's disease almost always shows a high serum-iron level.



*Plate 18.* — Addison-Biermer's disease (bone marrow) — following a 8-day course of liver therapy: 1. Basophilic megaloblast. 2. Polychromatic megaloblast. 3. Orthochromatic megaloblast. 4. Early polychromatic erythroblast (normoblast). 5. More mature polychromatic erythroblast (normoblast). 6. Orthochromatic erythroblast (normoblast). 7. Eosinophilic myelocyte. 8. Giant neutrophilic metamyelocyte. 9. Giant staff cell. 10. Macropolycyte.

*Note:* the coexistence of normoblastic and megaloblastic erythropoiesis; the basophilic megaloblast contains a nuclear fragment in the cytoplasm; the nuclei of polychromatic megaloblasts demonstrate fragmentation; two polychromatic megaloblasts are stippled.



*Plate 18.* — Addison Biermer's disease (bone marrow) following a 8-day course of liver therapy.



This symptom is accounted for by the presence of haemolysis and a less intense utilisation of iron by the bone marrow. Therapy by liver extracts or vitamin B<sub>12</sub> induces a decrease in the serum-iron level. The finding of low serum iron makes the diagnosis of pernicious anaemia doubtful, and directs suspicion to some other morbid process. Büchmann's case (according to Netoušek) is very demonstrative in this respect. In a classic syndrome of megaloblastic anaemia, confirmed by bone-marrow biopsy, the iron level was found to be low (38—41 gamma %). On autopsy, a carcinoma of the minor curvature of the stomach was found.

10. Neurologic examinations. Symptoms of degeneration in the posterior and lateral funiculi are nearly always absent in sprue or nutritional anaemia. They are markedly more benign in bothrioccephalus anaemia.

The remaining megalocytic or macrocytic anaemias may be ruled out on the basis of clinical observation.

### NONTROPICAL SPRUE

The megaloblastic syndrome is present only in certain cases of this disorder. Normocytic, hypochromic anaemia has been reported in several instances. Sprue was first described by A. Kriukov in 1922. In cases with a megaloblastic syndrome, the haematologic picture corresponds to that of Addison-Biermer's disease.

Differences in the peripheral blood picture, pertaining to the dimensions and the degree of haemoglobinization of red cells, are, however, noted in the literature. Because of the iron deficiency, which is more common in sprue, hypochromic erythrocytes may be present. Only in rare instances the anaemia is severe. Anisocytosis and poikilocytosis are less distinct than is the case in Addison-Biermer's disease. Megalocytosis is marked, the megalocytes being, of similar dimensions (isomegalocytosis). The mean corpuscular volume remains within normal limits. Because of impaired functioning of the spleen, Howell-Jolly bodies are often encountered.

Other symptoms (leukopenia with relative lymphocytosis, the presence of multisegmented granulocytes) do not differ from the picture of Addison-Biermer's disease.

In two cases of severe nontropical sprue, which the authors followed, the blood picture was characterised by marked anisocytosis, poikilocytosis and schizocytosis. In Plate 19 numerous megalocytes, polychromatic erythrocytes and stippled red cells may be seen. In both cases a number of basophilic and polychromatic megaloblasts were found. Several megaloblasts revealed an atypical segmented nucleus. The granulocytic series was characterised by a shift to the right.

The bone-marrow changes correspond to Addison-Biermers's disease. The marrow is markedly cellular and demonstrates a distinct promegaloblastic-megaloblastic reaction, together with the changes described above in the granulocytic series. Numerous giant metamyelocytes and staff cells are visible in the preparations. The megakaryocytes contain sporadically multisegmented nuclei.

In contradistinction to the assertions of Rohr, who claims the coexistence of normoblastic and megaloblastic erythropoiesis, the authors in their cases observed the exclusive presence of megaloblasts. Among the promegaloblasts, numerous giant forms were present. The nuclei of megaloblasts were atypical and varied in shape. Some cells contained two nuclei. In both cases, numerous mitotic figures of the red-cell series were present.

To assist differentiation from Addison-Biermer's disease, the following points should be considered:

1. Sprue — particularly in severe cases, bears the character of a wasting disease, the main complaints being referable to the alimentary system; Addison-Biermer's disease — the patients are usually well nourished ("bulky" and "flabby" appearance). In typical cases of sprue, the contrast between the general emaciation and the distended abdomen is strikingly noticeable.

2. The blood and bone-marrow findings are similar in both diseases.

3. Sprue — the onset is insidious, the course chronic; there is often a marked loss in weight, amounting to as much as 25 kg. in the course of a few months.

4. Sprue — abundant, foamy, grey or light yellowish stools (termed "white diarrhoea" by Alekseev); the stools show marked acidity.

5. Sore and painful tongue is common to both diseases; this symptom is inconstant.

6. The spleen and liver are usually not enlarged; in Addison-Biermer's disease, splenomegaly is often present; enlargement of the liver may be, however, encountered in cases with cardiac insufficiency.

7. Pallor of the skin, absence of subicteric tint; the skin is dry and the nails are brittle; symptoms of leukoderma are not observed; in sprue, areas of brownish pigmentation of the skin may be found.

8. In the majority of cases, free hydrochloric acid is present in the gastric juice; achlorhydria, if present, is not histamine-resistant.

9. X-ray examination reveals striking inequality in the width of small intestine segments and distortion of the mucosal pattern.

10. There is no bilirubinaemia.

11. The saponin fragility of red cells is normal.



12. The calcium level in blood is decreased, sometimes symptoms of tetany are present; these symptoms occur predominantly in nontropical sprue.

13. Osteoporosis and deformities of vertebral column are present.

14. A flat glucose-tolerance curve after an oral test dose of 100 gm.

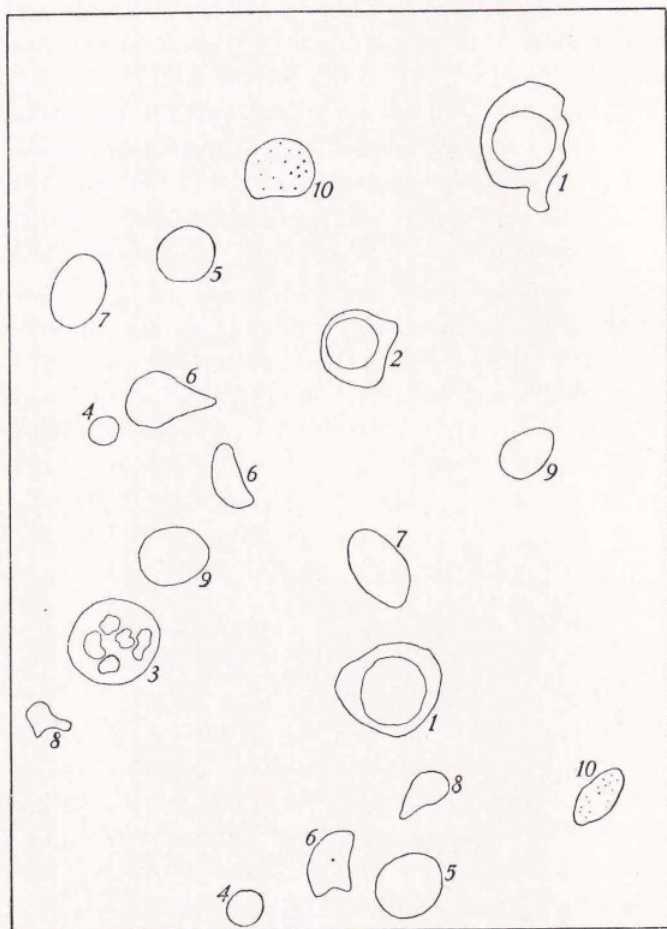
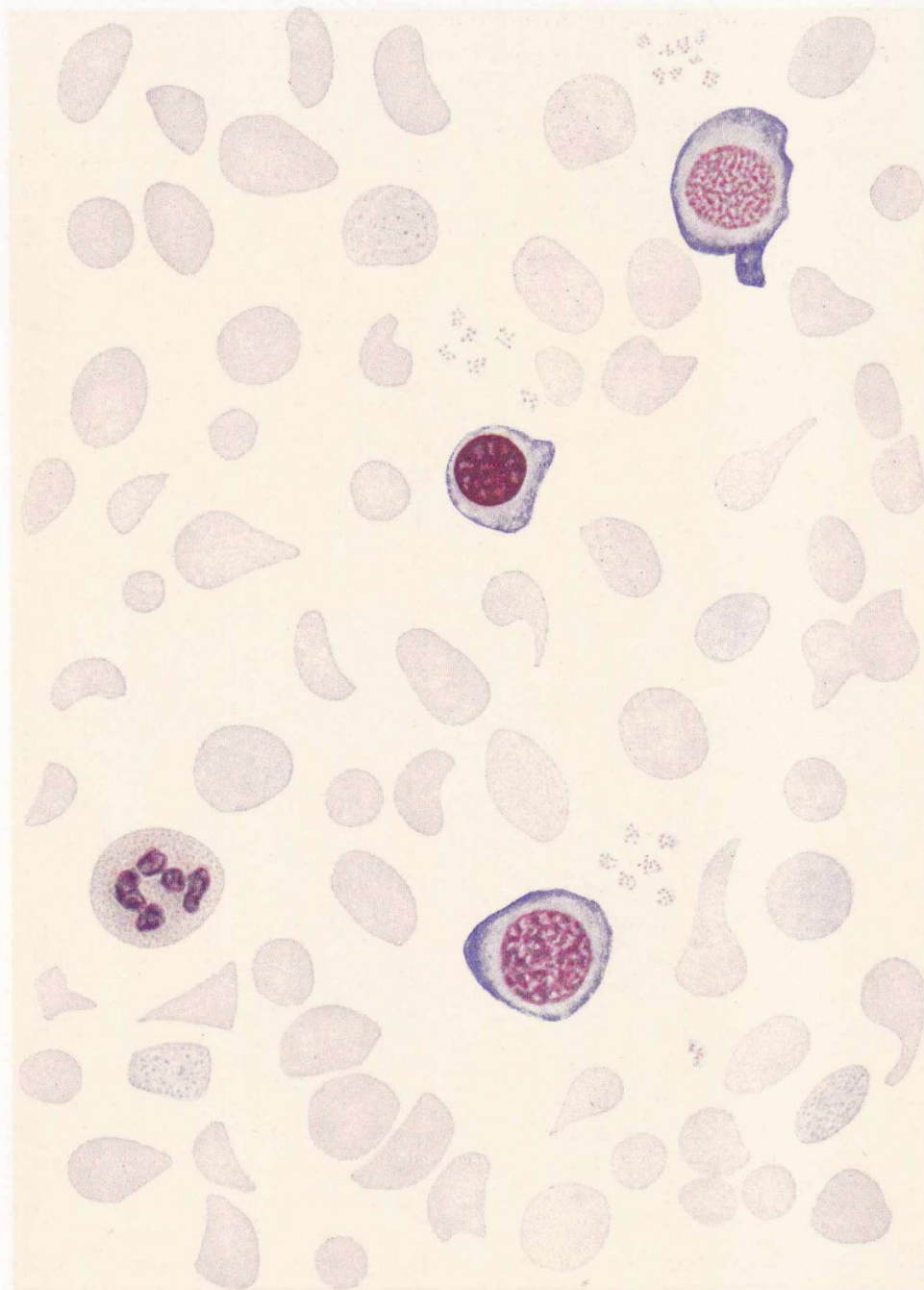


Plate 19. — Sprue (peripheral blood): 1. Basophilic megaloblast. 2. Basophilic erythroblast. 3. Macropolycyte (neutrophil with a hypersegmented nucleus). 4. Microcyte. 5. Macrocyte. 6. Poikilocyte. 7. Megalocyte. 8. Schizocyte. 9. Polychromatic erythrocyte. 10. Stippled erythrocyte.

The red cell picture is characterised by: marked *anisocytosis* (varied dimensions of erythrocytes), *poikilocytosis* (varied shape of erythrocytes), *megalocytosis* (the presence of large, oval red cells), *schizocytosis* (small red cells of bizarre outlines — “fragmented” red cells), *polychromatophilia* (red cells with a basophilic tinge in the cytoplasm), *basophilic stippling*.



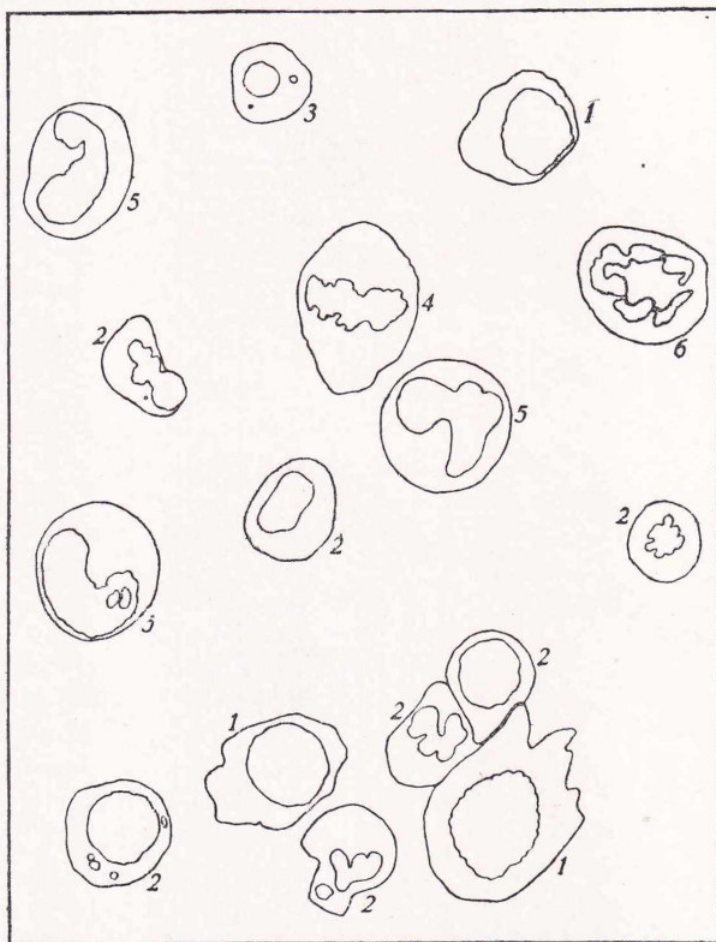
*Plate 19.* — Nontropical sprue (peripheral blood).



15. Normal or, more often, decreased cholesterol level.

16. Normal or decreased serum iron.

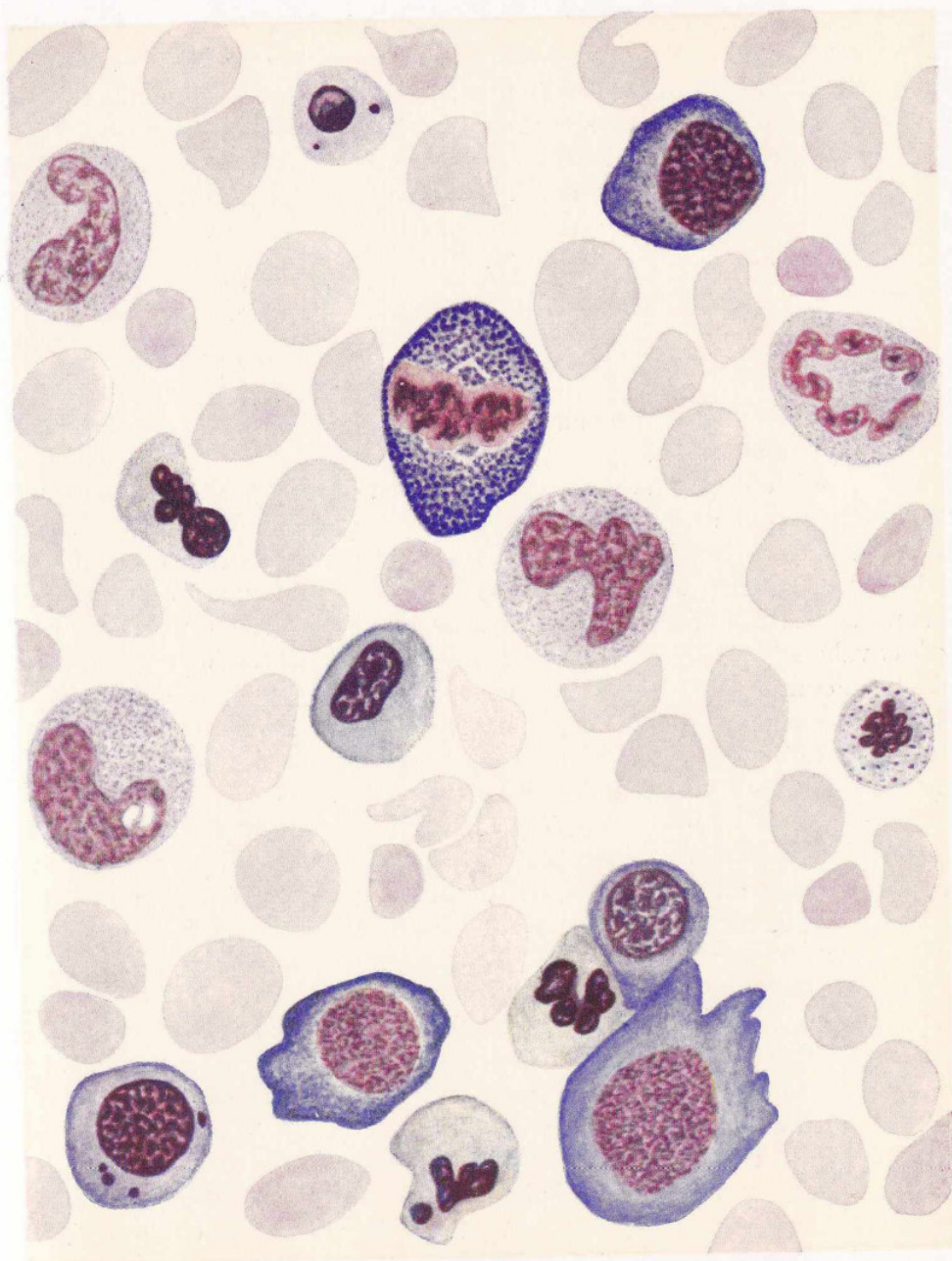
17. Neurologic symptoms of spinal-cord degeneration are met only exceptionally in sprue, on the other hand, peripheral nerve involvement is sometimes observed. These changes occur in connection with the vitamin deficiencies, which may associate themselves in the course of sprue (for example vitamin B<sub>1</sub> deficiency induces symptoms resembling beri-beri, Kassirsky).



*Plate 20.* — Nontropical sprue (bone marrow): 1. Early basophilic megaloblast. 2. Late basophilic megaloblast and polychromatic megaloblasts. 3. Orthochromatic megaloblast.

4. Megaloblast in mitosis. 5. Giant metamyelocyte. 6. Polymorphonuclear neutrophil.

*Note:* the nuclei of megaloblasts are markedly atypical; this phenomenon is not characteristic for sprue and may occur in all severe cases of any "acastlosis".



*Plate 20.* — Nontropical sprue (bone marrow).



In diagnosis of nontropical sprue, there should be borne in mind the possibility that the symptoms of this syndrome may be secondary. In such cases, the underlying disorder may be, for example, the abdominal form of Hodgkin's disease, lymphosarcoma or tuberculosis of retroperitoneal lymph nodes or generalised amyloidosis. These conditions may be responsible for deficiency of water- and lipidsoluble vitamins (Rodriguez-Molina).

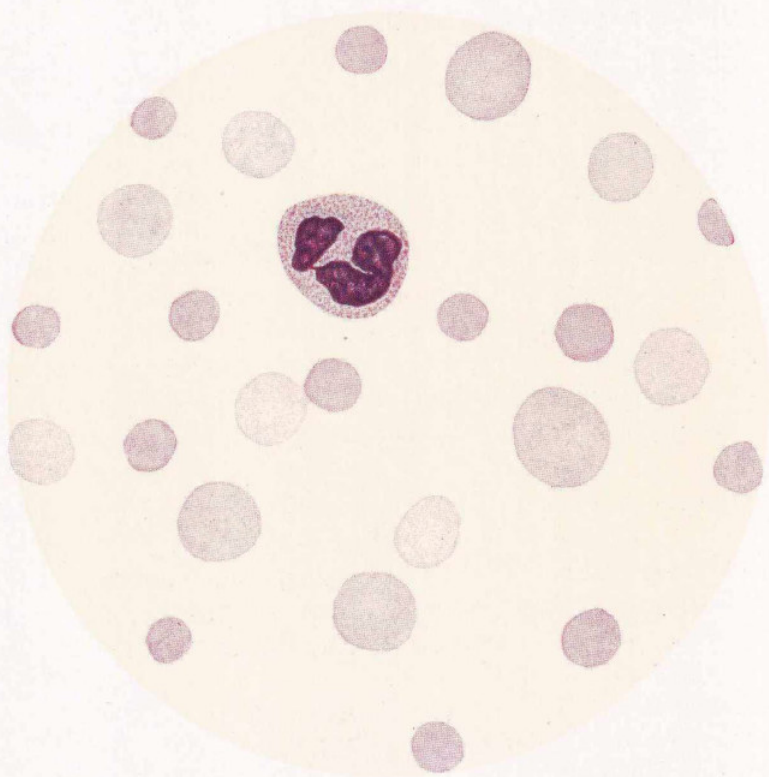
### CONSTITUTIONAL HAEMOLYTIC JAUNDICE (CHAUFFARD-MINKOWSKI)

#### *Hereditary Haemolytic Anaemia — Congenital Sphaerocytosis*

Among the congenital haemolytic anaemias, this syndrome is most commonly encountered.

The haematologic picture is usually characterised by mild, normocytic and normochromic anaemia, on the other hand, jaundiced cases without anaemia may be also encountered. In such cases compensatory erythropoietic mechanisms are operative and the loss of erythrocytes is well balanced. During a haemolytic crisis, the number of red cells may drop to low values (1,000,000—2,000,000 per cu.mm.). Examination of peripheral blood smears facilitates the diagnosis. Under the influence of unknown factors, the constitutionally less resistant erythrocytes change their shape. Owing to their plasticity, they swell and become thicker. A similar phenomenon may be observed under the influence of hypotonic salt solutions. Such cells, when observed in one plane (in smears under the microscope), possess a decreased diameter. These corpuscles stain darker and show no thinner central area. They are sphaerical in shape (sphaerocytes). During a haemolytic crisis the sphaerocytosis of the peripheral blood becomes more marked.

Plate 21 illustrates a blood smear from typical case. First of all, one is impressed by anisocytosis and polychromasia. The analysis of the blood picture shows that there are two erythrocyte types present: small and darkly stained ones (sphaerocytes) and large, greyish-pink red cells (macrocytes). In intravitaly stained preparations, the large forms contain the "*substantia reticulo-filamentosa*". During a haemolytic crisis, the reticulocytes may outnumber the sphaerocytes. These two cell types represent two separate phenomena viz. destruction and regeneration. Figuratively speaking, the sphaerocytes are decrepit, "senile" red cells, whereas the reticulocytes are on the threshold of life. The preponderance of one or the other of these forms depends on the degree of haemolysis and the regenerative activity of the bone marrow, as well as on the release of reticulocytes into blood stream.



*Plate 21.* — Congenital haemolytic anaemia (peripheral blood).



The type of anisocytosis, specific for haemolytic anaemia, results, when expressed graphically in a "biphasic" Price-Jones curve with two peaks, corresponding respectively to sphaerocytosis and macrocytosis.

The regenerative activity of the bone marrow may, in some instances, find its expression in leukocytosis with a shift to the left and an increase of the platelet count.

In the phases of severe anaemia, the bone marrow demonstrates a vivid erythropoietic activity. Entire fields of vision are strewn with erythroblasts at all developmental stages. The number of erythroblasts exceeds the number of the remaining bone-marrow cells and may even amount to as much as 90% (Aleksandrowicz). The regenerative activity varies with the degree of haemolysis and the severity of anaemia. Erythropoiesis is normoblastic. The maturation curve, as distinct from the case of siderotic syndromes, is normal. The number of mitotic figures is markedly increased. A shift to the left in the erythropoietic series may be observed in some instances (proerythroblastic reaction). It is conceivable that the sphaerocytes are formed outside the bone marrow; their presence in bone-marrow smears, may be the result of admixture of peripheral blood.

The granulocytes and megakaryocytes present no demonstrable morphologic changes.

The diagnosis of congenital sphaerocytosis is based on the following criteria:

1. Symptoms of jaundice in an individual with constitutional skeletal abnormalities; when remaining symptoms are present, the absence of these deformities does not necessarily rule out the diagnosis of congenital sphaerocytosis.

2. Positive indirect Hijmans van den Bergh's test.

3. The absence of bile pigments in urine.

4. Haematological findings (sphaerocytosis, reticulocytosis).

5. Increased osmotic fragility of red cells<sup>1</sup>; this test is of restricted and relative value; it may be negative; neither is it decisive as to other haemolytic syndromes; it may be positive in acquired haemolytic anaemia. A negative osmotic fragility test is thus not contradictory to the diagnosis of congenital sphaerocytosis. The results of this test vary with the phase of the illness. A more sensitive test consists in examination of osmotic fragility after previous incubation of red cells tested at 37°C. (98.6° F.). In cases of congenital haemolytic anaemia, the osmotic fragility of incubated red

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<sup>1</sup> The number of red cell fragility tests increases from day to day, many tests have been recently devised for the differentiation of haemolytic syndromes (mechanical fragility, heat fragility, acid fragility etc.). None of them is strictly specific and not one corresponds to the conditions of red-cell destruction *in vivo*.

cells is markedly increased. A control test in the same conditions, with normal erythrocytes, gives normal values. Coombs' test, or the test with trypaninised erythrocytes, may be helpful in differentiation between acquired and congenital haemolytic anaemias. These tests are positive in certain cases of the acquired variety. None of them is, however, strictly specific and further development of serologic diagnostic methods should be anticipated.

Furthermore, the determination of the survival of transfused erythrocytes is a valuable aid in establishing the diagnosis. In congenital haemolytic jaundice, as well as in other congenital haemolytic syndromes, the survival of donor red cells is usually normal; in the acquired variety, the survival time may be distinctly shortened. This test is recommended by those authors who ascribe primary importance in the pathogenesis of this disorder to congenital defects of erythrocytes. They claim that the rôle of the spleen is secondary. According to Crosby, the changes in the red-cell series may be attributed to faulty lipid metabolism. During the maturation of the erythrocyte, the lipid content of this cell decreases. The loss of lipids is greater in cases of congenital sphaerocytosis than in normal conditions. It may be assumed, that this induces changes in the molecular structure, and, in consequence, may be responsible for the abnormal shape of the red cell (sphaerocyte) and shortened survival.

Russian authors (Vlados, Janovsky) draw attention to the possibility of the rôle of haemolysins among the pathogenetic factors inducing changes in the red cells.

Their views are based on experimental work which demonstrated that, in some cases of congenital haemolytic jaundice blood transfusions and, in consequence, the determination of the period of survival of transfused erythrocytes, are not free from danger, in particular during periods of increased haemolysis. On the other hand, repeated transfusions of plasma to such patients sometimes exerts a beneficial effect diminishing the degree of haemolysis; a possible mode of action of transfused plasma could be attributed to inhibitory effect on haemolytic factors. Pretreatment with plasma opens the way for subsequent therapy with transfusions of red-cell suspensions.

6. Normal saponin fragility of red cells; decreased lysolecithin fragility (Singer).

7. Splenomegaly, during a haemolytic crisis in particular.

8. X-ray examination of the skeletal system (osteoporosis).

9. Chronic leg ulcer (inconstant symptom).

Sphaerocytosis with signs of marked erythropoiesis is not strictly specific for constitutional haemolytic jaundice. It may be present in other, not



only congenital, haemolytic syndromes. It may be found in acquired immuno-haemolytic syndromes, as well as in haemolytic syndromes induced by certain toxic factors. Sphaerocytosis may also appear sometimes in acute haemolytic anaemia of the Lederer-Brill type. This disorder is, however, characterised by severe anaemia and the constant presence of neutrophilic leukocytosis (in some cases exceeding 100,000) with a shift to the left. The myelogram may resemble the picture encountered in congenital sphaerocytosis.

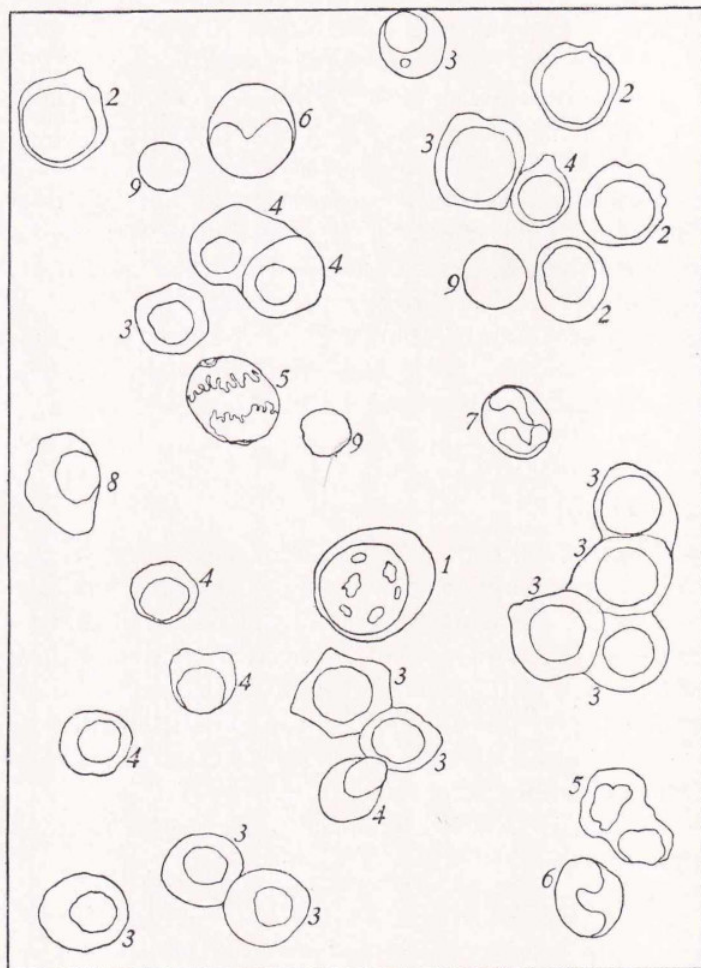
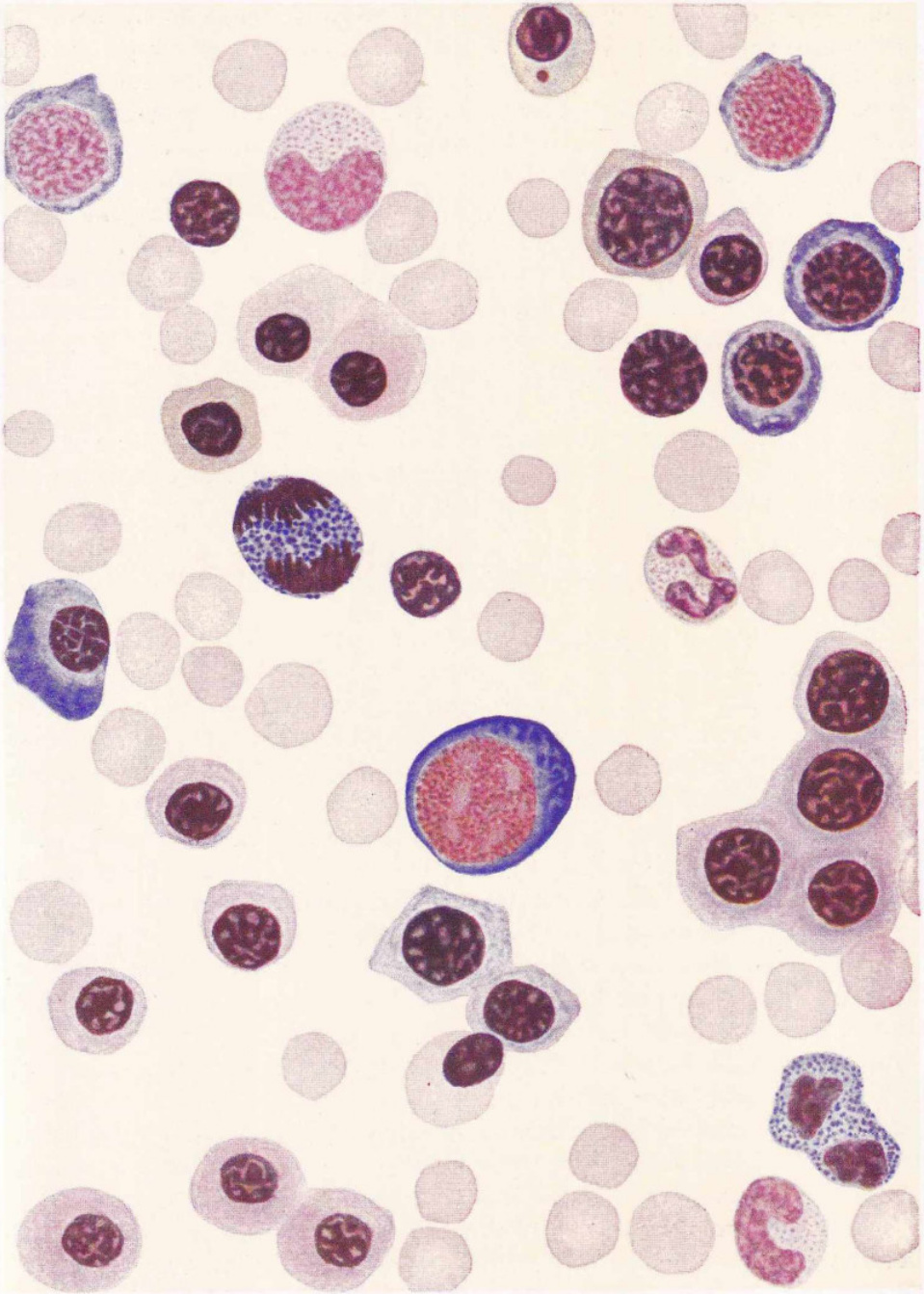


Plate 22. — Congenital haemolytic anaemia (bone marrow): 1. Proerythroblast. 2. Basophilic erythroblast. 3. Polychromatic erythroblast. 4. Orthochromatic erythroblast. 5. Erythroblast in mitosis. 6. Neutrophilic metamyelocyte. 7. Polymorphonuclear neutrophil. 8. Plasmocyte. 9. Naked nuclei.

*Note:* the presence of microsphaerocytes.



*Plate 22.* — Congenital haemolytic anaemia (bone marrow).



In recent years, the classical view concerning the symptoms of the haemolytic crisis (reticulocytosis, markedly stimulated erythropoietic activity of the bone marrow) has been challenged by Owren, Dameshek and Bloom, and others. These authors, Owren in particular, observed in their cases symptoms of aplasia or hypoplasia of the bone marrow (scarse cellularity of the bone marrow, arrested maturation in the erythroblastic series, reticulocytopenia in the peripheral blood and, in some instances, granulocytopenia and thrombocytopenia) during the onset of a haemolytic crisis. Owren goes so far as to claim that the crisis appears in consequence of marrow aplasia, and not as the result of increased haemolysis. He maintains, that this condition should be termed aplastic crisis. This interesting concept is supported by occasional findings in certain cases of congenital sphaerocytosis.

It is not unreasonable to assume that congenital haemolytic anaemia with sphaerocytosis represents a syndrome where various pathogenetic mechanisms are operative.

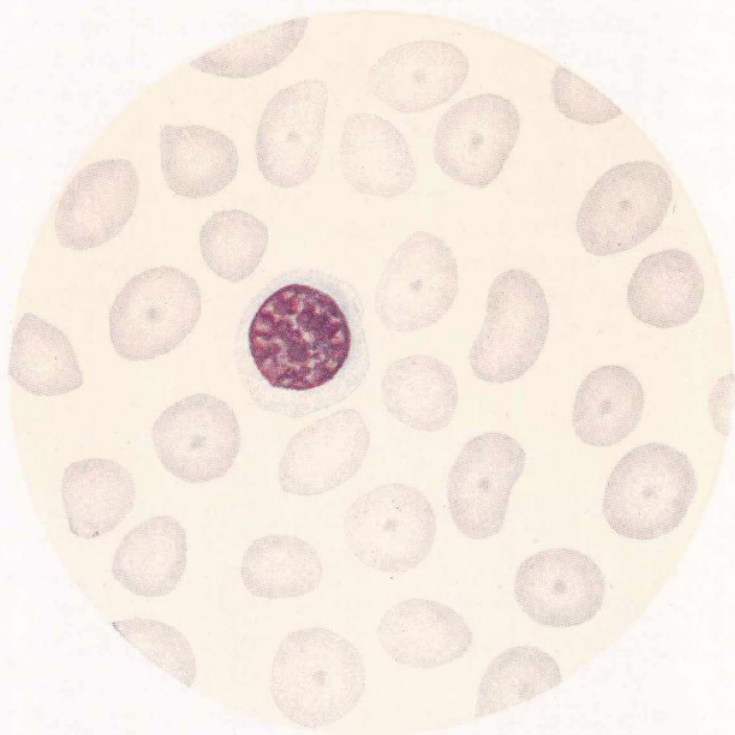
### COOLEY'S ERYTHROBLASTIC ANAEMIA

During the last few years a good deal of attention has been given in the haematologic literature to congenital haemolytic syndromes. Among this group of diseases a specific position should be allotted to the haemolytic syndromes occurring chiefly in the Mediterranean area. In a comprehensive review of these problems, Chini and Valeri emphasise the diversity of symptoms accompanying such syndromes, and propose a general denomination for them — Mediterranean haemopathies. Among them is Cooley's erythroblastic anaemia (Mediterranean anaemia, thalassaemia, target-cell anaemia, erythraemia familiaris chronica).

This syndrome occurs in Greek, Italian, Armenian and Spanish children. The form of disease observed in children is severe (*thalassaemia major*). Sporadic cases have been noted in children of other countries (England, Germany, Switzerland, Bulgaria).

An occult, mild form was reported during the last decade in juveniles and adults (*thalassaemia minor*).

Anaemia is distinctly hypochromic and is characterised by marked anisocytosis and poikilocytosis. The mean corpuscular volume is usually low (microcytosis), the shape of the cell is often oval. In spite of hypochromia, the administration of iron is ineffective. The cause of this phenomenon is not clear. On autopsy, iron deposits are found in internal organs. These deposits are, however, located within the reticulo-endothelial system. Congenital erythrocyte defects are responsible for disturbances in haemoglobin synthesis, as well as in iron utilisation.



*Plate 23.* — Cooley's erythroblastic anaemia (peripheral blood).

*Note:* the abundance of target cells.



Early haematologic symptoms may be present in infants in the fourth week of life. They manifest themselves in abnormal dimensions, shape and haemoglobin content of red cells. The disturbances in haemoglobin synthesis lead to paleness of erythrocytes and abnormal distribution of blood pigment in the cell. X-ray investigations indicate changes in the physico-chemical state of haemoglobin. In smears, pale, disc-like or oval erythrocytes with a narrow margin of haemoglobin on the periphery can be seen. These corpuscles are morphologically identical with those encountered in iron deficiency. The second type of the red cell is more characteristic of Cooley's anaemia. These cells are larger (macrocytes) and their haemoglobin is distributed in a specific pattern of two layers; it forms a disc in the central area and a rim on the periphery. The space between these two zones is colourless. Because of the resemblance they have been named "target" cells. Being extremely thin, they are also termed leptocytes. Their rôle is unknown. To Wintrobe they conveyed the impression of their membrane being too large for the contents.

Sporadic target cells may be found in healthy persons. They appear also in sickle-cell anaemia, as well as in other anaemias, in liver disorders, sprue as well as following splenectomy. It is claimed that they usually appear in the early phases of blood regeneration.

Plate 23 illustrates the peripheral blood picture during the early phase of Cooley's anaemia.

In the drawing, hypochromia, anisocytosis and numerous target cells should be noted.

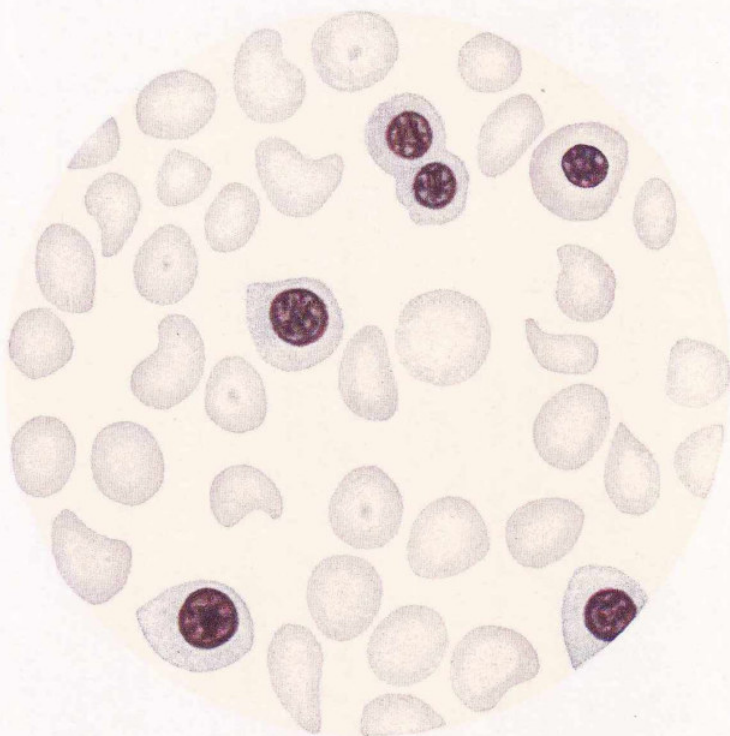
During later phases of the disease, erythroblasts appear in the peripheral blood. This phenomenon is caused by metaplasia of erythropoietic tissue in the liver and spleen. The number of erythroblasts may be high. The majority of them are at the polychromatic or orthochromatic stage. Karyorrhexis may often be observed, and more rarely, atypical erythroblasts with several nuclei. Signs of stimulated erythropoiesis are present, the number of reticulocytes increases.

Plate 24 illustrates the peripheral blood during a later stage of the disease.

Anisocytosis and poikilocytosis are more pronounced. Moreover, numerous schizocytes may be seen. Nearly all erythrocytes show distinct hypochromia. The presence of erythroblasts is the main feature.

The fully developed morbid picture is characterised by an increase in erythroblasts; basophilic stages and proerythroblasts appear among them. In severe cases, the blood picture may resemble di Guglielmo's erythromyelosis.

The release of erythroblasts into the peripheral blood is accompanied by neutrophilic leukocytosis with a shift to the left.



*Plate 24.* — Cooley's erythroblastic anaemia (peripheral blood). Late period.  
5 polychromatic erythroblasts and one orthochromatic erythroblast are to be seen.



Plate 25 illustrates the peripheral blood from a severe case of Cooley's anaemia (Wright's stain).

Numerous young erythroblasts and proerythroblasts are seen in the drawing. Some of the polychromatic erythroblasts are distinctly atypical (paraerythroblasts). They are of giant dimensions and possess a fine nuclear structure. The mature erythrocytes are characterised by anisocytosis, poikilocytosis, hypochromia and presence of target cells.

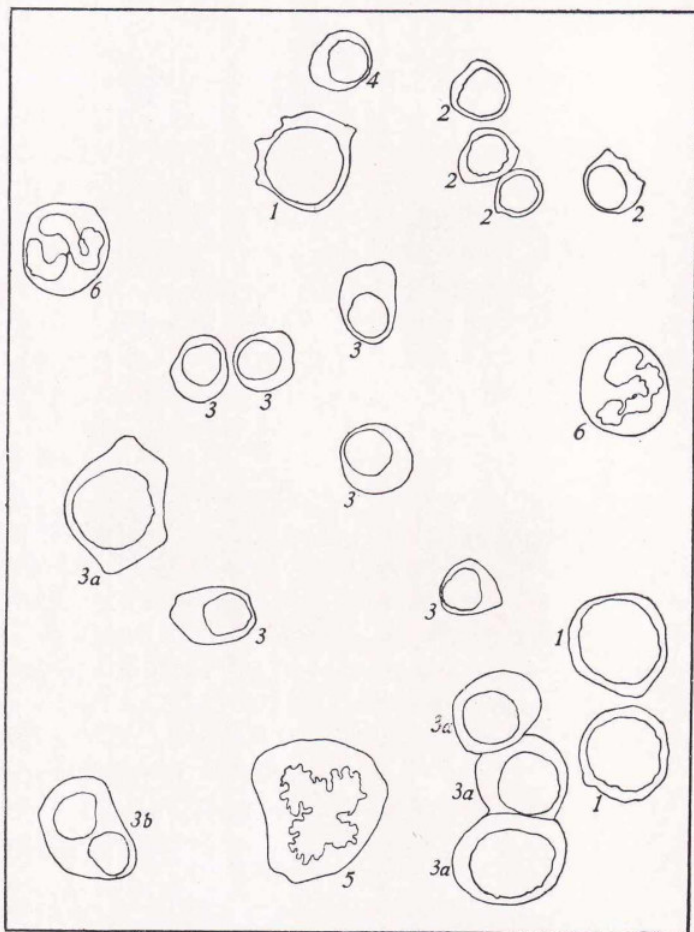
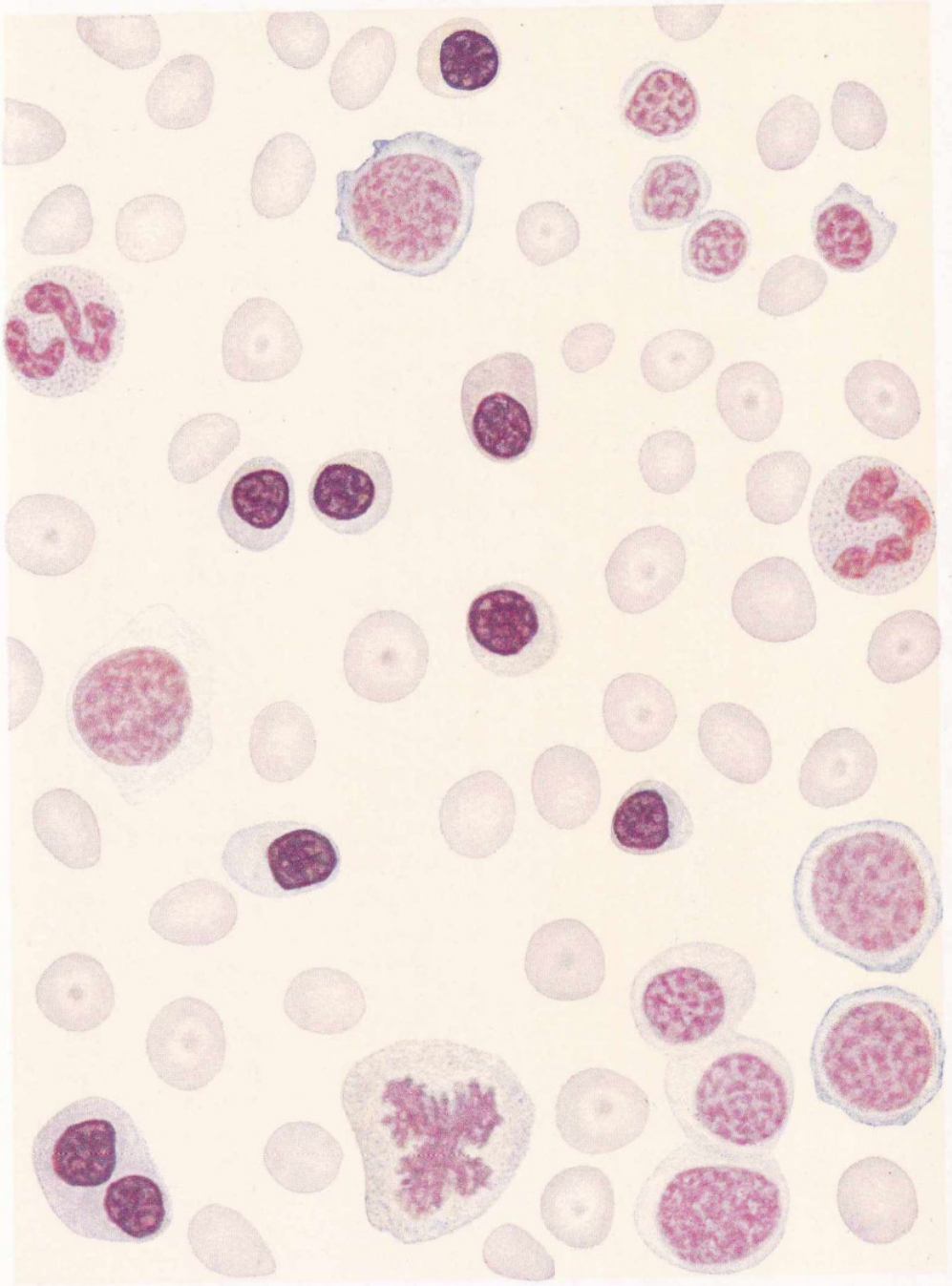


Plate 25.— Cooley's erythroblastic anaemia (peripheral blood, Wright's stain). Severe form: 1. Proerythroblast. 2. Basophilic erythroblast. 3. Polychromatic erythroblast. 3a. — polychromatic paraerythroblast (giant cell, characterised by abnormally fine nuclear structure). 3b. — binucleated polychromatic erythroblast. 4. Orthochromatoc erythroblast. 5. Pathological erythroblastic mitosis (probably multipolar; the chromosomes are blurred and clump together). 6. Polymorphonuclear neutrophil.

Note: numerous target cells.



*Plate 25.* — Cooley's erythroblastic anaemia (peripheral blood, Wright's stain). Severe form.



Marked hyperplasia of the erythropoietic tissue is present in the bone marrow. This phenomenon is held to accompany most haemolytic syndromes.

The diagnosis of Cooley's anaemia should be based on following symptoms:

1. The patient's extraction.
2. Constitutional skeletal abnormalities (mongoloid features).
3. Pale, dirty-yellowish skin.
4. Haematological findings (hypochromic anaemia, erythroblastosis, target cells).
5. Splenomegaly.
6. Skeletal abnormalities (widening of the marrow cavities, thinning out and decreased density of the compact mass).
7. Osmotic fragility often decreases (the erythrocytes do not haemolyse in solutions of low salt concentration); sometimes the fragility amplitude is broad (the beginning of haemolysis in 0.54% NaCl, the end in 0.03% NaCl). The target cells are osmotically more resistant.

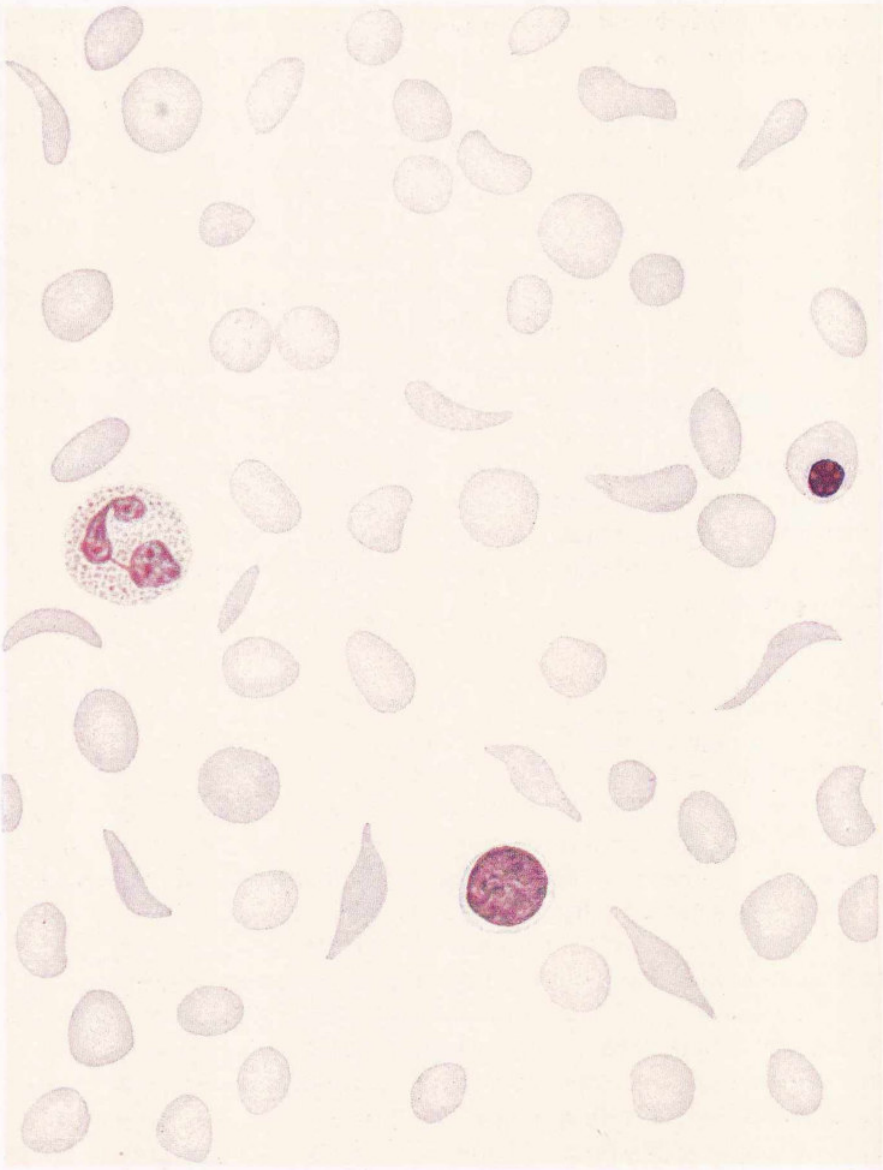
Another variety of constitutional haemolytic syndromes is sickle-cell anaemia.

### SICKLE-CELL ANAEMIA

The sickle-cell trait is observed in 7—28% of healthy Negroes. This trait was recently described also in several representatives of the white race. Atypical erythrocytes represent a constitutional trait not connected with a morbid process. In some individuals bearing this trait (0.6—9%) there develops, however, chronic haemolytic anaemia.

Sickle-cells may resemble elliptocytes; in shape, however, they are often semilunar with pointed ends. Some of them resemble oat grains. They are described in literature under various names (drepanocytes, meniscocytes, *hématies falciformes*).

The sickling phenomenon becomes manifest in oxygen deficiency and at low pH. In routine stained blood smears only occasional drepanocytes occur. To demonstrate their presence a special technique is required. A simple method is to cause local stasis by the compression of the finger for 5—10 minutes before taking the blood sample. The smears should be transferred without delay to a moist chamber (37°C, 98.6° F.) to retard drying. It is also practicable to prepare smears from venous blood collected under a layer of paraffin. The sealing off of a drop of blood under a coverslip for a few hours (sometimes up to 72 hours) at room temperature is often recommended. In the presence of oxygen, drepanocytes become reversed to normal shape.



*Plate 26.* — Sickle-cell anaemia (peripheral blood).



The cause of haemolytic phenomena in some of the sickle-cell trait bearers is unknown. Drepanocytes show a markedly increased osmotic resistance.

The anaemia is usually severe and normochromic. The mean corpuscular volume is normal; in rare instances slight macrocytosis may be detected. The signs of haemolysis are accompanied by an erythropoietic response, manifesting itself by reticulocytosis and peripheral erythroblastosis. Reticulocytes are of normal shape, or oval. During periods of intensive erythrocyte destruction, neutrophilic leukocytosis with a shift to the left may be observed, as well as thrombocytosis.

Plate 26 represents the peripheral blood from a case of sickle-cell anaemia.

The smear was prepared from venous blood collected under a layer of paraffin.

The red-cell picture is characterised by anisocytosis, poikilocytosis and the presence of drepanocytes.

Some of the erythrocytes are hypochromic.

The myelogram resembles the picture usually presented by other haemolytic syndromes.

Diagnostic criteria:

1. Constitutional skeletal abnormalities.
2. Chronic anaemia.
3. Haematological findings.
4. Bilirubinaemia.
5. Decreased osmotic fragility of red cells.
6. Normal or retarded erythrocyte-sedimentation rate in spite of anaemia; the sickle shape of erythrocytes interferes with rouleaux formation — this symptom is specific for drepanocytosis.
7. Paroxysmal episodes of abdominal distress (stabbing pain in the epigastrium or in the left or right region of the upper abdomen).
8. Pains in the joints or bones suggestive of "rheumatic" complaints.
9. X-ray examinations of the skeletal system.
10. Splenomegaly; in advanced stages atrophy of the spleen.
11. Chronic leg ulcer (inconstant symptom).
12. Neurologic symptoms (drowsiness, coma, convulsions, palsies, signs of paresis, headaches, nystagmus, temporary or permanent blindness); these symptoms are attributed to thrombotic accidents.

Recently the existence of several varieties of human haemoglobin (Haurowitz, Pauling, Singer and Singer, and others) has been demonstrated. These varieties of haemoglobin differ physico-chemically in many respects — as for example in amino-acid composition, electrophoretic mobility, alkali-denaturation resistance etc. They differ also in their antigenic properties. X-ray diffractions methods have demonstrated that the molec-

ular orientation of haemoglobin in abnormal red cells differs from that in normal erythrocytes (Ponder and coll.). This phenomenon may be attributed to the presence of abnormal haemoglobin.

It seems reasonable to assume that in congenital haemolytic syndromes, disturbances in metabolism of the whole organism may occur. In Mediterranean haemopathies and in sickle-cell anaemia, they may result in faulty haemoglobin synthesis and, in consequence, in abnormal haemoglobin production. It is possible that in congenital sphaerocytosis faulty lipid metabolism is involved. According to the data from recent investigations, similar phenomena may occur in some of the acquired haemolytic anaemias or in syndromes in which haemolytic mechanisms are operative (for example Addison-Biermer's disease — according to Owren the megalocytes present in this disease contain foetal haemoglobin). It may be deduced that the abnormal shape of erythrocytes institutes only the morphologic sign of metabolic disturbances involving the organism, followed by functional disorders of the red-cell series. The possibility of the influence of the neurohumoral regulation on these phenomena should be also borne in mind.

## HAEMOLYTIC DISEASE OF THE NEWBORN

### *Erythroblastosis foetalis*

The presence of small numbers of erythroblasts (200 to 2,000 per cu.mm.) in the peripheral blood of the newborn, especially in cases of premature births, is held to be a normal phenomenon in the first days of life. Marked erythroblastosis is, on the other hand, a pathologic symptom occurring in several clinical syndromes. The observations made during the past ten years have shed new light on the rôle of the Rh factor in immunological relationship between the mother and the foetus. These investigations demonstrate that one of the causes of the erythroblastosis in the newborn lies in the presence of a serological Rh incompatibility.

Haemolytic disease in the newborn comprises three apparently separate syndromes — *hydrops foetalis*, *icterus gravis neonatorum* and *congenital anaemia of the newborn*. According to Levine, the manifold clinical manifestations of this disease may be explained by the iso-immunisation theory. It is reasonable to assume that the appearance of *hydrops foetalis* is provoked by a prolonged action of potent iso-antibodies, congenital anaemia of the newborn being the result of a shorter period of iso-immunisation. More frequently encountered is *icterus gravis neonatorum*, which, according to this concept, should be regarded as an intermediate condition. All these three syndromes have increased haemolysis



and abnormal haematopoiesis as a constant common feature. It should be emphasised that clear pictures of one of these syndromes do not always appear, cases presenting features of each of this triad are frequently encountered. The most outstanding haematological trait is instituted by the presence of marked erythroblastosis in the peripheral blood to be regarded as a sign of the abnormal function of the blood-forming and blood-destroying tissues. The number of erythroblasts in the peripheral blood ranges from 10,000 to 100,000 per cu.mm. This phenomenon is caused by compensative metaplasia of the erythropoietic tissue in the liver and the spleen.

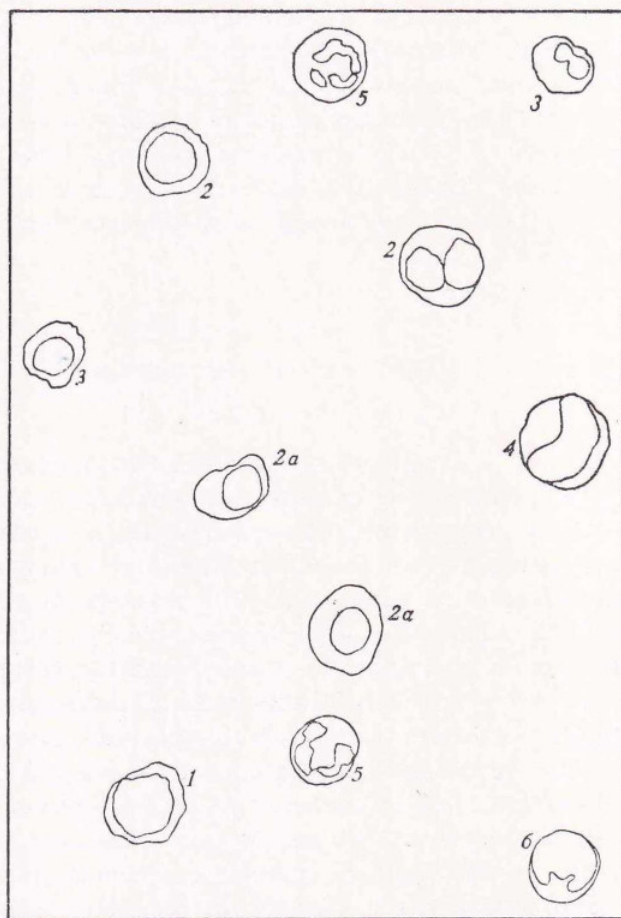


Plate 27. — *Erythroblastosis foetalis* (peripheral blood): 1. Basophilic erythroblast. 2. Polychromatic erythroblast. 2a. —late polychromatic erythroblast. 3. Orthochromatic erythroblast. 4. Neutrophilic metamyelocyte. 5. Segmented neutrophil. 6. Monocyte.

Note: the presence of erythroblasts in peripheral blood, polychromatophilia of red cells, macrocytosis and the shift to the left in granulocytes.

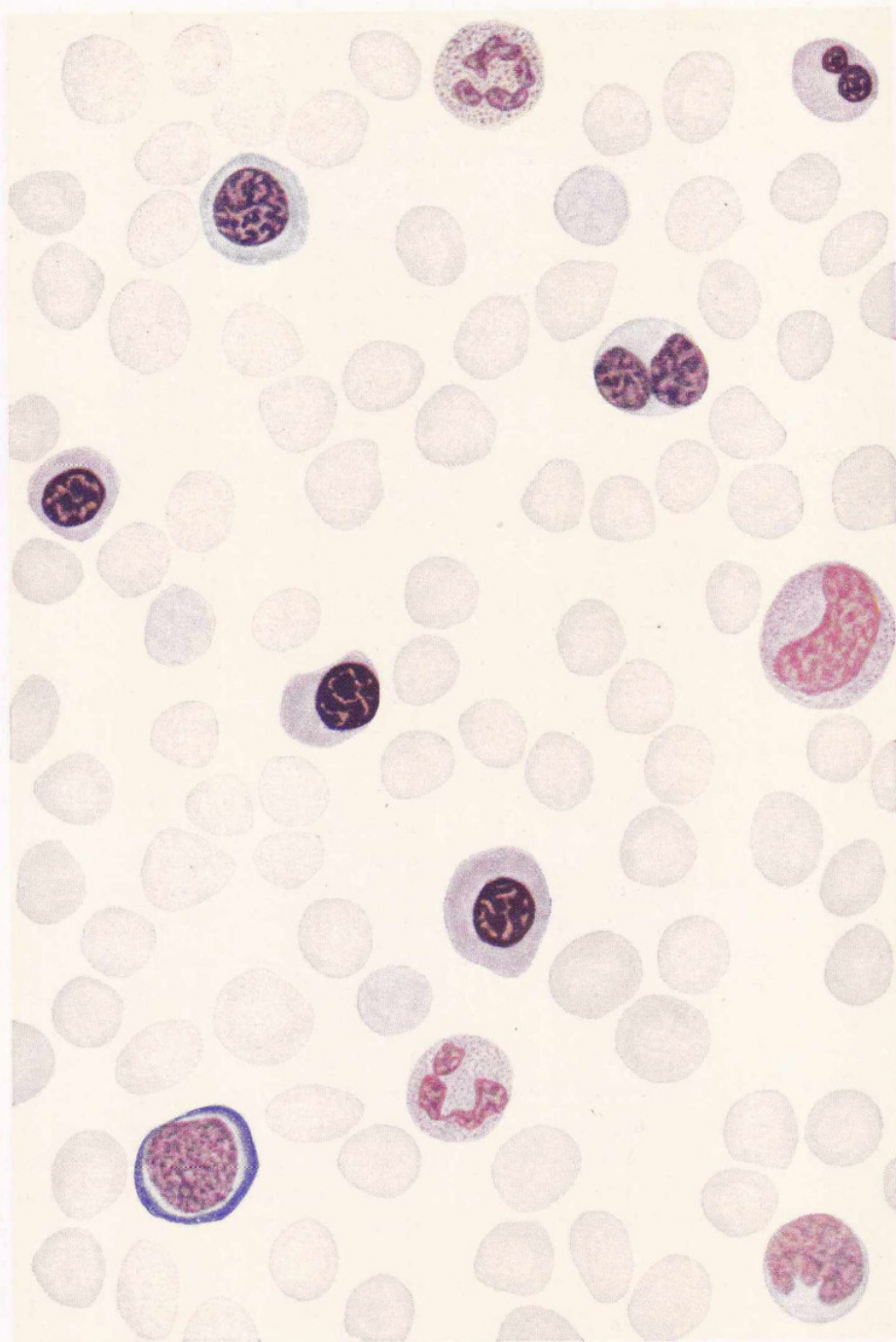


Plate 27. — *Erythroblastosis foetalis* (peripheral blood).



The erythroblasts encountered in the peripheral blood represent all stages of the maturation cycle. The erythrocytes are normochromic, and the colour index is often raised above 1.0. The shape of the red cells remains normal. There appears, however, a marked degree of anisocytosis with macrocytosis. The presence of macrocytes is explained by the increased number of reticulocytes. This symptom is important in the recognition of *erythroblastosis foetalis*. The Price-Jones curve shows the presence of two peaks (a biphasic curve). The mean corpuscular volume is also increased. The fact that polychromatophilia and red cells containing nuclear fragments are found, points to intense erythropoietic activity.

Neutrophilic leukocytosis (raised to 30,000) with a shift to the left may often be observed. The platelets may, in severe cases, be greatly reduced in number, an occurrence which leads to the appearance of the clinical picture of thrombocytopenic purpura. The pathogenesis of this symptom may also be explained by the action of immunological mechanisms.

Plate 27 illustrates the peripheral blood picture in a case of *erythroblastosis foetalis*.

The following characteristic traits should be noted:

1. Numerous erythroblasts.
2. Normochromia and macrocytosis of the red cells.
3. Moderate anisocytosis and an insignificant degree of poikilocytosis.
4. The presence of immature neutrophilic granulocytes (metamyelocytes and myelocytes).

The diagnosis should be based on the above given haematological picture and the following additional findings:

1. Symptoms of jaundice and anaemia in the newborn.
2. Hepato- and splenomegaly.
3. Familial history (the course of preceding pregnancies or abortions).
4. Serologic demonstration of Rh incompatibility between the blood of the mother and of the child, examination of Rh factor in the blood of the father.
5. The demonstration of antibodies in the blood of the mother.

In differential diagnosis, various conditions, such as congenital syphilis (serological tests, roentgenological examination of the skeletal system), infections during the prenatal period or after birth, congenital abnormalities of the liver or bile ducts, atelectasis of the lungs, spontaneous haemorrhages or bleeding induced by trauma and congenital heart disease should be taken into account.

Other blood disorders, such as leukaemia, idiopathic thrombocytopenia and constitutional haemolytic jaundice are only rarely encountered in the newborn. Severe anaemia, enlargement of the liver and spleen are not present in simple *icterus neonatorum*.

## REFRACTORY ANAEMIA — APLASTIC ANAEMIA PANCYTOPENIA

*Total insufficiency of the bone-marrow tissue (Tempka) —  
Anaemia aplastica — Anaemia anhaemopoëtica — Anaemia para-  
lytica — Anaemia aregenerativa — Panmyelophthisis*

This disorder is characterised by anaemia, usually accompanied by granulocytopenia and thrombocytopenia. Certain haematologists therefore consider the terms aplastic or aregeneratory anaemia not entirely adequate. The former term indicates the changes in the erythrocytic system only and fails to appreciate the participation of the leukocytic and megakaryocytic systems in the morbid process. The latter suggests that the anaemia is the result of blood losses leading to exhaustion of the blood-forming tissues. Panmyelophthisis suggests the presence of an acellular bone marrow, when biopsy shows that is not always the case. In numerous instances the bone marrow is cellular or even hyperplastic, but the cells present are immature and functionally less efficient. Other terms, such as refractory anaemia or insufficiency of the entire bone-marrow tissue, are sometimes used. The latter term is to be preferred, because it covers bone-marrow aplasia in an anatomical sense, and implies a functional insufficiency as well.

The clinical picture of this disorder may vary considerably and does not depend on its aetiology (exposure to toxic or infectious agents, post-irradiation injury, hormonal disturbances, sequence of another blood disease, unknown factors). It is, however, very rare for the injury to be limited to the erythrocytic system only.

In recent years, much attention has been given to the rôle of immunological mechanisms in the pathogenesis of certain haematological syndromes characterised by a diminished number of red cells, white cells and platelets (pancytopenia). Such investigations have given rise to a new branch of haematology — immuno-haematology. The scope of immunological investigations was in the initial phase limited to the action of antibodies on red cells; at present immuno-haematological investigations embrace also the action of antibodies on the leukopoietic and megakaryocytic systems (Dacie; Dameshek and Schwartz; Dameshek; Gasser; Hassig; Heilmeyer; Maier; Dausset; Evans and coll., Moeschlin and coll.; Chevalier; Bernard and Ely; Ackroyd; Fleck; Lille-Szyszkowicz and Gi-biński; Ławkowicz, Lille-Szyszkowicz and Zawadzki; van Loghem and coll., Boerman, Bodd and Loutit; Neber and Dameshek and others).

The occurrence of pancytopenia in the course of spleen disorders, such as tuberculosis, sarcoidosis etc. has also been recorded in the literature.



For a long time, the anaemia may remain the sole symptom of the disease and granulocytopenia with thrombocytopenia develop at a later stage. In certain cases, thrombocytopenia may be the initial symptom, granulocytopenia and anaemia developing at an advanced stage of the disease. Cases in which granulocytopenia was the initial symptom have also been reported.

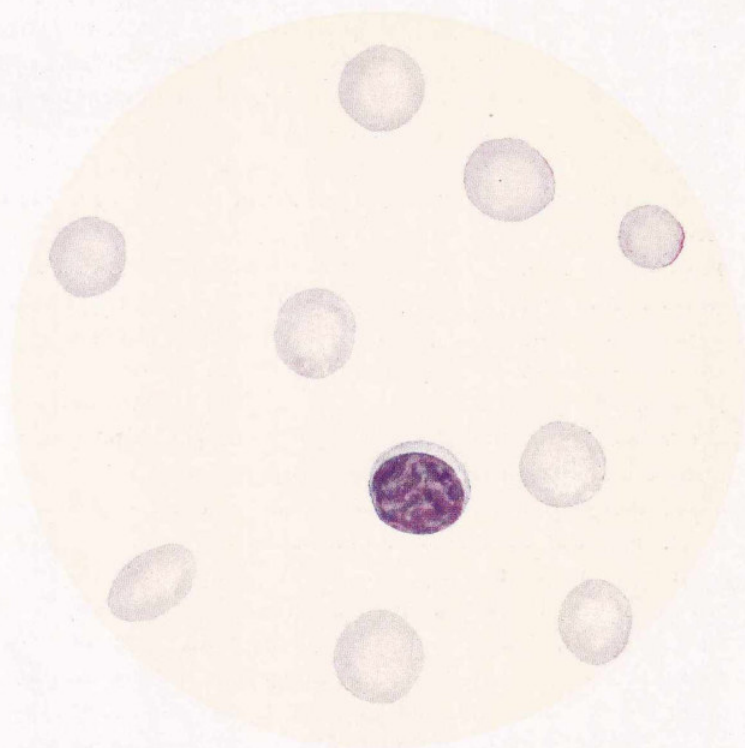
The peripheral blood picture is in typical cases characterised by a proportional decrease in the number of red cells and in the haemoglobin level. In consequence, the colour index remains around 1.0. In spite of severe anaemia, the red cells retain their normal shape and size. Staining properties undergo no changes. The mean corpuscular volume is normal. Anisocytosis and poikilocytosis are very slight. The lack of such signs of regenerative activity of the erythropoietic tissue as macrocytosis, reticulocytosis, polychromasia or basophilic stippling, should be regarded as highly significant. There is also an absence of nucleated red cells. The presence of sporadic reticulocytes points to the preservation of a certain degree of erythropoietic activity.

In the leucocytic picture granulocytopenia with relative lymphocytosis (up to 90 %) is present. Such cases should be differentiated from acute aleukaemic leukaemia (undifferentiated cell leukaemia). In this type of leukaemia granulocytopenia may also be present and the micromyeloblasts may closely resemble lymphocytes. It is, of course, understood that the peripheral blood picture does not suffice for correct diagnosis. On the other hand, a single biopsy demonstrating the presence of an acellular bone marrow cannot rule out the diagnosis of acute leukaemia, which may develop at any later period (two cases observed by one of the authors and sixteen cases observed by Heilmeyer). A decrease in platelets is also a characteristic feature in the picture of aplastic anaemia. Giant platelets or platelets with pathologic granulation are sometimes encountered.

Plate 29 illustrates the peripheral blood smear from a case of aplastic anaemia (red-cell count — 1,200,000, haemoglobin 25%) The normal appearance of the red cells (shape, size and colour) as well as the lack of any sign of erythropoietic activity should be noted.

The bone-marrow picture may be extremely varied. Heilmeyer classifies the changes observed into five groups:

1. Total aplasia of the bone marrow with only sporadic lymphoid reticulum cells and lymphocytes persisting.
2. Aplasia of the erythropoietic tissue, the granulopoietic series remaining more or less preserved and characterised by a shift to the left.
3. A hypoplastic bone marrow with relative lymphocytosis.
4. A cellular bone marrow with a shift to the left in both the erythropoietic and the granulopoietic series.



*Plate 28.* — Aplastic anaemia (peripheral blood).



5. A decrease in the number of erythroblasts, granulocytes and megakaryocytes with a simultaneous reticulum-cell reaction.

In the present state of knowledge, it is difficult to determine whether these bone-marrow pictures represent separate and different disorders, or are but varieties of the same disease. The variety of bone-marrow pictures may be explained by a different pathogenetic mechanism or by a variable intensity of injury by aetiological factors which are operative. According to this concept, the most severe form of injury becomes manifest as a result of total destruction of bone-marrow elements viz. total aplasia in an anatomical sense. In such cases, it is possible to speak of an "empty" bone marrow, because besides a few reticulum cells and lymphocytes, the normal bone marrow elements are absent. Such cases are, however, rare.

Aplastic anaemia does not always appear as a consequence of bone-marrow aplasia. It may also be the result of deep qualitative disturbances in the blood-forming tissues. This may lead to degenerative changes, to maturation disorders or to impaired release of mature elements into the blood stream. In spite of its being manifold and variable in appearance, the bone-marrow picture is of value in establishing the prognosis in aplastic anaemia. That is to say, the biopsy enables the state of the individual blood-cell series to be estimated and makes it possible to gain a deeper insight into the course of the morbid process.

In extreme cases, with total aplasia of the blood-forming tissues, the bone marrow is thin, jelly-like, containing no pulp particles. The paucity of cellular elements in bone-marrow smears is, under low power magnification, striking. Under immersion nucleated elements are rarely encountered. In addition to a few erythroblasts or granulocytes, nuclei of degenerated cells or faintly visible, delicate, chromophobic basket-cells (remnants of degenerated nuclei) are present. On the other hand, a striking feature is instituted by the increased number of reticulum cells, first of all of the so-called large and small lymphoid reticulum cells and lymphocytes, and in some instances of plasmocytes also. In aplastic or hypoplastic bone marrow, tissue basophils (mast-cells, "Gewebsmastzellen") are sometimes encountered. These are not present in normal myelograms (Bremy, Fadem, Leitner, Rohr, Undritz). A case has been reported with 7.1 % of tissue basophils in the myelogram (Fadem).

Plate 29 illustrates the bone marrow from a case of an aplastic syndrome in the course of disseminated tuberculosis. On the drawing, taken from several fields of vision, faintly outlined cells with greyish-blue, agranular cytoplasm are seen. The nuclei are round or oval, showing some resemblance to the nuclei of lymphocytes. The nuclear structure is, however, more delicate and less compact (large reticulum lymphoid cells). Cer-

tain of these cells contain single nucleoli. One small lymphoidal reticulum cell and one polychromatic erythroblast are shown on the drawing.

In other instances, the bone marrow contains cells of the erythropoietic and granulopoietic series, but it is distinctly hypocellular. Moreover the cells demonstrate qualitative changes. The erythroblasts have "frayed" outlines, their cytoplasm is irregularly stained and may contain vacuoles or coarse basophilic granules. The nuclear structure is often blurred, the arrangement of chromatin is abnormal, or the shape of the nucleus is atypical. In some of these cells lack of correlation between the degree of maturity of the cytoplasm and of the nucleus may be present.

Plate 30 illustrates the bone-marrow picture in the course of chronic, progressive aplastic anaemia. The drawing presents atypical, degenerated erythroblasts (paraerythroblasts) and basket-cells.

The cells of the granulopoietic series also show such degenerative changes, as vacuolation, pathological granulation, abnormal segmentation of the nucleus, and lack of correlation between the degree of maturity of the nucleus and of the cytoplasm.

The number of megakaryocytes is decreased. Usually these cells cannot be demonstrated in the bone-marrow smears.

In cases in which the bone marrow is cellular, the preponderance of immature erythroblasts and granulocytes together with the lack of megakaryocytes is of diagnostic value.

It is not in every case that the entire bone-marrow tissue undergoes aplasia. Aplastic foci may be situated close to foci of an abnormal or hyperplastic blood-forming tissue. Therefore, repeated biopsies (in addition to the sternum, the spinous processes, pelvic bones, ribs and other bones may be selected as the site of diagnostic puncture) are highly recommended in such cases.

The examination of histological sections obtained by trephination may be helpful in certain cases. In sections, the reciprocal relationship of the bone-marrow elements may, because of the preserved structure, be more readily evaluated.

The following criteria may serve as an aid to establishing the diagnosis.

1. The haematological syndrome: refractory anaemia, granulocytopenia and thrombocytopenia.
2. In cases, where the aetiological factor is known a meticulous previous case history may be helpful (infection<sup>1</sup>, exposure to toxic substances, irradiation).

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<sup>1</sup> One of the authors obtained a complete recovery, following tonsillectomy, in a case of severe bisystemic cytopenia (erythrocytopenia 900,000 per cu.mm. and granulocytopenia 1,000 per cu.mm. in a 30-year old female). The patient suffered from chronic, purulent tonsillitis.



3. An increased erythrocyte-sedimentation rate. This is observed in all severe cases. The presence of this symptom is probably caused by dysproteinaemia. Moreover, this symptom may depend on reticulum-cell reactions, especially of the plasmocytes.

4. A high or normal serum-iron level. This may be present also in Addison-Biermer's disease: in the latter syndrome, however, the serum iron decreases, by contrast with aplastic anaemia, in the course of liver extract or vit. B<sub>12</sub> therapy.

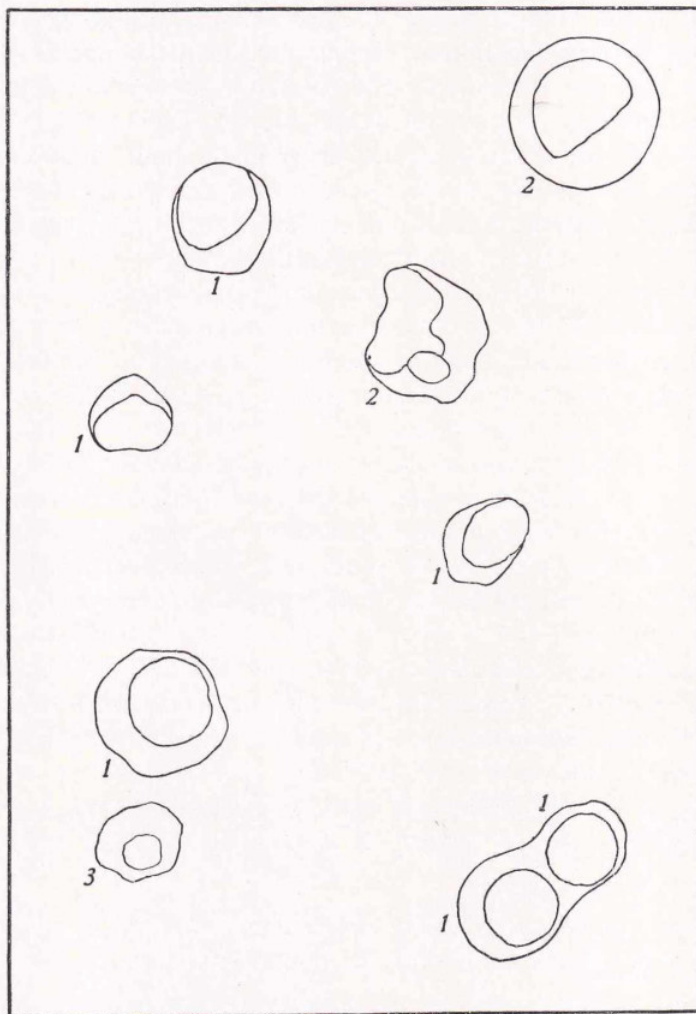
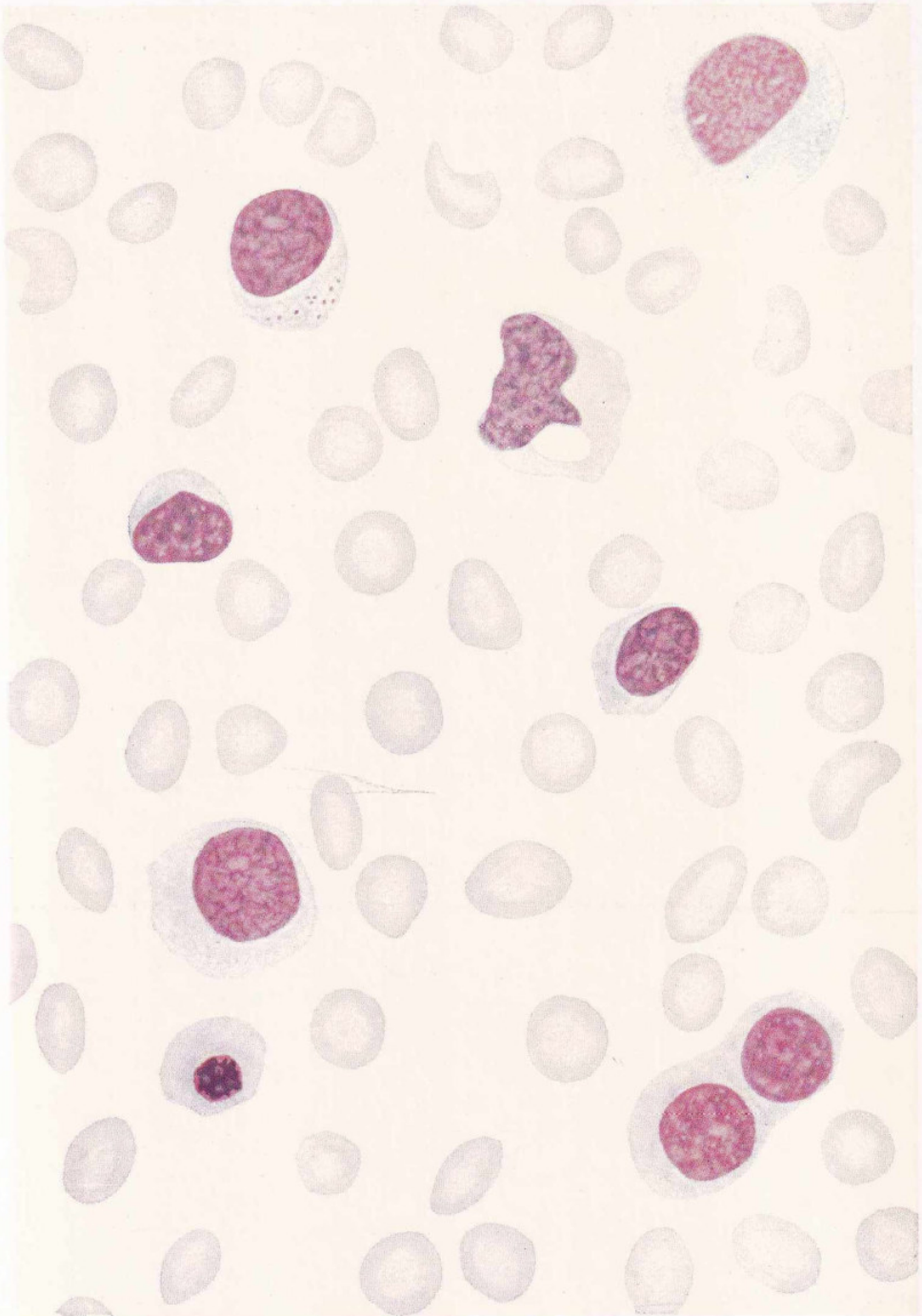


Plate 29.—Aplastic anaemia (bone marrow). Increase in reticulum cells: 1. Lymphoid reticulum cells. 2. Atypical reticulum cells (these may be changed to such an extent as to suggest a neoplastic disease, *e.g.* lymphosarcoma). 3. Polychromatic erythroblast.



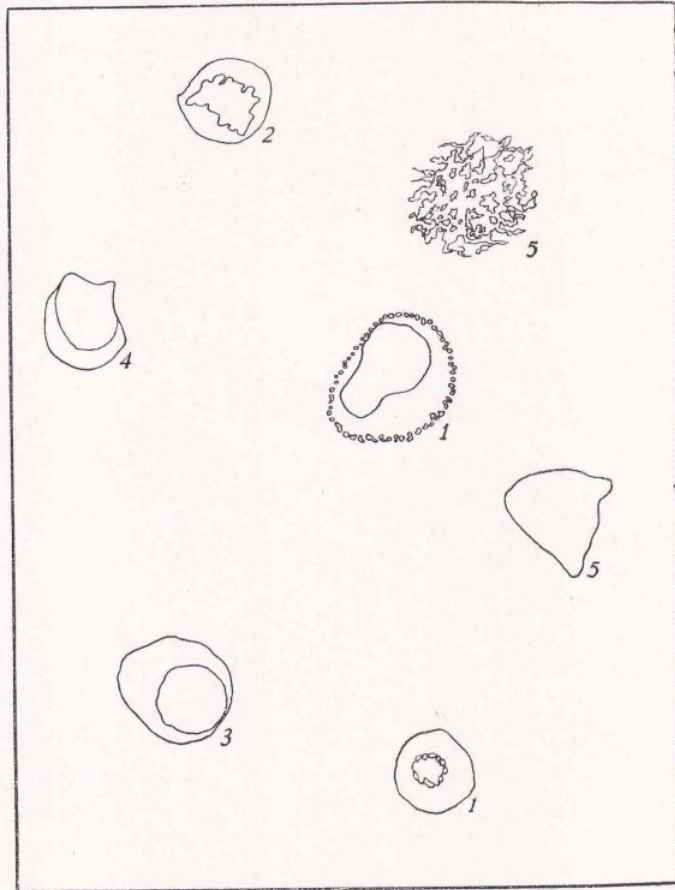
*Plate 29.* — Aplastic anaemia (bone marrow). Increase of reticulum cells.



The syndrome of anaemia, granulocytopenia and thrombocytopenia may also be observed in Addison-Biermer's disease. The presence of this disorder may be ruled out on the basis of other symptoms, such as the lack of achlorhydria, neurologic disturbances and of megaloblastic erythropoiesis.

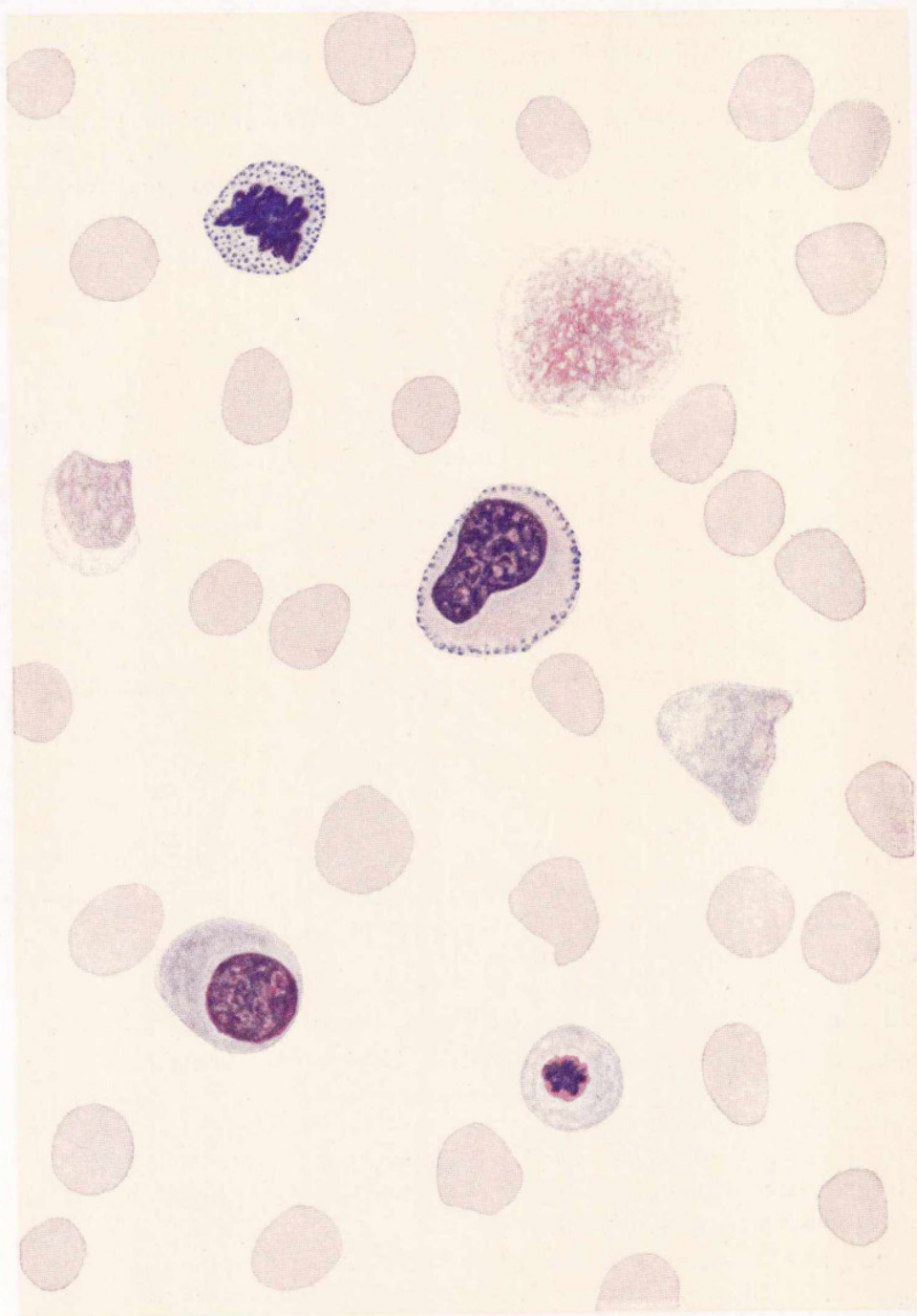
The anaemia in Werlhof's disease (idiopathic thrombocytopenic purpura) being developed in consequence of blood loss, is usually hypochromic and accompanied by leukocytosis. Numerous megakaryocytes are encountered in the bone marrow.

The syndrome of agranulocytosis is rarely accompanied by severe anaemia or thrombocytopenia.



*Plate 30.*—Aplastic anaemia (bone marrow). Paraerythroblasts: 1. Paraerythroblast. 2. Atypical erythroblastic mitosis. 3. Polychromatic erythroblast. 4. Lymphoid cell. 5. "Basket-cell" — remains of a nucleus.

Bone-marrow preparations are usually markedly less cellular, as suggested by the drawing.



*Plate 30.* — Aplastic anaemia (bone marrow). Paracrythroblasts.



Differentiation between the aplastic anaemia and the latent form of myelosis or lymphadenosis may be very difficult. It is not easy to decide if bone-marrow hyperplasia is caused by regenerative activity or if it is a sign of a preleukaemic state. Splenomegalia and enlargement of the peripheral lymph nodes may assist in diagnosis, but these symptoms do not always appear in latent leukaemias. Prolonged clinical observation and repeated biopsies produce decisive data.

## OROYA FEVER

*Carrión's Disease — Tropical Macrocytic Anaemia  
(Bartonellosis)*

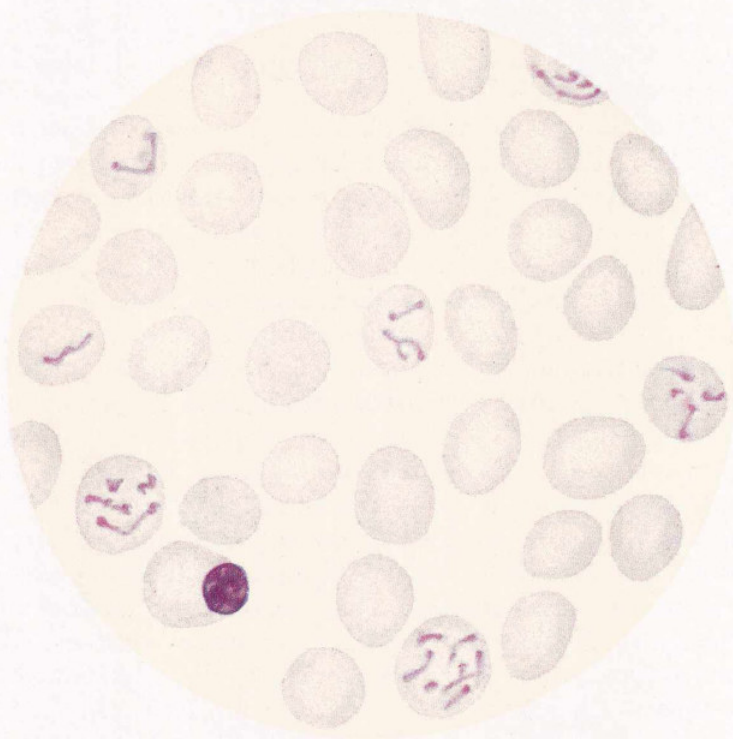
This disease is encountered in endemic foci in the valleys of the West Andes. The most striking clinical feature is the severe haemolytic anaemia, which may lead to a marked drop in erythrocytes, even to below 1,000,000 in the course of a few days. Anaemia is macrocytic, hypochromic or normochromic. The rapid erythrocyte destruction is associated with signs of stimulated haematopoiesis. A microorganism, *Bartonella bacilliformis*, is the causative factor. It is transmitted by certain *Diptera* (*Phlebotomus verrucarum* or *Phlebotomus noguchii*).

In Giemsa-stained smears the bartonellae appear as rods or granules present within the red cells or adhering to the surface of those cells. Some of the organisms resemble rods with club-like thickenings at both ends. They occur singly or in clusters sometimes aggregating in chains, which may be Y- or V-shaped.

The bartonellae are very common in the animal kingdom, in particular in rodents and ruminants. Their presence has also been recorded in dogs. The animals are infected by insects (fleas, lice or ticks). The infection is latent and becomes manifest only after splenectomy. Severe haemolytic anaemia develops after the removal of the spleen and numerous bartonellae may be demonstrated in the blood. The rate of mortality is high.

Among the bartonellae only *Bartonella bacilliformis* is pathogenic in man and is responsible for Carrión's disease. This microorganism may be cultivated in vitro. On the other hand, the cultivation of *B. muris*, inducing severe haemolytic anaemia in splenectomised rats, is extremely difficult. Such cultures have up to date been obtained only by Marmoston-Gotes and Perla, and by Ławkowicz.

The place of bartonellae in bacterial classification is so far a moot point. Their morphological, biological and epidemiological properties support the hypothesis of their being related to the rickettsia group (Ławkowicz 1938, Gieszczykiewicz 1939).



*Plate 31.* — Oroya fever (Bartonellosis). Peripheral blood.



Plate 31 illustrates the peripheral blood from a case of Oroya fever.

Clusters of *Bartonella bacilliformis* may be seen in the red cells. There is also an orthochromatic erythroblast present.

A case of bartonellosis in Poland was described by Aleksandrowicz, Kowalczykowa and Przybylkiewicz (1954).

## ERYTHROCYTOSIS

An increase of the red-cell count above normal may be encountered in various conditions. Among these the most frequent are: oxygen deficiency at high altitudes or in experimental conditions at low barometric pressure, in the newborn (as a sign of anoxia), emphysema or silicosis of the lungs, chronic bronchitis, certain forms of congenital heart disease, mitral stenosis, circulatory insufficiency without detectable heart defects, pulmonary artery induration, Ayerza's disease, cor pulmonale syndrome, deformities of the spine, carbonmonoxide poisoning, hyperthyroidism, certain spleen diseases and loss of blood plasma with subsequent rise in concentration of blood corpuscles.

In the past few years, considerable attention has been given to the appearance of erythrocytosis in persons, not subjected to the action of the factors mentioned above. Such cases do not conform to the pattern of typical polycythaemia. Among other differences, splenomegalia is absent in such cases. These patients are often emotionally unstable, they frequently show a tendency to hypertension, coronary disease and hyperacidity of the gastric juice. The Russian school of physiopathologists has drawn attention to the rôle of the central nervous system in the regulation of the functions of the internal organs. The participation of the central nervous system in the pathogenesis of erythrocytosis should be borne in mind. The influence of the neuro-humoral regulation is generally known and in these conditions it may be easily detected by clinical observation.

## ERYTHRAEMIA — POLYCYTHAEMIA VERA

Among similar syndromes erythraemia (*polycythaemia rubra vera idiopathica*, Vaquez's disease, Vaquez-Osler's disease) or latent, chronic erythromyelosis should be singled out.

In this disease the hyperplasia is not confined to the erythrocytic system, but also takes place in the megakaryocytic and granulocytic series (*panmyelohyperplasia*).

The increase in the red-cell count, up to as much as 10—14 millions per cu.mm. is the outstanding haematological feature. The degree of increase in concentration of blood corpuscles may be estimated by the haematocrit index, or by the determination of blood viscosity. Cases in which the haematocrit index reached a value of 92, have been reported. Lower values ranging from 65 to 75 are, however, more frequently encountered. The mean corpuscular volume is usually normal or slightly decreased. The haemoglobin level is usually high (120—170 %) but is not always in proportion to the increase in the red-cell count. Therefore, the colour index may be less than 1.0. In blood smears, slight anisocytosis excepted, the red cells retain their normal appearance. In some instances, microcytosis may develop. In cases in which the colour index is low, erythrocytes are hypochromic. Sometimes, a few polychromatic cells or stippled erythrocytes may be detected. Erythroblasts are even less frequently encountered. The reticulocyte count may be increased but rarely exceeds 20‰.

The increased activity of the bone marrow is expressed by leukocytosis with a shift to the left, as well as by thrombocytosis. An important diagnostic indication is the increase in the number of basophilic and eosinophilic granulocytes.

The bone marrow is cellular and, according to the majority of investigators, shows signs of hyperplasia in the red-cell series, as well as in the granulopoietic and megakaryocytic systems. The reaction of the megakaryocytic system — an increase in megakaryocytes — should be regarded as significant.

The absence of this symptom, however, does not invalidate the diagnosis.

Numerous polychromatic and orthochromatic erythroblasts (normoblasts) are encountered in bone-marrow smears. Less mature forms are also found, entirely normal in appearance and showing no signs of maturation trouble or degeneration.

A shift to the left in the granulocytic series with increased numbers of promyelocytes and myelocytes is often present.

Plate 32 illustrates the bone marrow from a case of erythraemia. The numerous polychromatic and orthochromatic erythroblasts should be noted. Moreover, numerous granulocytes and one megakaryocyte can be seen.

The following list of symptoms may be helpful in establishing the diagnosis of erythraemia:

1. A specific colour of the patient's skin and mucous membranes (polycythaemic rubor); this may be particularly conspicuous on the skin of the face, and the distal parts of the extremities.



2. Haematologic findings: an increase in red cell, granulocyte and thrombocyte counts.

3. Enlargement of the spleen (a symptom present in about 75 % of patients).

The Russian authors (Ch. Ch. Vlados, B. P. Shvedsky) distinguish four clinical forms of polycythaemia:

- a) an early form without splenomegaly (*polycythaemia simplex*);
- b) Vaquez's form characterised by splenomegaly;
- c) a form characterised by arterial hypertension, the spleen not being enlarged (Gaisböck's disease);
- d) a form characterised by both arterial hypertension and enlargement of the spleen.

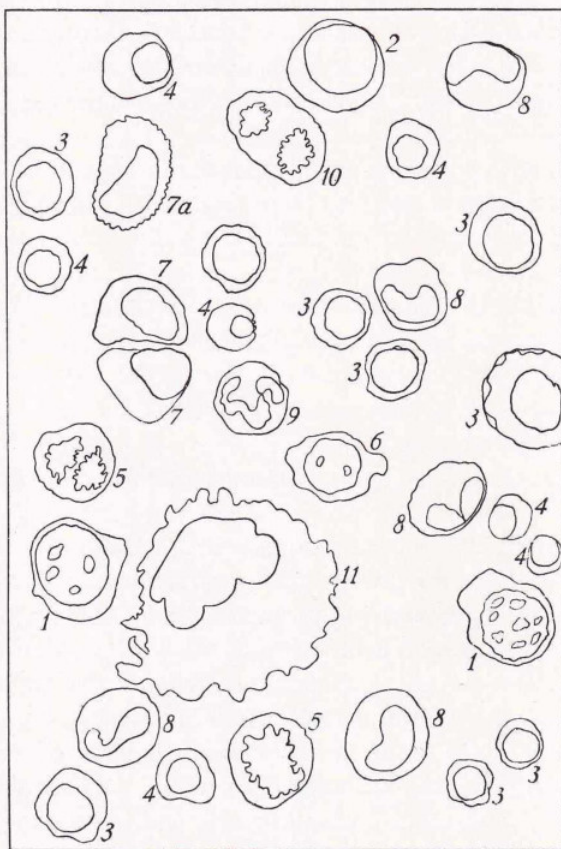


Plate 32. — Polycythaemia vera (bone marrow): 1. Proerythroblast. 2. Basophilic erythroblast. 3. Polychromatic erythroblast. 4. Orthochromatic erythroblast. 5. Erythroblast in mitosis. 6. Myeloblast. 7. Neutrophilic myelocyte. 7a. — eosinophilic myelocyte. 8. Neutrophilic metamyelocyte. 9. Polymorphonuclear neutrophil. 10. Granulocyte in mitosis. 11. Megakaryocyte.

Note: the marked cellularity of the bone marrow and the presence of three mitoses.

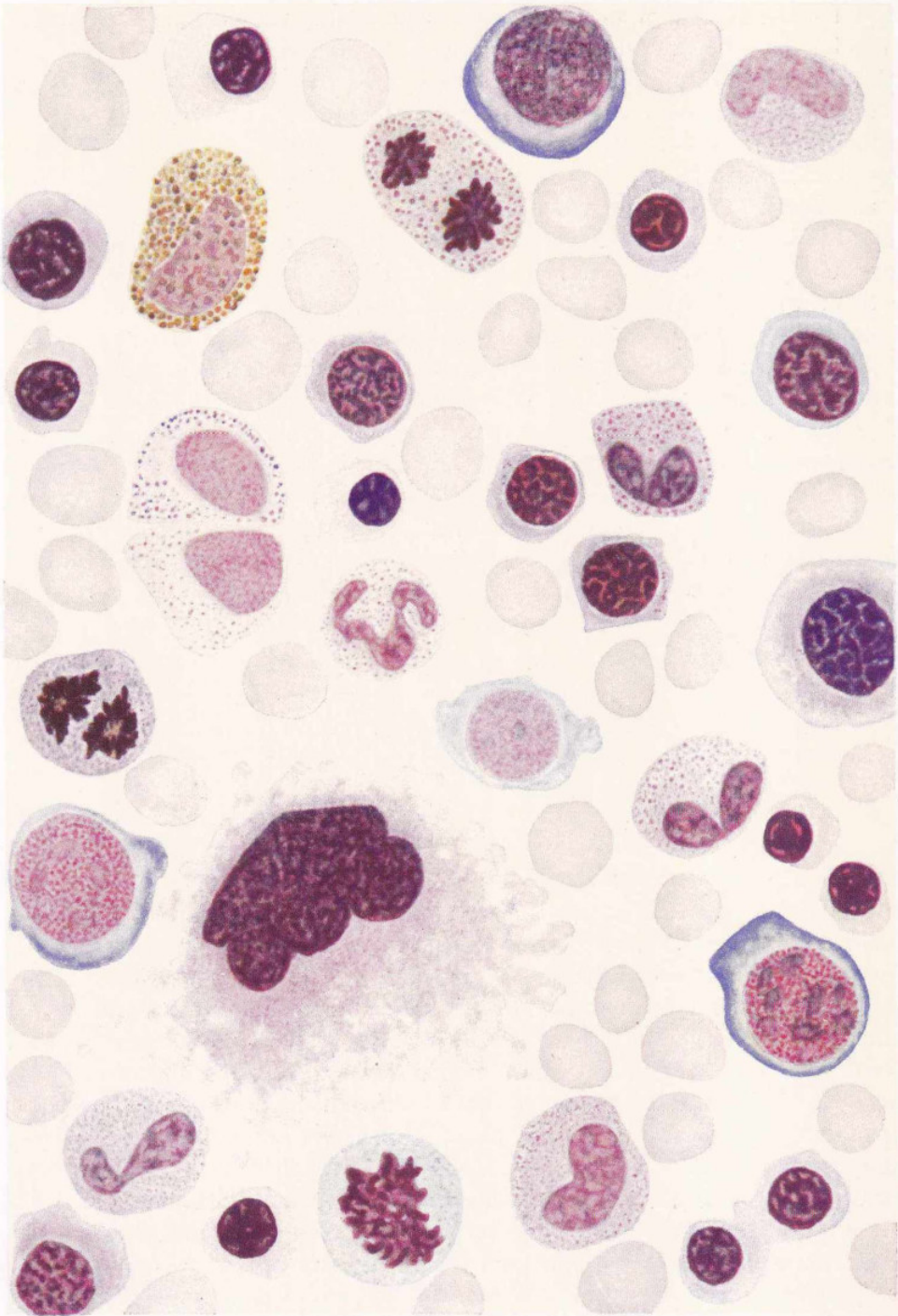


Plate 32.—Polycythaemia vera (bone marrow).



4. Increase in basal metabolic rate (inconstant) and an increase in uric-acid level in the blood.
5. Decrease in serum-iron level.
6. Marked delay in sedimentation rate of the red cells.
7. Cardio-vascular disturbances.
8. Neurologic signs.
9. Changes in the eyegrounds.
10. An increase of total blood volume (this symptom is not specific for polycythaemia vera, it is encountered also in erythrocytosis).

In secondary forms of polycythaemia (viz. in polycythaemia of known aetiology — erythrocytosis) the symptoms of increased blood-forming activity are limited to the erythrocytic series and do not, as in the primary form (polycythaemia vera) comprise all three main myeloid-cell series. In secondary forms, there may even be no evidence of stimulation of erythropoiesis to any great extent.

Secondary forms may be differentiated from primary polycythaemia by means of oxygen-saturation determinations of arterial blood. In polycythaemia vera (Vaquez's disease), the oxygen saturation of arterial blood is normal, in secondary forms it is distinctly decreased.

## ACUTE ERYTHROMYELOSIS IN NEOPLASTIC FORM

### *Proerythroblastoma*

In the literature of the last twenty years numerous clinical observations on abnormal hyperplasia of the blood-forming tissues have been reported. It is now accepted that, in addition to the commonly encountered and generally known myeloses and lymphadenoses, there exist many other varieties of pathological proliferation of the cells of the blood-forming tissues — many varieties of so-called leukaemia.

Di Guglielmo was the first to report the occurrence of acute uncontrolled proliferation of the erythrocytic series — a disease which he termed *erythraemia acuta* (acute erythromyelosis). A chronic variety of erythromyelosis with a course similar to that of chronic leukaemia has been since described. Both the acute and chronic varieties are always fatal. The disease is characterised by anaemia and atypical erythroblastic proliferation with metaplasia in the liver and spleen, which leads to the enlargement of these organs. There may be present in the peripheral blood from 5 to 122 erythroblasts per hundred leukocytes. More mature erythroblasts preponderate in the chronic variety, and in the acute type — immature forms.

Erythroblasts and their stem-cells may, like myeloblasts in the course of acute myelosis, reveal a wide range of abnormalities in the course of acute di Guglielmo's disease. Di Guglielmo considers these changes as a sign of deep dysplastic processes taking place in the erythropoietic tissue (paraerythroblasts). In none of the cases described by di Guglielmo and other authors were the deviations from normal morphology so marked as to make the recognition of erythroblasts as such impossible. Such profound changes are, however, encountered in cases in which the erythropoietic tissue undergoes profound degenerative changes, resembling neoplastic transformation.

One of the present authors (together with N. Włodarski and J. Gewont) observed a 52-years old female with orthochromatic anaemia, refractory to liver therapy, granulocytopenia, thrombocytopenia and achlorhydria. The spleen was not palpable.

About 50 % of the nucleated bone-marrow elements consisted of atypical cells, extremely varied in appearance. The outstanding features of these cells were: vacuolation of both nucleus and cytoplasm and a shift in the ratio of nucleus to cytoplasm in favour of the cytoplasm. Such features are generally known to be characteristic of neoplastic cells. A detailed examination of bone-marrow smears revealed a decrease in granulocytes and absence of megakaryocytes. Signs of erythroblastic proliferation were present. Some of the erythroblasts showed vacuoles in the cytoplasm or in the nucleus. A few typical proerythroblasts with vacuoles in the cytoplasm were encountered together with vacuolated polychromatic or orthochromatic erythrocytes. Degenerative changes were found also in erythroblasts in mitosis (plate 33).

The findings described above were taken to be evidence of a genetical relationship between the profoundly changed, abnormal cells and degenerated erythroblasts, as also of pathological transformation of the erythropoietic tissue. Taking the myelogram into account, it was assumed that the atypical cells correspond to pathologically changed proerythroblasts.

These facts suggest the presence of an anarchic, uncontrolled proliferation concerning in particular the erythropoietic tissue, accompanied by dysplastic changes, abnormal mitoses and arrested maturation. Such a phenomenon affecting a single-cell type is characteristic of neoplastic growth.

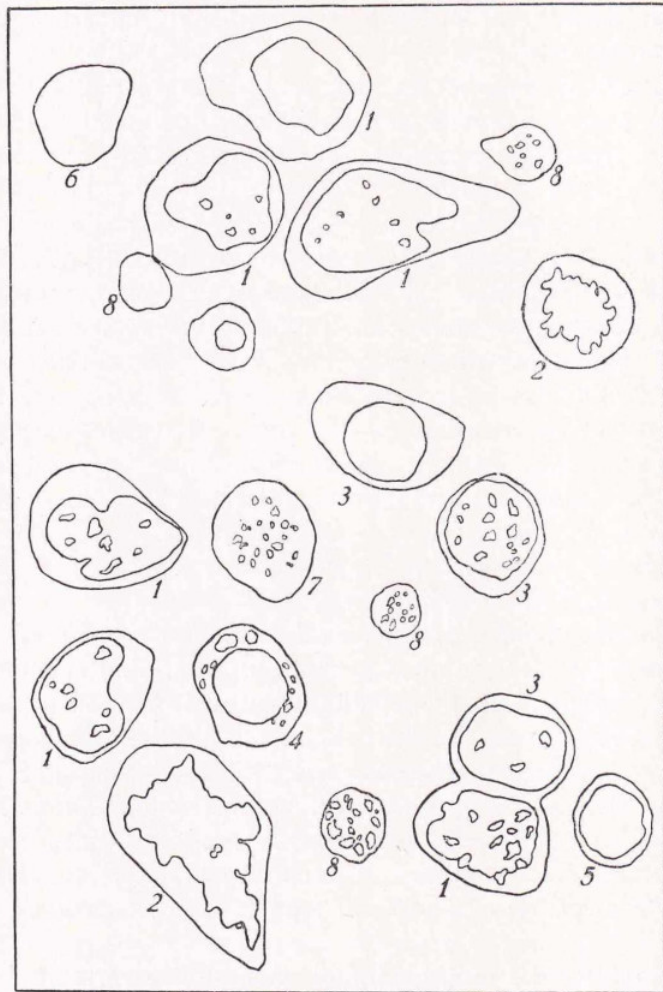
By contrast with acute di Guglielmo's disease, there were, in the case described above, only a few erythroblasts present in the peripheral blood. Moreover, no signs of erythroblastic metaplasia were found in the spleen, liver and lymph nodes.

The morphological features of atypical cells, symptoms of widespread osteolytic process conform, together with the rapid and fatal course of

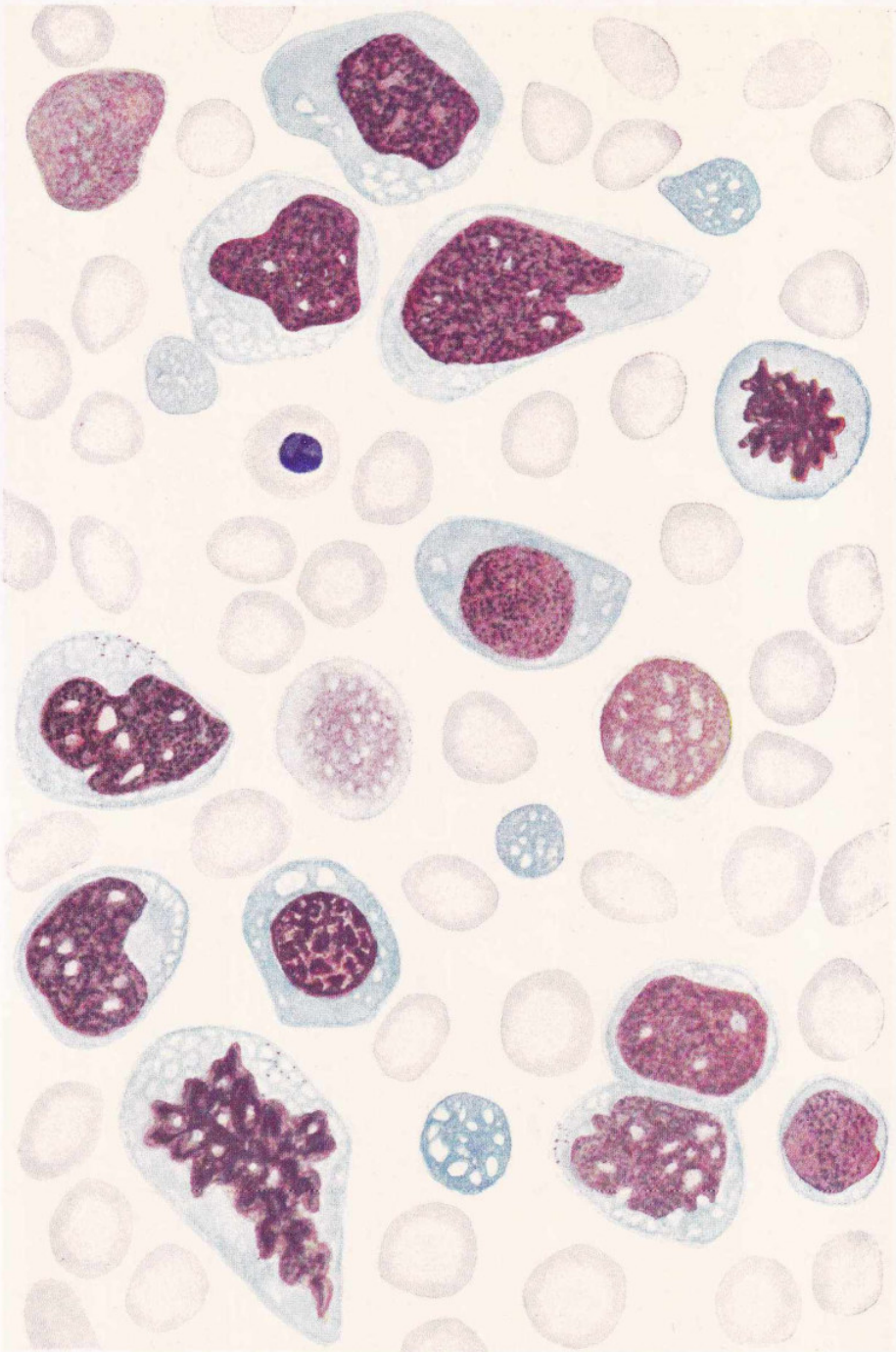


the disease, suggested the diagnosis of a neoplastic proliferation of the erythropoietic tissue (*proerythroblastoma*). During the terminal phase of the disease the presence of atypical proerythroblasts has been also demonstrated in peripheral blood smears.

A similar phenomenon of release of tumour cells into the peripheral blood stream is known to occur in the course of chloroma, plasmocytoma or lymphosarcoma.



*Plate 33.* — Proerythroblastoma (bone marrow): 1. Atypical vacuolated cell. 2. Atypical cell in mitosis. 3. Atypical proerythroblast. 4. Vacuolated basophilic erythroblast. 5. Atypical basophilic erythroblast. 6. Basket-cell (nuclear remnant). 7. Atypical orthochromatic erythroblast with a blurred, indistinct nucleus, marked vacuolation and indistinct cytoplasm. 8. Vacuolated basophilic erythrocytes.



*Plate 33.* — Proerythroblastoma (bone marrow).





### CHAPTER III

## THE GRANULOCYTIC SYSTEM

### THE DEVELOPMENTAL CYCLE OF GRANULOCYTES

The stem-cell of the granulocytic series is usually called myeloblast (granulocytoblast). It is a cell from 12 to 20  $\mu$  in diameter, with a large, oval or round nucleus. By comparison with proerythroblasts, the nuclear structure is much more finer. The nucleus contains from 2 to 6 sky-blue vesicular nucleoli (Plate 34).

In some instances, the outline of the nucleus is irregular and may be indented. The myeloblasts differ in this respect from proerythroblasts, in which the nuclei are usually round or oval. The agranular cytoplasm stains light blue or greyish-blue. A narrow border, darker in shade, may usually be found on the periphery of the cell. The cytoplasm of the stem-cells of the red-cell series stains more deeply. More important, however, in the recognition of myeloblasts, is the absence of granules in the cytoplasm. Nevertheless, in the course of acute myelosis, the myeloblasts may reveal the presence of cytoplasmatic inclusions — short, azurophilic rods called Auer's bodies.

Peroxidase or oxidase reactions are in myeloblasts usually negative. In more mature forms, however, a positive result may be obtained. The presence of peroxidase-positive granules in myeloblasts is inconstant and, in fact, rarely encountered. On the other hand, small sudanophilic granula, as well as a faint, diffuse sudanophilia may often be demonstrated in the cytoplasm of myeloblast by the use of Sudan black B. The few myeloblasts present in normal bone-marrow smears amount usually to from 1—2% of all nucleated elements.

The morphologic features of myeloblasts may, in pathologic conditions, reveal marked changes. Atypical small myeloblasts (*micromyeloblasts*) — cells misleading by similarity to lymphocytes — or, large myeloblasts with abnormal, lobulated nuclei may appear in bone marrow and peripheral blood smears.



The next, subsequent, developmental stage of granulocytes is the promyelocyte (progranulocyte, myelocyte A).

Promyelocytes are cells larger than their precursors — the myeloblasts. Dimensions may, however, vary within wide limits. The chromatin forms a reticular, fine structure and is evenly distributed throughout the nucleus. From one to four nucleoli may, in some instances, be seen. The cytoplasm is abundant and gradually becomes acidophilic. “Young” promyelocytes are basophilic — in later stages the cytoplasm acquires a pink hue. The presence of a large azurophilic granulation in the cytoplasm is the most striking feature of the promyelocyte and gives it its specific appearance.

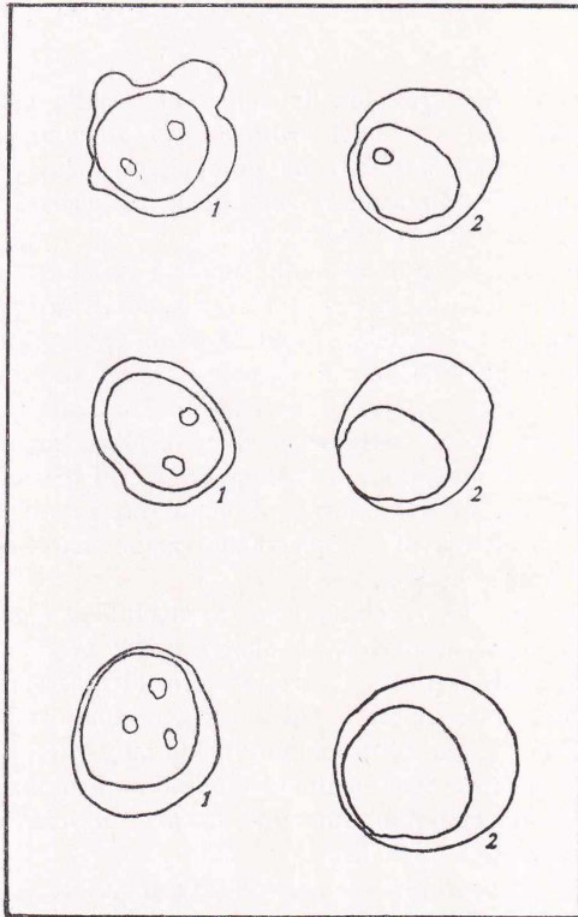
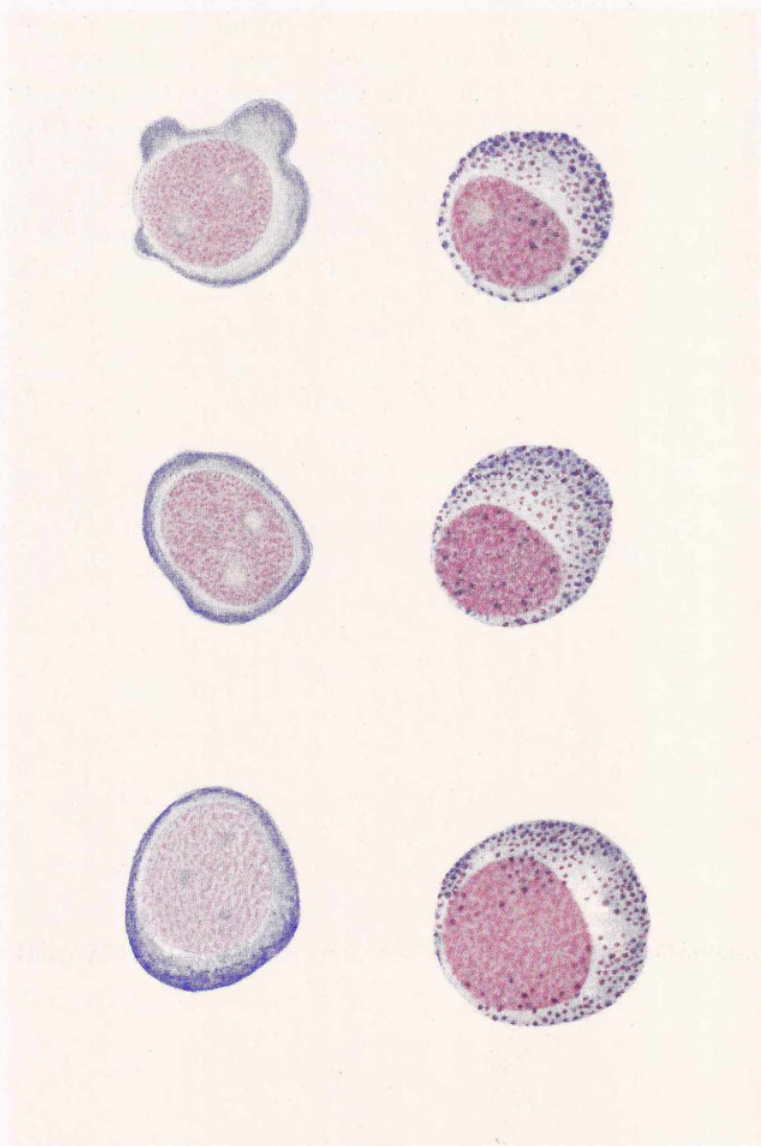


Plate 34. — The developmental cycle of granulocytes: 1. Myeloblasts. 2. Promyelocytes.

Note: nucleoli in all myeloblasts and in one of the promyelocytes; the characteristic coarse azurophilic granulation of promyelocytes.



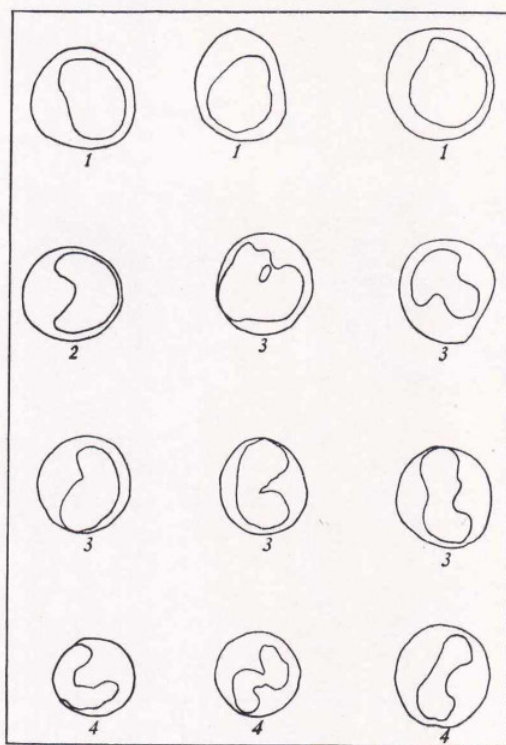
*Plate 34.*—The developmental cycle of granulocytes.



As maturation progresses, the nuclear chromatin becomes more coarse. The shape of the nucleus remains round or oval. In other instances, however, the nucleus may be kidney-shaped or sausage-shaped and is eccentrically located. At this stage, the nucleoli usually disappear. In this phase of development the cell is termed myelocyte. The cytoplasm undergoes striking changes, the cytoplasm granulation becomes differentiated. The azurophilic granules disappear and are replaced by one of the three varieties of "specific granules" neutrophilic, eosinophilic or basophilic. The kind of granules present — neutrophilic, eosinophilic<sup>1</sup>, basophilic enable the respective myelocytes to be identified.

Intermediate stages are often encountered between the promyelocyte and myelocyte. Cells, containing azurophilic granulations and at the same

<sup>1</sup> J. Aleksandrowicz claims that eosinophilic granulocytes originate directly from mesenchymic cells, and do not pass through the stage of common granulocyte stem-cells. In accordance with this view this author terms eosinophilic granulocytes — mesenchymocytes.



*Plate 35.* — The developmental cycle of neutrophilic granulocytes: 1. Myelocyte. 2. An intermediate form between a myelocyte and a metamyelocyte. 3. Metamyelocyte. 4. Staff cell.

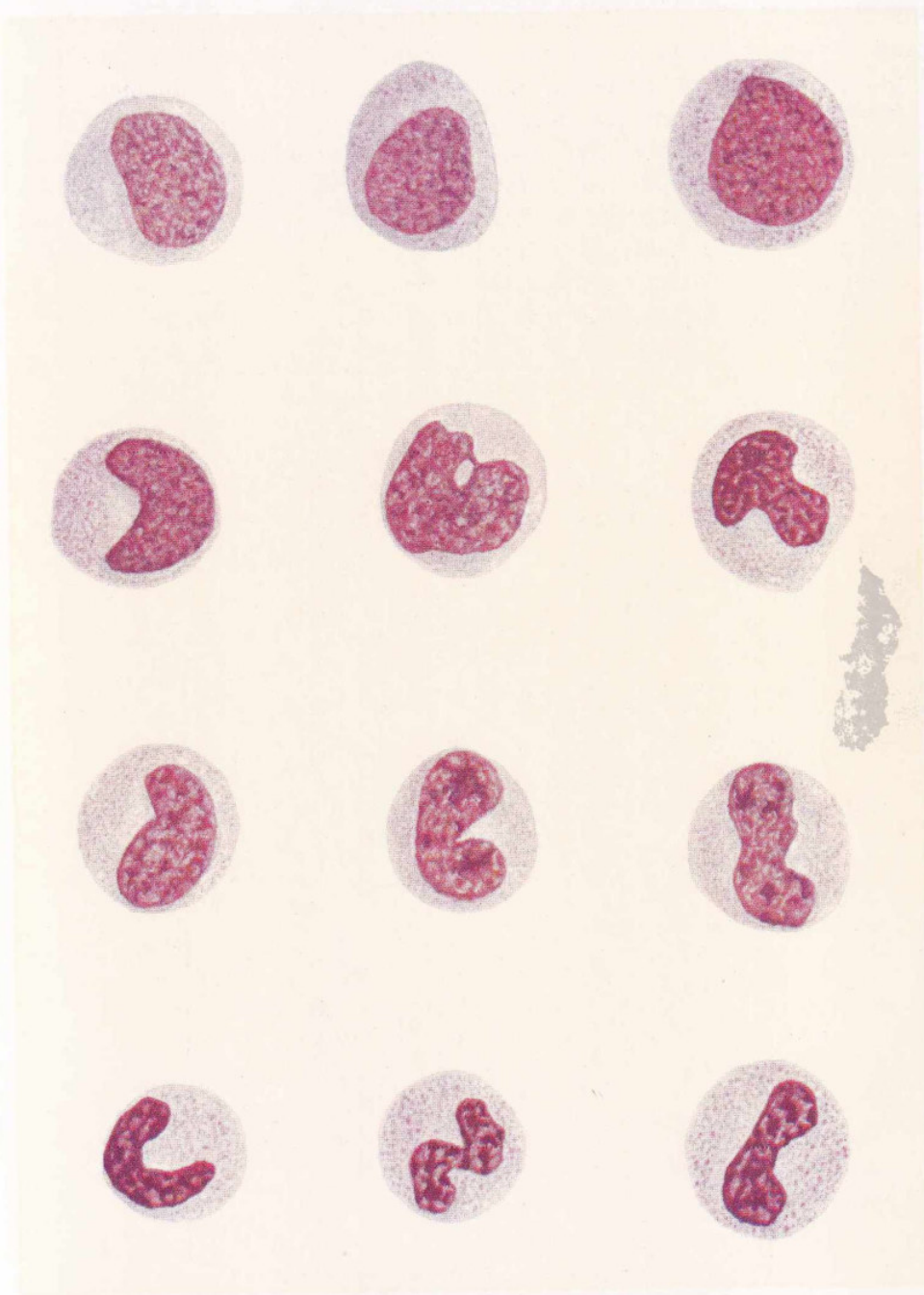


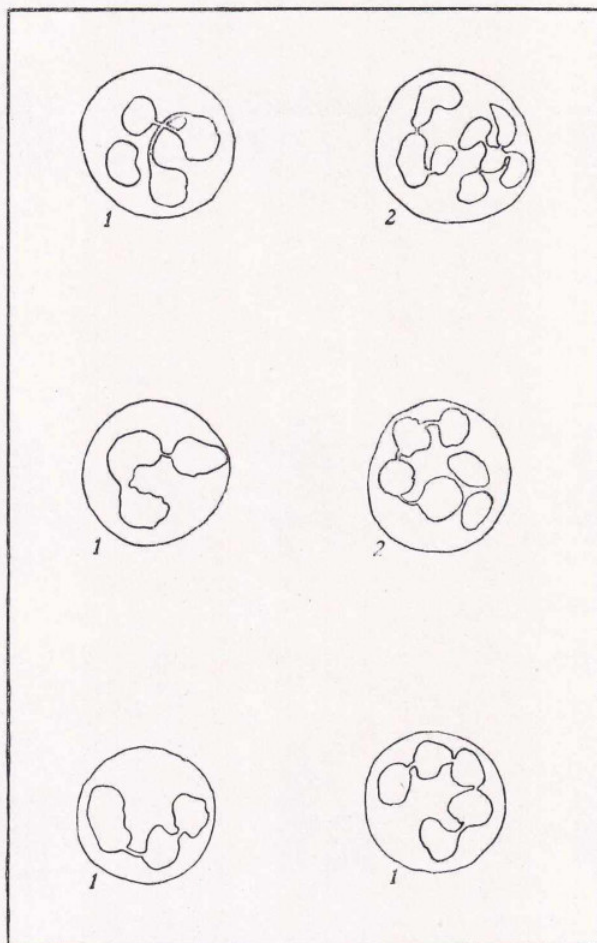
Plate 35. — The developmental cycle of neutrophilic granulocytes.



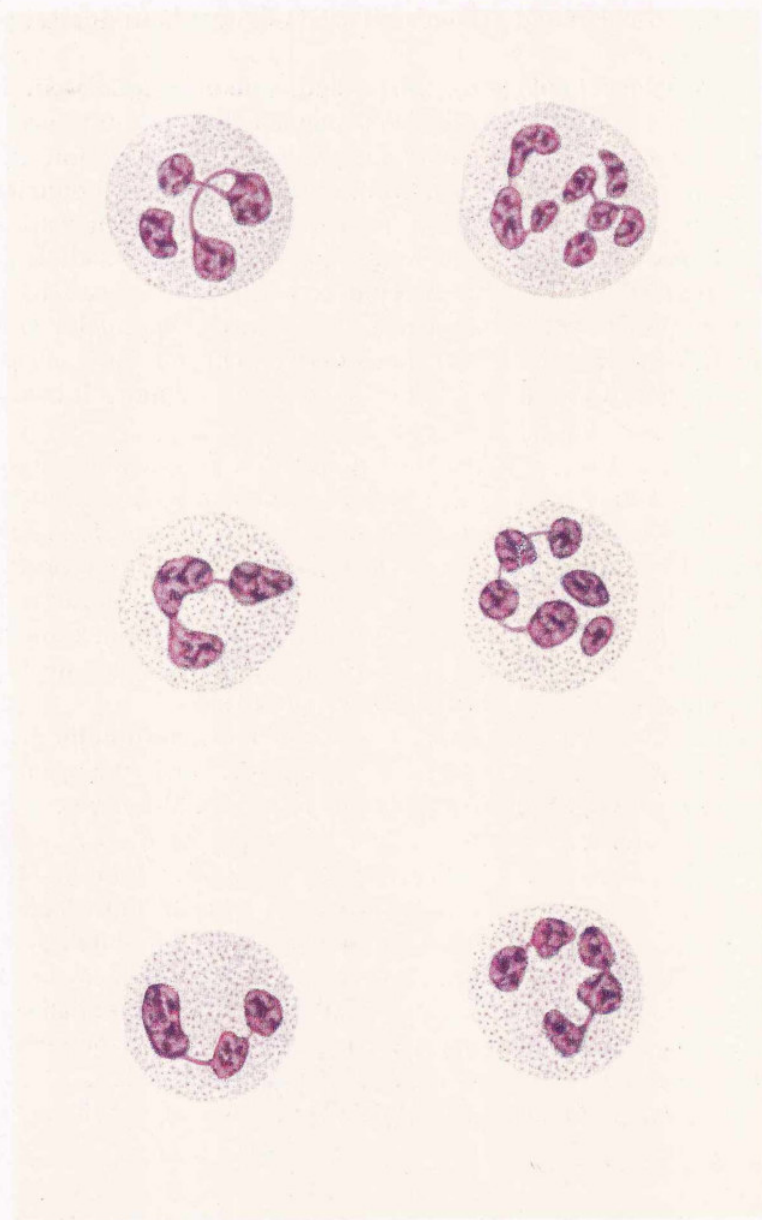
time one of the "specific" granulations (neutrophilic, eosinophilic or basophilic), may be found in the bone marrow. Only cells with distinctly differentiated granules should be considered as mature myelocytes.

The next developmental stage, the metamyelocyte ("juvenile form") has a markedly different nucleus. Nuclear chromatin is coarse, although it retains certain features of immaturity. The nucleus resembles a thick, curved rod (horse shoe or kidney-shaped). The granulation is definitely neutrophilic, basophilic or eosinophilic.

The nucleus acquires a more slender shape, its chromatin clumps together, giving it a pycnotic appearance. Such cells are termed staff cells



*Plate 36.* — The developmental cycle of neutrophilic granulocytes. Mature forms: 1. Polymorphonuclear ("segmented neutrophil"). 2. Granulocyte with a hypersegmented (multi-segmented) nucleus. Such cells are, when large, termed macropolycytes.



*Plate 36.* — The developmental cycle of neutrophilic granulocytes.  
Mature forms.



(band-forms or stabs) and are encountered commonly in normal peripheral blood smears.

In fully mature granulocytes, the nucleus becomes lobulated. The lobes are joined by chromatin strands. As long as these strands are thick the granulocyte is called a staff cell. With progressing indentation, the strands become thin, thread-like. Such cells are termed polymorphonuclear granulocytes (segmented granulocytes). There are usually from 2 to 4 nuclear lobes. Granulocytes with bilobed nuclei and granulocytes with three nuclear lobes are the most frequently encountered in the peripheral blood (35% and 41% respectively). Granulocytes with four lobed nuclei are less numerous (17%). Granulocytes called macropolycytes or multisegmented granulocytes (Plate 36), with nuclei possessing 5 or more lobes, may also be present (1 to 1.9%).

The number of nuclear lobes should be taken into account in differential counting. The term "shift to the left" in granulocytes describes the condition in which there is an increase in staff cells exceeding 5%. The expression "shift to the right" implies in granulocytes, that there is an increase in multisegmented granulocytes. Most laboratories, however, attach insufficient importance to the occurrence of this phenomenon. Nevertheless, the presence of the "shift to the right" in granulocytes may be helpful in recognition of certain haematologic syndromes.

The functions and biological properties of the neutrophilic granulocytes comprise: amoeboid movement, phagocytosis, and the production of proteolytic, lipolytic, as well as glycolytic enzymes. Moreover, the presence of oxidases (concerned in the oxygen transfer) and of peroxidases (concerned in the liberation of oxygen from peroxides and the transfer of oxygen to readily oxygenated substances such as benzidine) may be demonstrated in the cytoplasm of neutrophilic and eosinophilic granulocytes, as well as, in some instances, in basophilic granulocytes. On the basis of these properties, cytochemical tests for the differentiation between granulocytes and other leukocytes, were elaborated. In general, granulocytes are oxidase-positive, as well as peroxidase-positive. It would seem that the positive reaction is due to the presence of specific granulation.

#### ACIDOPHILIC GRANULOCYTES (EOSINOPHILIC GRANULOCYTES, EOSINOPHILS)

The presence of eosinophilic granules may, in some instances, be demonstrated even in the promyelocyte. Such a cell may be seen on Plate 37. It has a distinctly basophilic cytoplasm containing numerous azurophilic and several eosinophilic granules.

As differentiation proceeds, the coarse eosinophilic granules become more numerous. The nucleus is oval, round or kidney-shaped and, usually, excentric (eosinophilic myelocyte). Some greyish-blue or dark blue granules are also present in the cytoplasm. Eosinophilic myelocytes, with a preponderance of such granules and only a few eosinophilic granules, may be encountered. The dark basophilic granules of eosinophils should be not confused with the specific basophilic granulation. The latter, by contrast with the former, stain metachromatically with toluidine blue in alcoholic solutions.

Gradually, the dark granulation is replaced by the specific eosinophilic granules. In some instances, however, the dark granules remain for a long time in the cytoplasm of more mature cells. The maturation cycle is similar to that found in neutrophilic granulocytes. Eosinophilic metamyelocytes, eosinophilic staff cells and segmented eosinophils can be distinguished. Multisegmented eosinophils (eosinophilic macropolycytes) are only rarely encountered. Eosinophils with a bilobed nucleus are the most frequent in their occurrence. The shape of the nucleus institutes the most characteristic feature of the cell. Its lobes are symmetrical, oval or pear-shaped and connected by a thin, thread-like chromatin bridge.

The chromatin is less abundant in the nuclei of mature eosinophilic granulocytes and of finer texture than in neutrophilic granulocytes. The granules usually completely fill the cytoplasm, which is, in consequence, invisible. The granules are coarse and strongly refractile. They may be compared to grains of caviare. In colour, they are pink or brownish-red (in Wright's stain, bright yellow).

The peroxidase and oxidase reactions give positive results. In Sudan black B preparations some granules are uniformly stained or possess a sudanophilic lipidal outer layer around an inner sudanophobic core. There are complex proteids in eosinophilic granules along with iron and phosphorus. It has been also shown that eosinophils contain histamine. It is generally held that eosinophils are concerned in disintegration and removal of body proteins, as well as in detoxication of foreign proteins.

In normal conditions, the number of eosinophils amounts from 2 to 4%.

Eosinophilic reactions (increase in eosinophils) are frequently observed in allergic or anaphylactoid states.

Plate 37 illustrates the different developmental stages of eosinophils.

Plate 39 illustrates eosinophilia in a bone-marrow smear. Numerous eosinophils at different developmental stages may be seen. The plasmocytic reaction, accompanying certain eosinophilic syndromes (Tempka), should be noted.

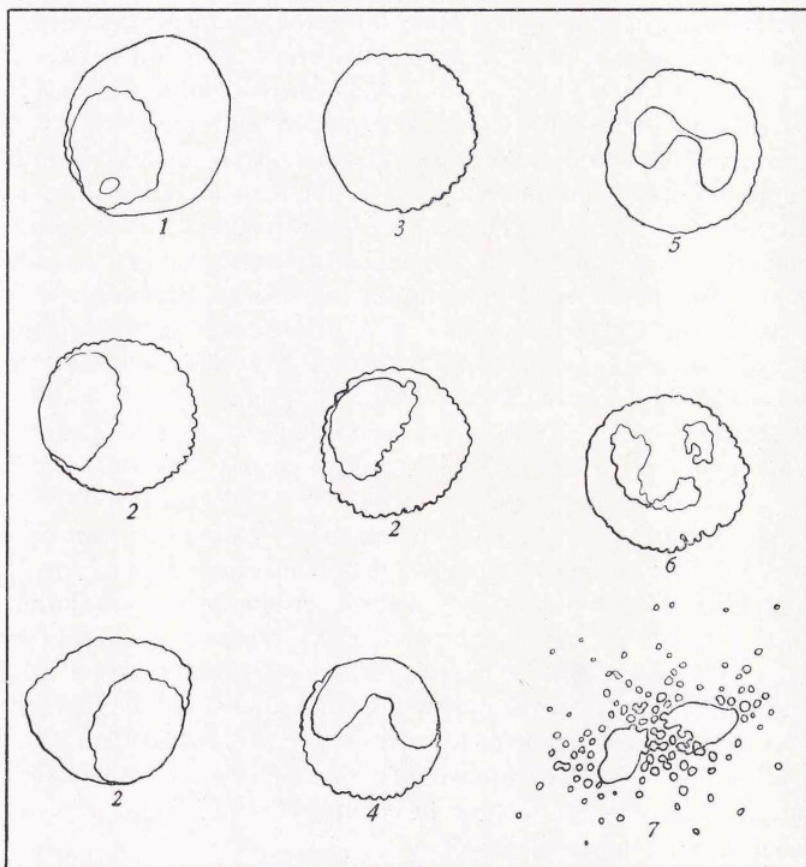
In cases of eosinophilia in the peripheral blood, several possibilities should be considered. Eosinophilia may appear as a result of an allergic



reaction. This may be attributed to an increased sensibility to foreign proteins or other antigens, as well as to decomposition products of body proteins. Among the syndromes accompanied by eosinophilia, the following are the most important from clinical point of view:

1. Allergic disorders, such as bronchial asthma, hay-fever, urticaria, angioneurotic oedema.

2. Parasitic infestation, in particular trichinosis and echinococcus disease; in intestinal infestation (for example oxyuriasis) eosinophilia is less constant.



*Plate 37.* — The developmental cycle of eosinophilic granulocytes: 1. Eosinophilic promyelocyte. 2. Eosinophilic myelocyte. 3. Eosinophilic metamyelocyte. 4. Eosinophilic staff cell. 5. Mature eosinophil with a typical bilobed nucleus. 6. Eosinophil with a hypersegmented nucleus. 7. A desintegrating eosinophil.

*Note:* among the specific eosinophilic granulation the dark granules present in the cytoplasm of several cells. Slight differences in the technique of staining may cause the eosinophilic granules to vary in colour—from darkish pink to yellowish-brown.

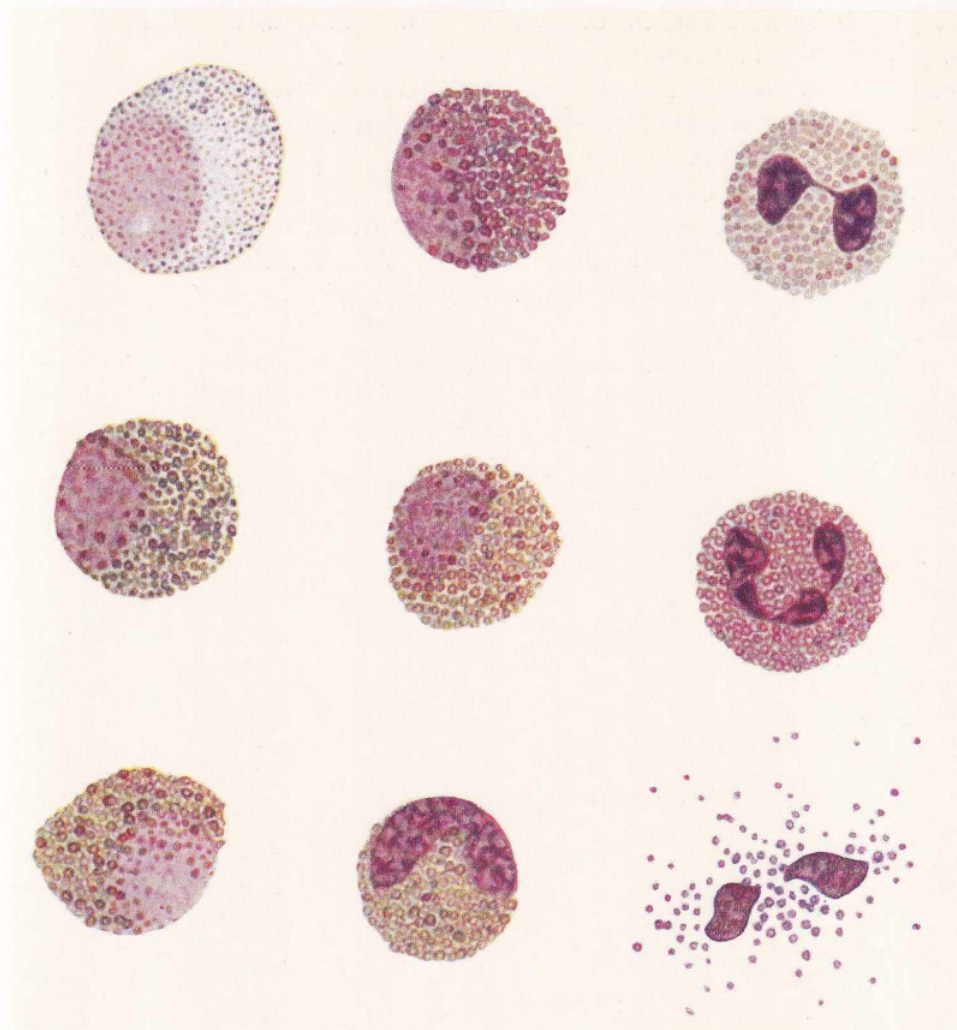


Plate 37. — The developmental cycle of eosinophilic granulocytes.



3. Certain skin diseases connected with infection or skin-irritation (*pemphigus*, *dermatitis herpetiformis*, *scabies*, *eczema*, *prurigo*).

4. Certain acute infectious diseases (scarlet-fever, rheumatoid diseases); moreover, during the initial period of gonorrhoea, local eosinophilia may be found in the pus.

5. Loeffler's syndrome and tropical eosinophilia.

6. Certain diseases of the haematopoietic system. Chronic eosinophilic myelosis, polycythaemia, Addison-Biermer's disease, Hodgkin's disease<sup>1</sup> (malignant lymphogranuloma) — inconstant symptom.

<sup>1</sup> In some instances, a marked eosinophilia may be the only symptom of Hodgkin's disease. In a case of Hodgkin's lymphogranuloma, observed by one of the authors, eosinophilia preceded other symptoms and lymph-node enlargement by many months.

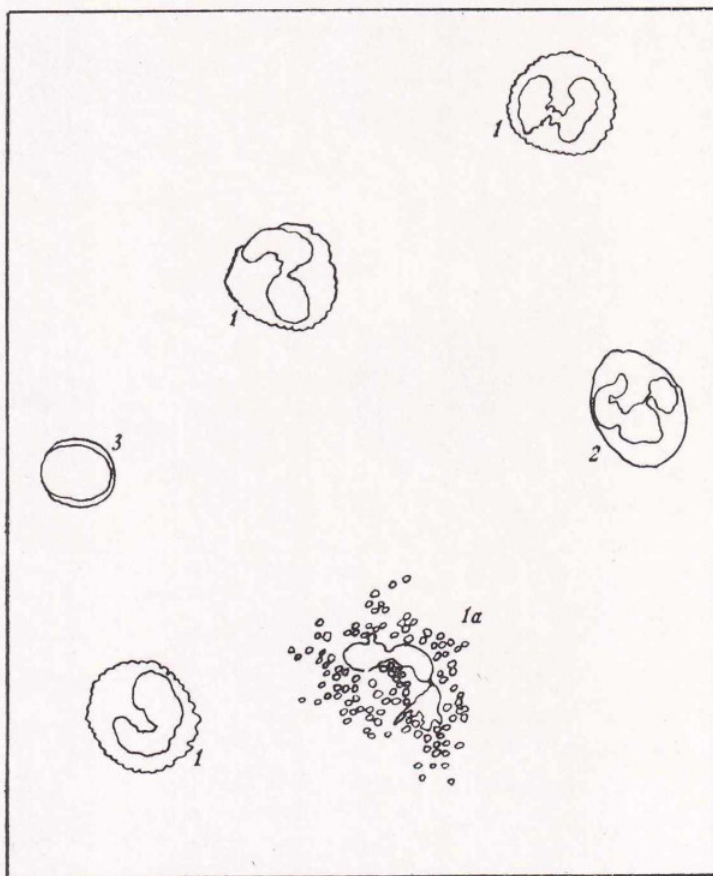
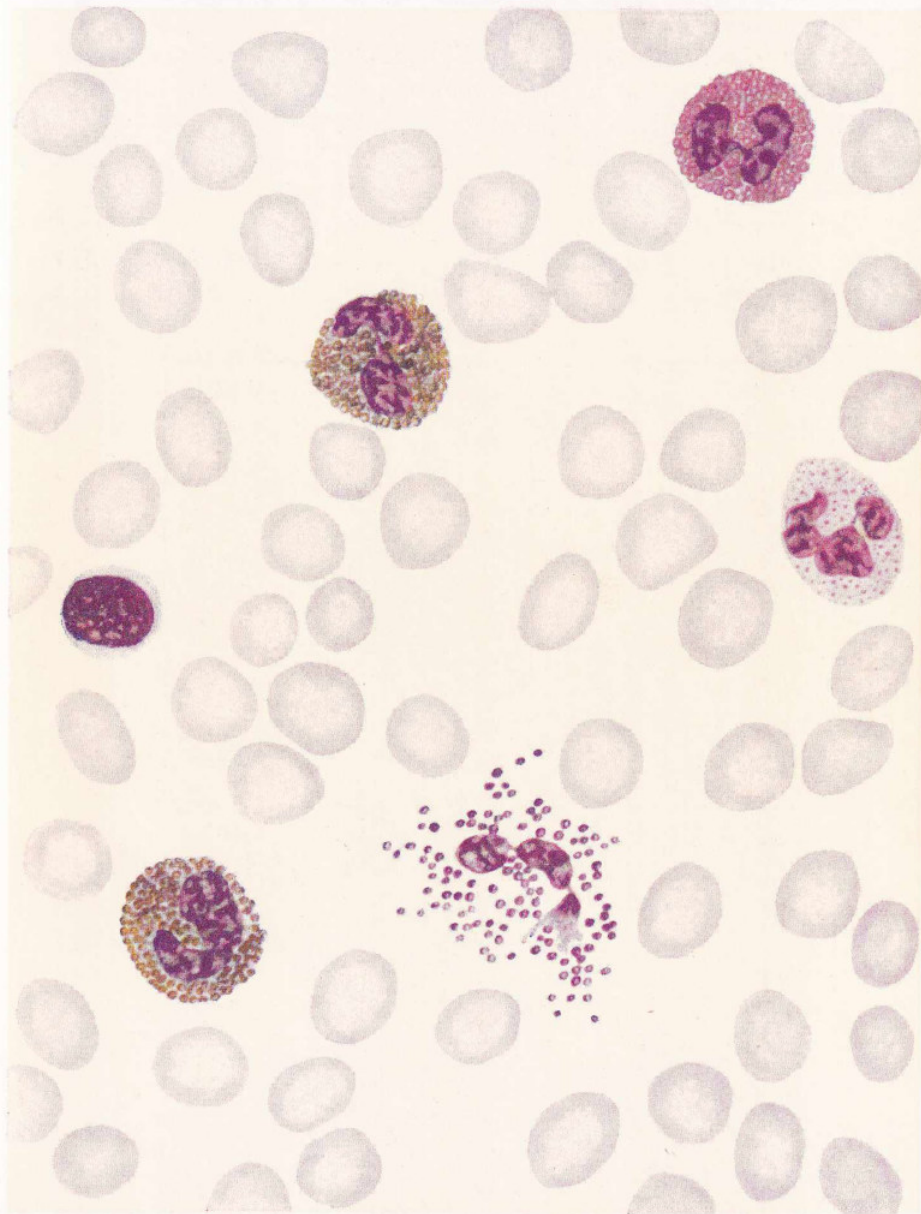


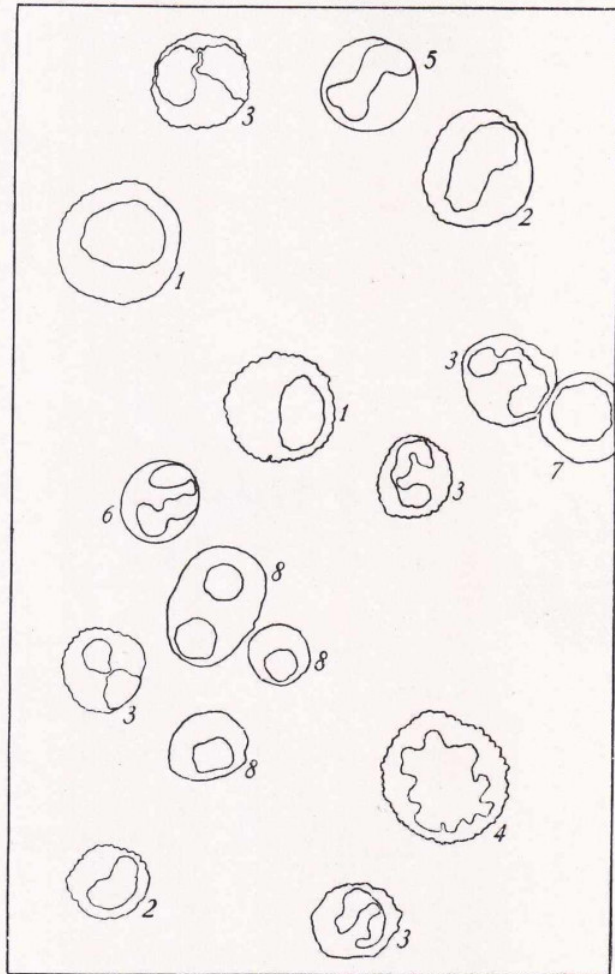
Plate 38. — Eosinophilia in peripheral blood: 1. Mature eosinophils. 1a. — damaged eosinophil. 2. Neutrophilic granulocyte. 3. Lymphocyte.



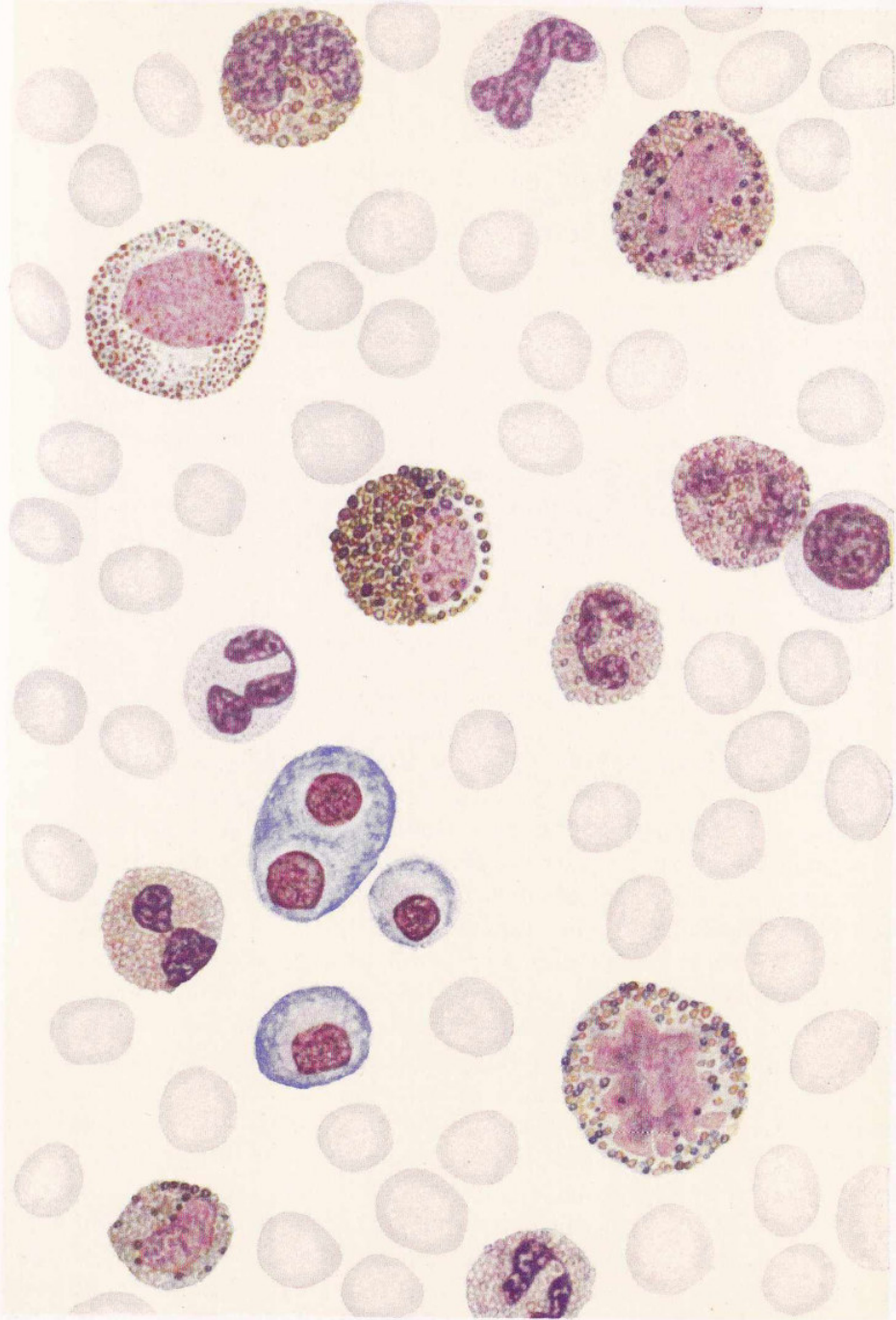
*Plate 38.* — Eosinophilia in peripheral blood.



7. After splenectomy.
8. Eosinophilia may be the initial symptom of irradiation injury (by X-rays or radium) to the haematopoietic system.
9. Marked eosinophilia of obscure origin in the peripheral blood or the bone marrow may considerably precede the development of a chronic neutrophilic myelosis (myelogenous leukaemia).
10. Constitutional and familial eosinophilia.
11. Syndrome of persistent eosinophilia, splenomegaly and hyperleukocytosis.



*Plate 39.* — Bone-marrow eosinophilia: 1. Eosinophilic myelocyte. 2. Eosinophilic metamyelocyte. 3. Mature eosinophils. 4. Eosinophil in mitosis. 5. Neutrophilic metamyelocyte. 6. Mature neutrophils. 7. Polychromatic erythroblast. 8. Plasmocyte.



*Plate 39.* — Bone-marrow eosinophilia.



12. After the administration of certain drugs (liver extracts, barbiturates — in particular nirvanol — digitalis, strophanthine, salicylates, arsene, camphor, mercury salts, phosphorus, pilocarpine, streptomycin and other antibiotics).

13. Orach poisoning.

14. Neoplastic diseases (inconstant symptom).

15. *Periarteriitis nodosa*.

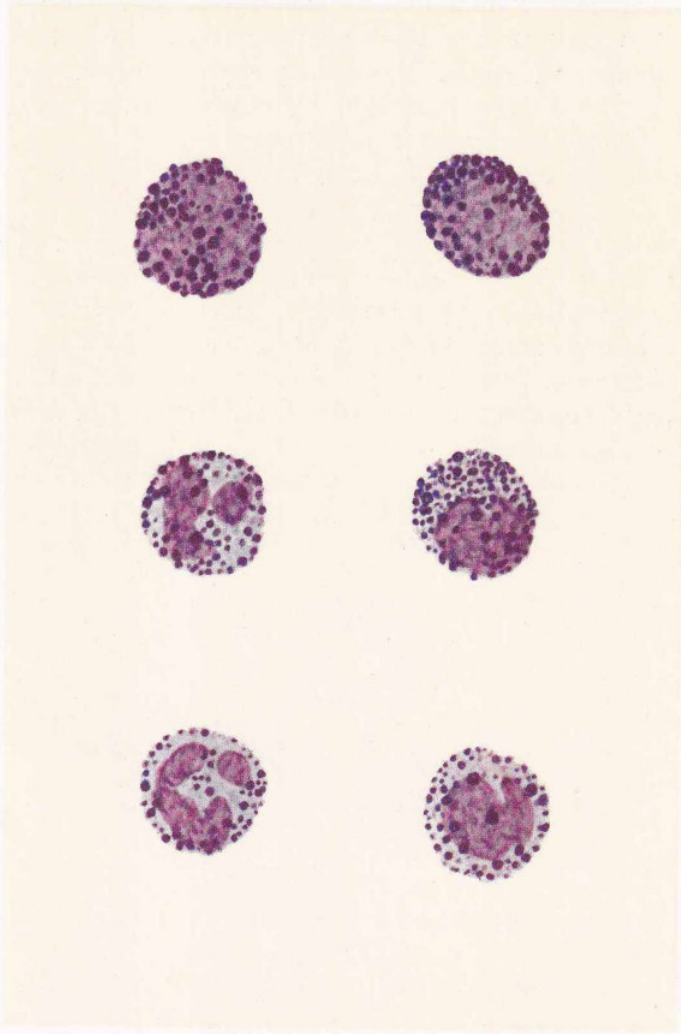
16. Ulcerative colitis.

Eosinophilia in the peripheral blood is usually accompanied by eosinophilia in the bone marrow. Nevertheless, marked eosinophilia in the bone marrow may occur without an increase of eosinophils in the peripheral blood. The occurrence of local tissular eosinophilia is generally known. Such eosinophilia may be present in a wide variety of disorders. Local eosinophilia in tuberculous pleural effusions, in eosinophilic granuloma and in granulomatous lymph nodes should be noted.

The above examples demonstrate, that the absence of eosinophilia in the peripheral blood does not rule out the presence of an allergic reaction.

### BASOPHILIC GRANULOCYTES (BASOPHILS)

The basophilic granulocytes are the least studied among blood elements, and their rôle, in consequence, is not definitely known. In normal conditions, the percentage of such granulocytes in peripheral blood smears amounts to approximately 0.5 %. Most investigators have classified them as myelocytes and mature forms. Nevertheless basophilic cells with kidney-shaped nuclei, similar to the nucleus of metamyelocytes, may be found in smears. The nucleus of basophils contains less chromatin than the nuclei of other granulocytes, and therefore stains less intensively. Furthermore, the nuclei of basophils are of loose structure. The cytoplasm is faintly acidophilic; it contains a variable amount of coarse dark violet or almost black granules of different sizes. These granules are similar to blots of China ink. They dissolve readily in water, and may be dissolved during staining, leaving empty spaces in the cytoplasm. Such "vacuoles", irregular, "bizarre" nuclear outlines, and the leptochromatic character of the nucleus, facilitate the recognition of basophils. Poor visibility of the nucleus, on the other hand, often makes it impossible to establish the developmental stage reached by the cell. It stains lightly and its outlines may be obscured by superimposed granulation, which often fills the whole cell. Additional difficulties arise out of the irregularity of nuclear shape. It may be inferred that basophils exhibit individual maturation phenomena, which cannot be classified in a manner similar to those observed in neutro- or eosinophils. In some instances, basophils



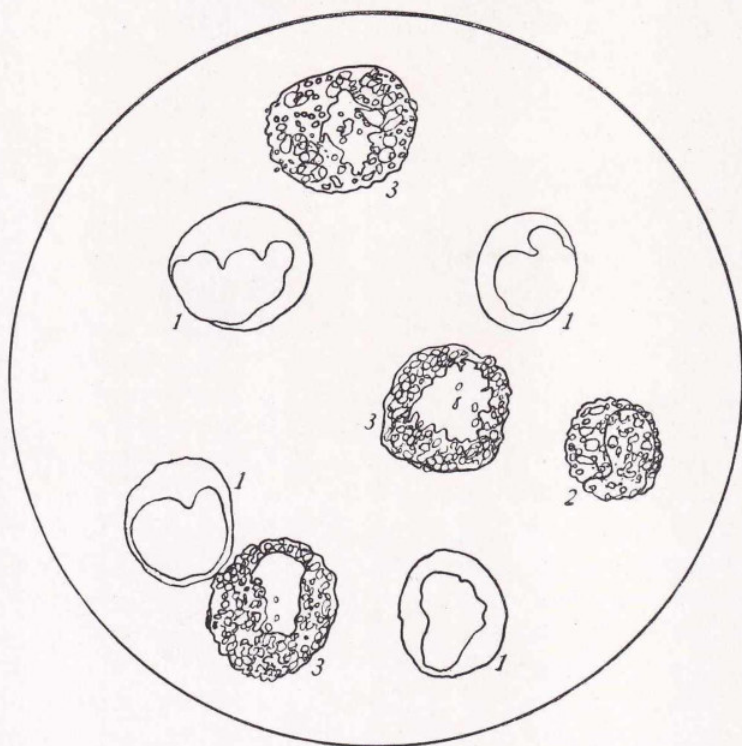
*Plate 40.* — Basophilic granulocytes (May-Grünwald-Giemsa stain).

*Note:* the "empty", round spaces in the cytoplasm, corresponding to dissolved granulations.

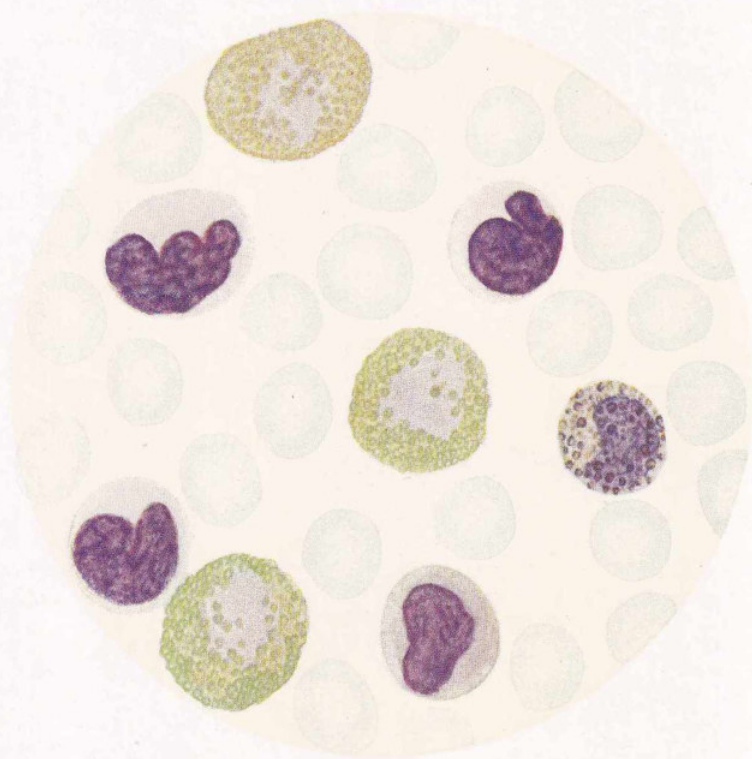


with kidney-shaped or round nuclei may be encountered in peripheral blood smears, whilst eosinophils and neutrophils do not demonstrate any signs of immaturity. Such basophilic granulocytes should, on purely morphological grounds, be classified as myelo- or metamyelocytes. It is, however, difficult to explain in these cases the presence of immature granulocytes in peripheral blood, limited to the basophilic variety only.

The shape of nuclei of basophils may be readily observed in peroxidase preparations. The basophilic granules are peroxidase-negative (Plate 41). Only in rare instances are solitary, minute peroxidase-positive granules present in basophils. The nuclear shape becomes "unmasked" and is clearly visible. Some nuclei resemble those seen in myelocytes or metamyelocytes. Other ones are irregularly lobulated, in a manner differing markedly from the characteristic segmentation of the nuclei of neutrophilic or eosinophilic granulocytes.



*Plate 41.* — Basophilic granulocytes. Peroxidase reaction (Graham). 1. Peroxidase-negative basophils. 2. Basophil containing a few peroxidase-positive granules. 3. Neutrophils (distinctly peroxidase-positive granulation).



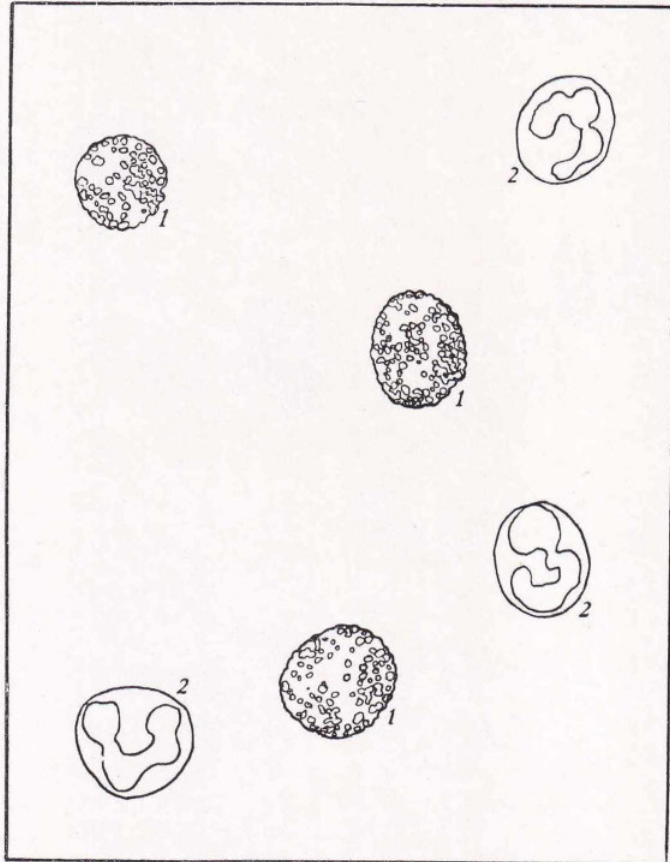
*Plate 41.* — Basophilic granulocytes. Peroxidase reaction (Graham).



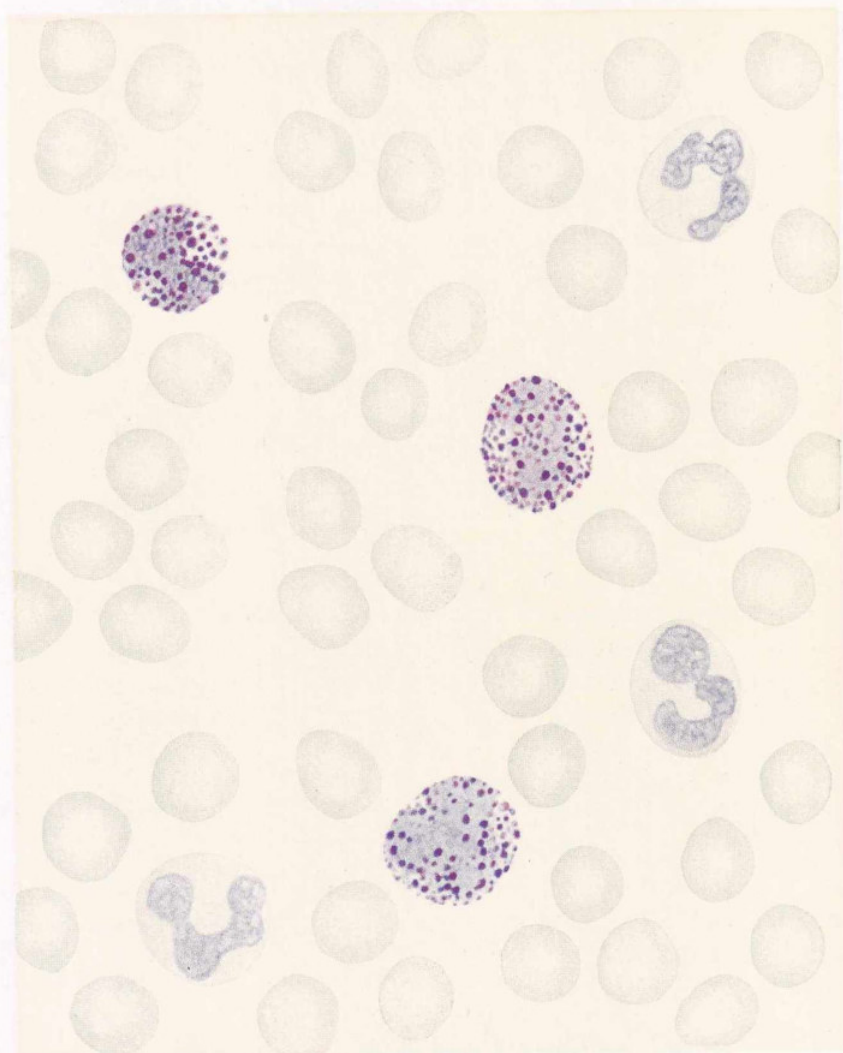
The basophilic granulation is, when stained by alcoholic solutions of toluidine blue or thionine blue, metachromatic. These blue stains impart a pinkish-violet colour to the basophilic granulation (phenomenon of metachromasia). The remaining leukocytic varieties of granulation are not metachromatic (Plate 42).

In intravital preparations basophils are motile. Their granulation, when stained by neutral red, is reddish-brown (chestnut).

Basophilic granulocytes should not be identified with basocytes (tissue basophils, mast-cells) commonly encountered in the neighbourhood of capillaries, in lymph nodes and in proper connective tissue. The morphology of both these cells is similar although there are differences. Mast-cells are larger, granulation is uniform; the nucleus is smaller, round



*Plate 42.* — Basophils (toluidine blue stain): 1. Basophilic granulocytes — the specific granulation stains metachromatically. 2. Neutrophilic granulocytes — the specific granulation cannot be discerned.



*Plate 42.* — Basophils (toluidine blue stain).



and excentric. Mast-cells are present also in the intestine walls and in chronic inflammatory foci. An increase in mast-cells is not paralleled by an increase in basophilic granulocytes.

The rôle of mast-cells is not definitely known. Homlgren, Jorpes and Wilander have expressed the opinion that these cells may be concerned in the production and storage of heparin. This assumption is based on the fact that metachromatic properties are common to both heparin and basophilic granulation. It should be noted, however, that the phenomenon of metachromasia is not specific for heparin, and may be demonstrated in various cells and tissues.

An increase in basophilic granulocytes may be helpful in recognition of certain haematopoietic disorders. The highest percentage of basophils so far reported was 83% observed during the terminal phase in a case of chronic myeloid leukaemia (Joachim).

An increase of basophils is frequently encountered in the course of this disease. The highest percentage observed by the present authors was 19% in peripheral blood. This symptom may be helpful in differentiation between leukaemia and a myeloid reaction with a high leukocytosis.

Furthermore, an increase in basophils may be observed in the course of polycythaemia, Hodgkin's disease, chronic haemolytic syndrome following splenectomy, X-ray irradiation injury, after administration of foreign proteins, idiopathic thrombocytopenia, chronic sinusitis, smallpox and chickenpox.

Several cases of basophilic leukaemia have been reported in literature. Such a syndrome was described among others by Polish investigators, Tempka and Hanicki. Leukaemia of this type is extremely rare. Up to the year 1949, only 12 reports on cases of basophilic leukaemia had been published (V. Hule).

## PEROXIDASE REACTIONS

The technique of peroxidase reactions is less elaborate than in the case of oxidase reactions and, therefore, more suited to everyday practice.

### PEROXIDASE REACTION (GRAHAM)

#### *Reagents:*

1. 40% formaldehyde (formalin);
2. 96% aethyl alcohol (or absolute alcohol);
3. 40% aethyl alcohol;
4. 3% hydrogen peroxide solution;
5. Benzidine;

*Procedure:*

(a) freshly made, air-dried smears are fixed from 0.5 to 1 min. in a mixture of 1 volume of formalin with 9 volumes of 96% alcohol;

(b) the slide is rinsed in tap water;

(c) the slide is placed in a Petri dish and the reagent solution is allowed to act for from 4 to 5 min.; reagent solution — a few benzidine crystals are dissolved in 10 ml. of 40 % alcohol and 0.02 ml. of 3 % hydrogen peroxide is added. The Petri dish should be gently tilted in all directions;

(d) the slide is rinsed in tap water;

(e) the blood film is counterstained by Giemsa solution.

*Results:* the granulation of neutrophilic and eosinophilic granulocytes (beginning from the stage of promyelocyte) is positive viz. brownish-gold or yellow-green. Myeloblasts, lymphoblasts, lymphocytes, megakaryocytes, plasmocytes, erythroblasts and platelets are negative.

Monocytes are usually faintly positive, negative results in their cytoplasm are, however, often obtained. Immature monocytes are negative.

Plate 43 illustrates the peroxidase reaction in a bone-marrow smear.

## PEROXIDASE REACTION (SATO)

*Reagents:*

1. 0.5 % aqueous solution of copper sulphate;
2. Benzidine . . . . . 0.2 Gm.;  
    3 % hydrogen peroxide . . . . . 4 drops;  
    distilled water . . . . . 200.0 cu.mm.;
3. 1 % aqueous solution of safranine.

*Procedure:*

(a) the copper sulphate solution is allowed to act on fresh, air-dried, unfixed smears for from 1 to 1.5 min.;

(b) the reagent is poured off (the smear should not be rinsed!) and the blood film is covered with the reagent 2 (benzidine solution) for 2 min.;

(c) the smears are thoroughly rinsed in tap water;

(d) counterstain with safranine (approximately 2 min.).

*Results:* the peroxidase-positive granulation is dark blue. Plate 44 illustrates the peroxidase reaction in a bone-marrow smear prepared by the Sato method.

## SUDAN BLACK B STAIN (PROCEDURE OF CZERSKI)

*Reagents:*

1. Absolute methyl alcohol;
2. Saturated solution of Sudan black B in 70% aethyl alcohol;
3. 1% aqueous solution of methyl green;



*Procedure:*

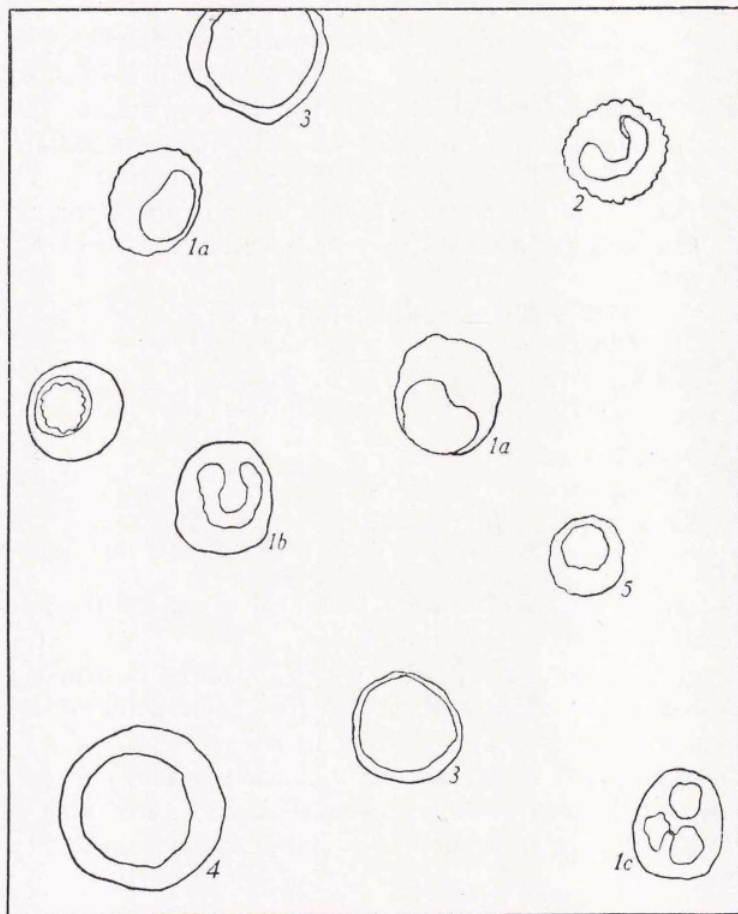
(a) an old smear (stored for 2-3 weeks) or a smear stored for from 24 to 48 hrs. in a refrigerator (from  $+4$  to  $+6^{\circ}\text{C}$ ) is fixed by methyl alcohol during 3 min.;

(b) staining with Sudan black B solution during 20 (or even up to 40) min.

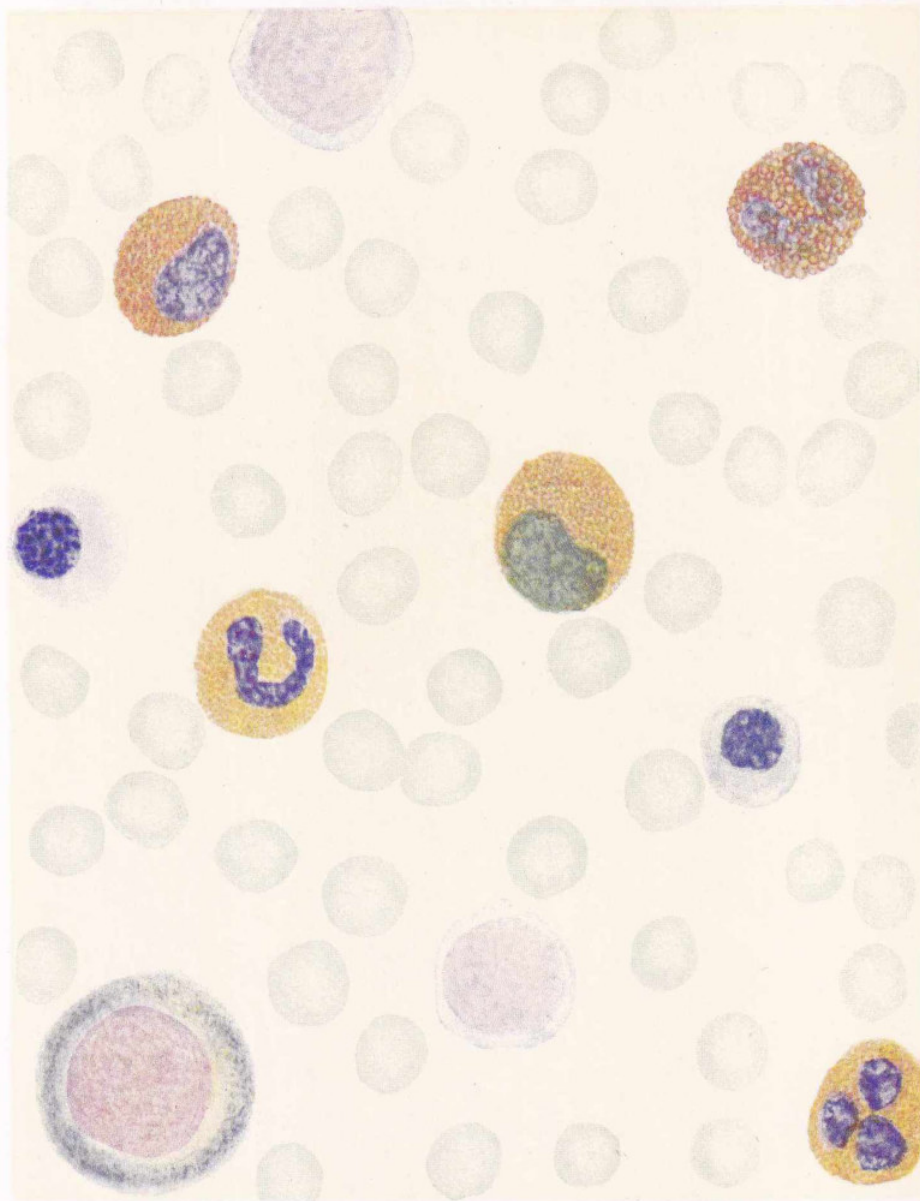
(c) rinsing with 70% aethyl alcohol;

(d) nuclear counterstain with methyl green;

The results and their interpretation see text (chapters on leukaemia).



*Plate 43.* — Peroxidase reaction in the bone marrow (Graham's method): 1. Neutrophilic granulocytes containing peroxidase-positive granules in the cytoplasm; 1a. — myelocyte; 1b. — staff cell; 1c. — polymorphonuclear. 2. Eosinophilic granulocyte with a segmented nucleus — the specific granulation is distinctly peroxidase-positive. 3. Myeloblast — these cells are peroxidase-negative. 4. Proerythroblast. 5. Polychromatic erythroblast. The cells of the red-cell series are peroxidase-negative.



*Plate 43.*—Peroxidase reaction in bone marrow (Graham's method).



## SHIFT TO THE LEFT IN GRANULOCYTES

Various factors may cause the release of immature granulocytes into the peripheral blood.

Among the most frequent causes of a shift to the left are:

1. Acute infections, especially those caused by *Staphylococci*, *Streptococci*, *Pneumococci*, *Corynebacterium diphtheriae*, *Diplococci* of epidemic encephalitis, *Bacterium coli*; among other diseases, smallpox and typhus fever should be mentioned; certain chronic infections, such as pulmonary tuberculosis.
2. Noxious action of certain endo- or exogeneous toxins;
3. Severe haemorrhages;
4. Surgical intervention, particularly abdominal operations;
5. Neoplastic disease, particularly accompanied by necrosis and marked malignant proliferation;

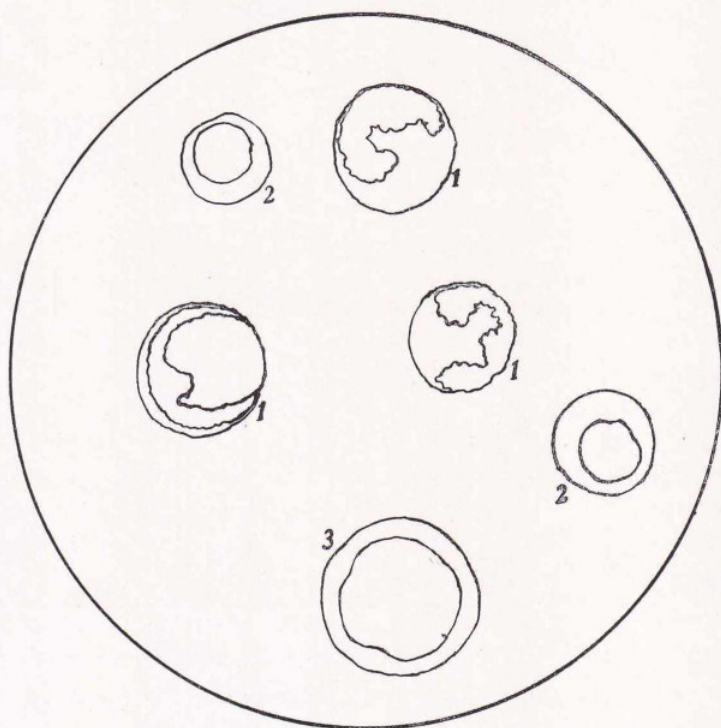
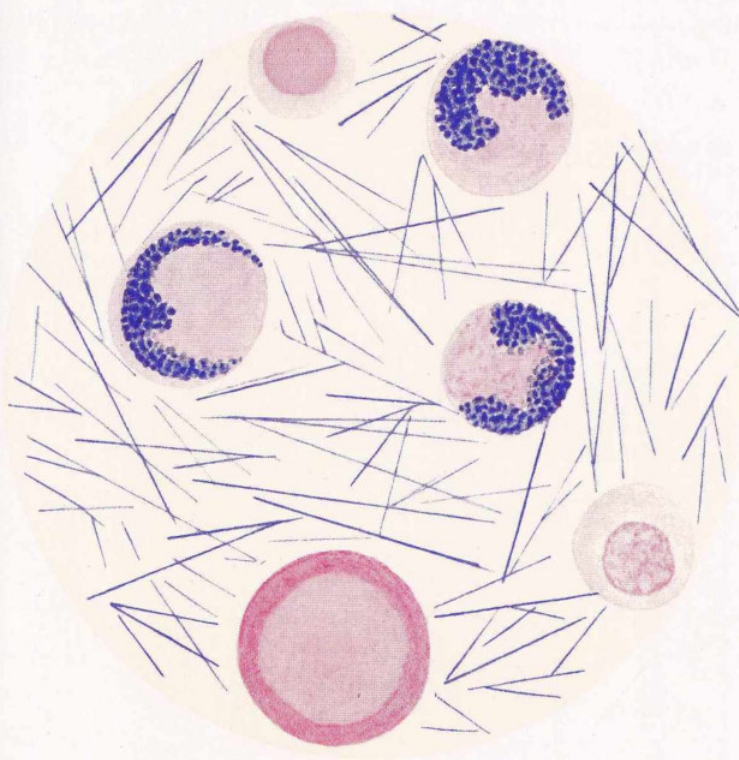


Plate 44. — Peroxidase reaction after Sato (bone marrow). The peroxidase-positive granules are deep blue: 1. Granulocytes — peroxidase-positive. 2. Erythroblasts — peroxidase-negative. 3. A peroxidase-negative stem-cell (precise recognition of this cell is difficult).

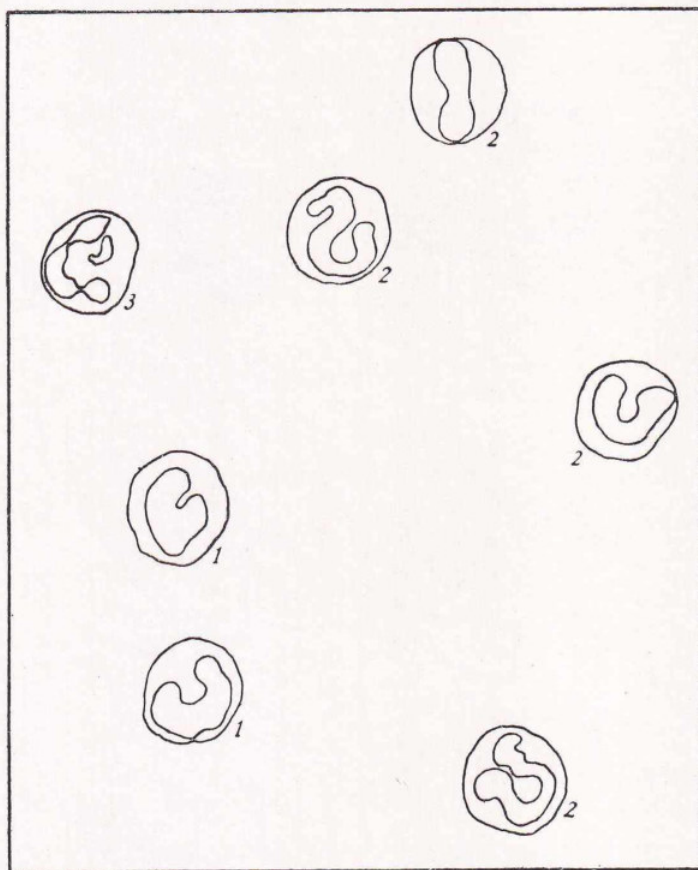


*Plate 44.* — Peroxidase reaction after Sato (bone marrow).



6. Myocardial or pulmonary infarction;
7. Chronic myelogeneous leukaemia;
8. Polycythaemia;
9. Strenuous physical exertion; labour in obstetric patients; prolonged vomiting; paroxysmal tachycardia; convulsive seizures;
10. In the newborn as a physiological phenomenon.

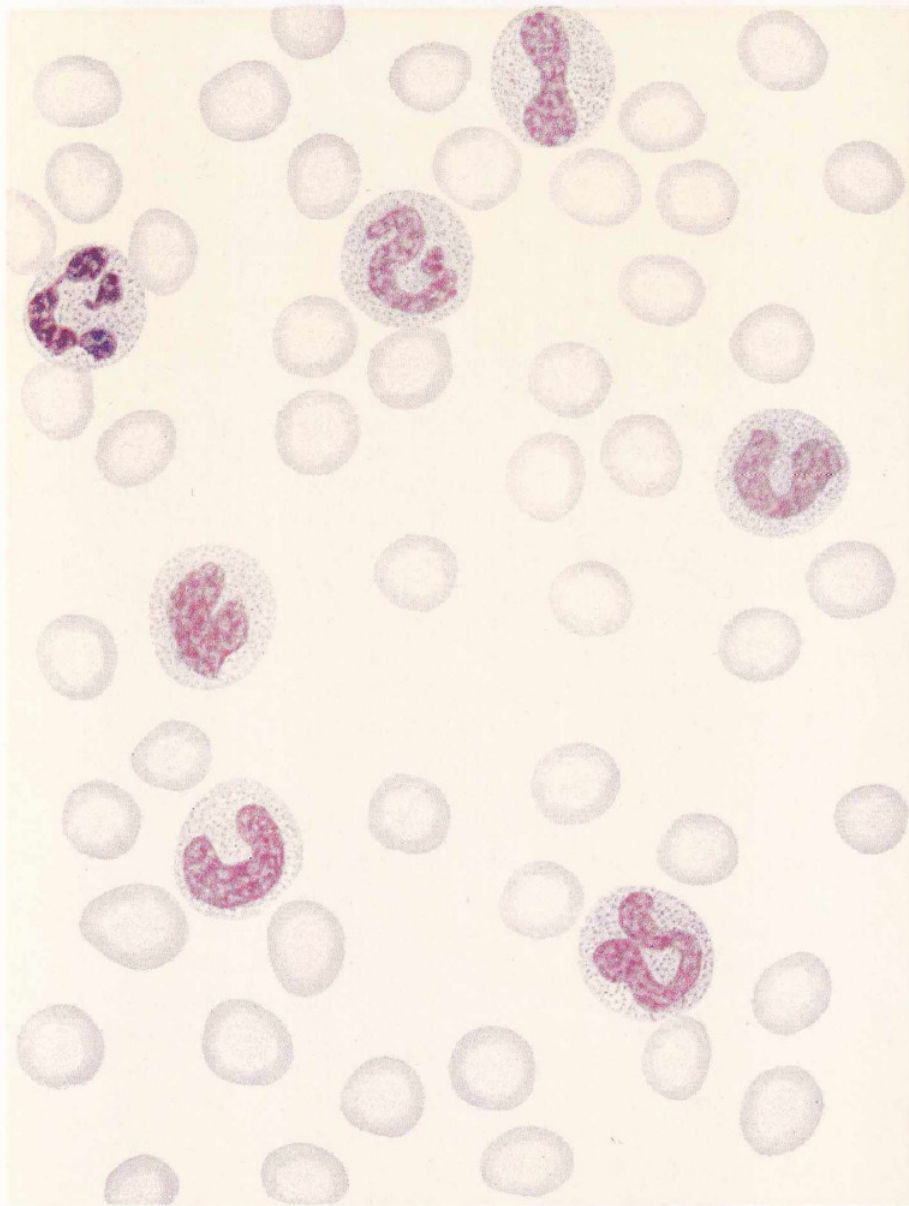
Neutrophilic leukocytosis is usually accompanied by a shift to the left. In the course of certain acute infections, the percentage of staff cells may exceed 50 %. In addition to staff cells, metamyelocytes or even less mature



*Plate 45.* — Shift to the left in peripheral blood: 1. Neutrophilic metamyelocyte ("juvenile" neutrophil). 2. Neutrophilic staff cells. 3. Polymorphonuclear neutrophil.

The transition from a metamyelocyte to a staff cell is gradual. The classification of an intermediate cell as one of the stages mentioned is subjective and liable to vary with individual haematologists. The nucleus of typical metamyelocytes is usually thicker and more fine in structure. The appraisal of such subtle differences is possible only in perfectly executed smears.

cf Plate 46 — Peüger-Huët's anomaly — an apparent shift to the left.



*Plate 45.* — Shift to the left in peripheral blood.



forms may be encountered. The "shift to the left in granulocytes" is of importance in pulmonary tuberculosis. The appearance of this symptom may indicate that the morbid process is becoming activated and shows a spreading tendency. Such a conclusion may, of course, be drawn only on the basis of serial blood examinations and clinical observation.

Plate 45 illustrates the shift to the left in granulocytes in a peripheral blood smear.

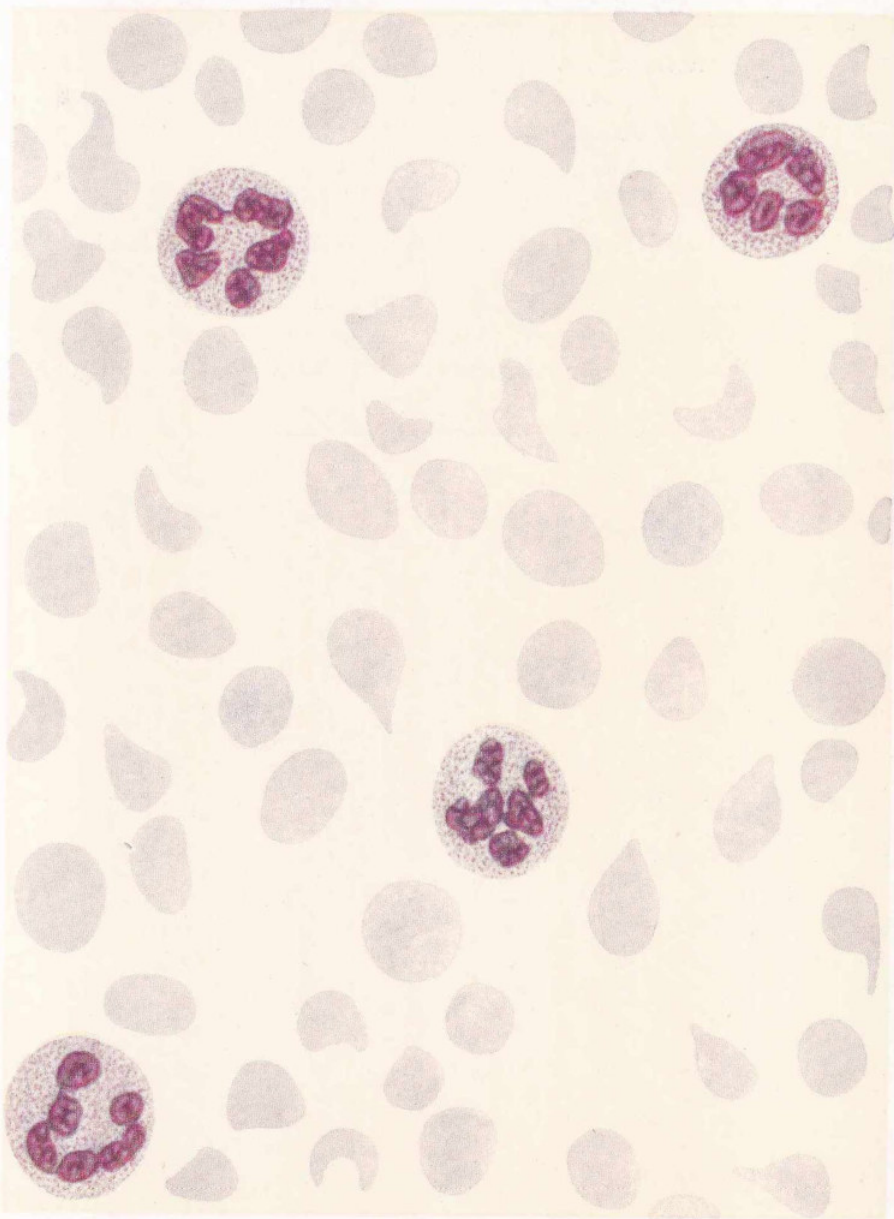
In addition to staff cells, one metamyelocyte, characterised by a markedly less mature nucleus, can be seen.

### SHIFT TO THE RIGHT IN GRANULOCYTES

The occurrence of numerous granulocytes with hypersegmented nuclei was reported by Undritz as a familial anomaly. The shift to the right in granulocytes is of diagnostic importance in Addison-Biermer's disease. This symptom often persists for long periods in the course of pernicious anaemia, despite treatment. Furthermore, this symptom may be present in other "acastloses", nontropical sprue with megaloblastic erythropoiesis, diseases of the liver, chronic sepsis (a rare symptom), chronic hypochromic anaemia, and in some instances in X-ray or radium injury. Recently, Kiderly has stressed the occurrence of a shift to the right in granulocytes in patients with carcinoma of the stomach, and following splenectomy. After the removal of the spleen, this phenomenon is transitory.

It is held that the shift to the right institutes a sign of hypoplastic granulopoiesis. Multisegmented granulocytes develop, according to this view, directly from promyelocytes or so-called "giant" metamyelocytes. Furthermore, it is claimed that the shift to the right in peripheral blood is accompanied by a shift to the left in the bone marrow. There arises the question: can it be assumed that there exists a rigid relationship between the shape of the nuclei of mature granulocytes (band-form, segmented or multisegmented) and the degree of the granulopoietic activity of the bone marrow (hypo- or hyperplastic)? Or, on the other hand, is the deciding factor the developmental stage, at which segmentation of the nucleus begins? The question remains, as yet, unanswered and constitutes a moot point.

A number of authors claim that the degree of nuclear segmentation is dependent on the age of the granulocytes. The observations of Czerski, Łaukowicz and Porembińska indicate, however, that the ageing of granulocytes is accompanied by other morphological signs. These consist in a marked pycnosis of the nucleus and a tendency of nuclear lobes to clump together. Thus the nucleus assumes a round or oval shape and only careful examination by phase-contrast microscopy, or of preparations stained by specific nuclear stains, reveals the presence of lobes in the nuclei.



*Plate 46.* — Shift to the right in granulocytes.



Moreover, Porembińska observed a specific, isolated response of multisegmented granulocytes to vit. B<sub>12</sub> treatment in cases of Addison-Biermer's disease. This phenomenon consists in an increase, even up to 600 %, of the initial count of multisegmented granulocytes after the administration of vit. B<sub>12</sub>. This may be taken to indicate that multisegmented granulocytes represent a separate strain of these cells.

### PELGER-HUËT'S ANOMALY OF THE GRANULOCYTES

This occurs rarely and is considered as a familial transmitted anomaly. The peripheral blood changes consist in the appearance of numerous neutrophilic staff cells, metamyelocytes or even myelocytes. Segmented

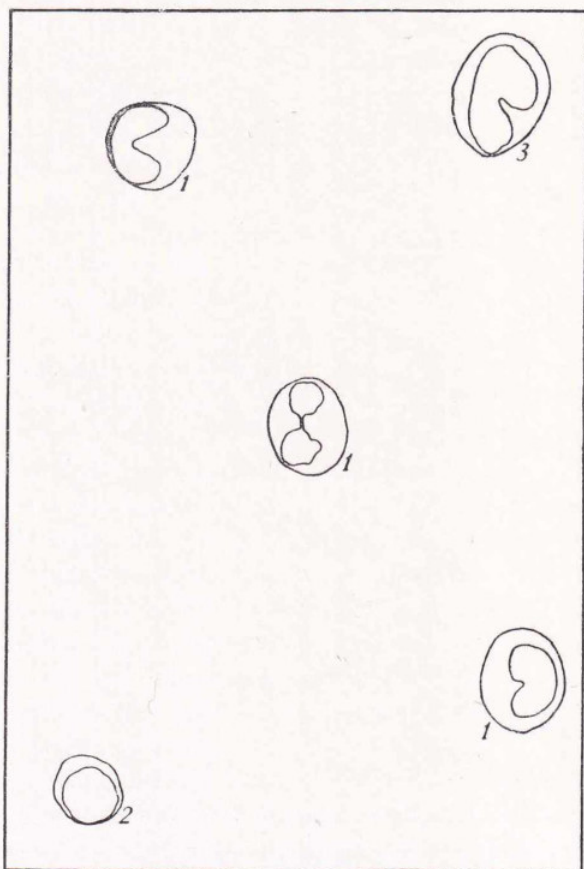


Plate 47. — Pelger-Huët's anomaly of granulocytes: 1. Neutrophils. 2. Lymphocyte. 3. Monocyte. Pelger's anomaly may be differentiated from a shift to the left on the basis of nuclear structure of granulocytes (cf Plate 45).

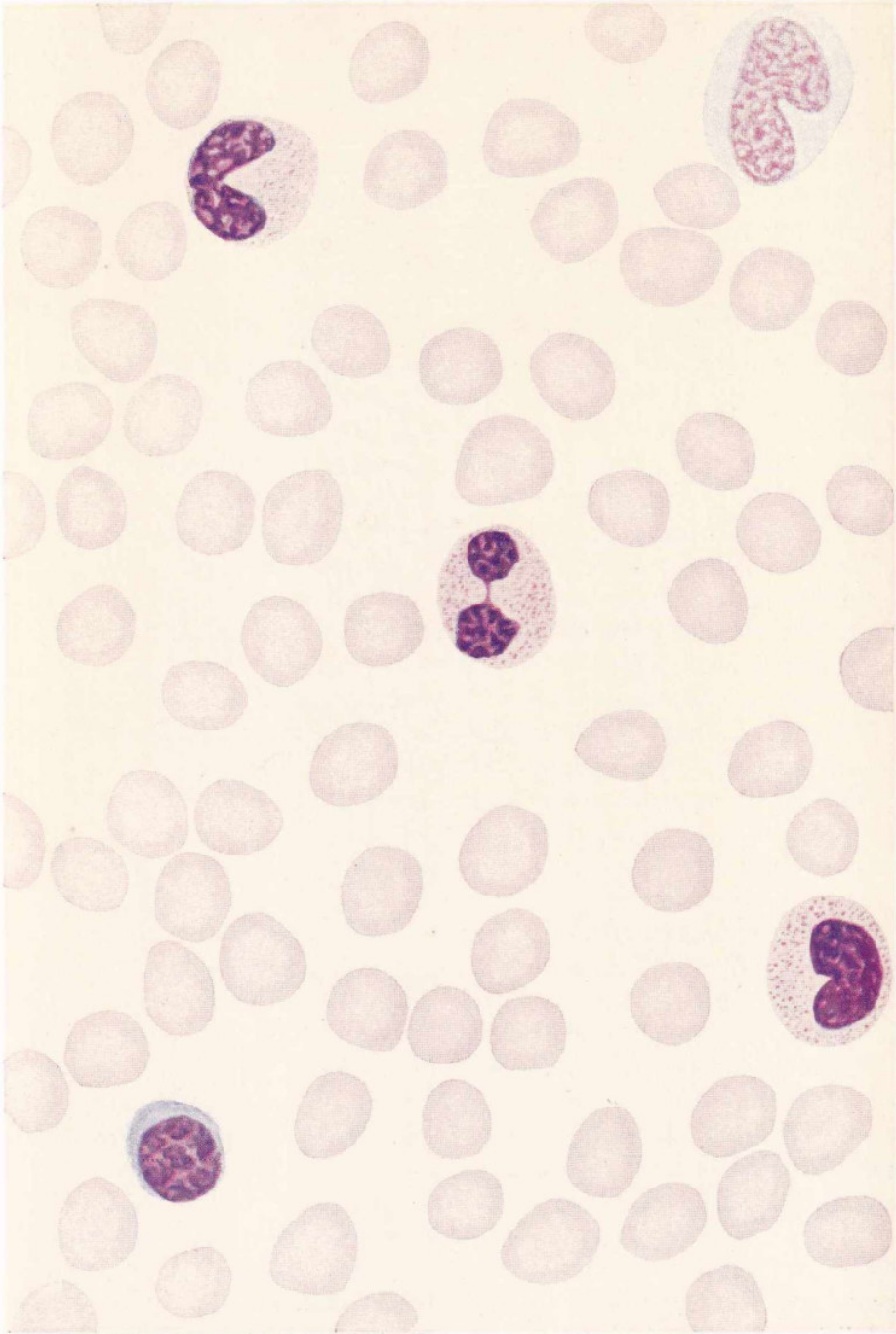


Plate 47. — Pelger-Huët's anomaly of granulocytes.



nuclei of granulocytes are usually bilobed, the lobes being joined by thin chromatin strands. Analogical deviations are observed in eosinophils. A casual examination of the blood picture suggests the presence of a shift to the left (a pseudo-regenerative blood picture). This phenomenon is, however, caused by a congenital hyposegmentation of granulocytic nuclei, and not by a regenerative response of the bone marrow.

A detailed examination of granulocytes demonstrates the contrast between the immature shape of the nucleus and its pycnotic and coarse structure. The nuclei, which on the basis of shape should be classified as metamyelocytic, contain abundant chromatin, as seen in mature cells. The anomaly may also concern the basophils and sometimes monocytes, which demonstrate abnormal nuclei of bizarre shape and coarse chromatin strands.

The white-cell count is usually normal. This nuclear anomaly is observed in otherwise completely normal individuals. Among Polish authors, Śmigielski, Ławkowicz and Włodarski, Szymkiewicz, as well as Trojanowska and Czajka have reported the occurrence of this anomaly.

Plate 47 illustrates a peripheral blood smear from a case of Pelger-Huët's nuclear anomaly of the granulocytes.

The shape of nuclei of two neutrophils corresponds to that of metamyelocytic nuclei. The nuclear structure, however, corresponds to that of mature nuclei, and is ever hyperpycnotic. The granulocyte in the middle of the drawing has a bilobed nucleus. Neutrophils with more nuclear lobes are rarely encountered in Pelger-Huët's anomaly.

Note the abnormal nuclear structure of the lymphocyte.

## QUALITATIVE CHANGES OF THE CYTOPLASM OF GRANULOCYTES

In most laboratories, the examination of the white-cell picture is limited to the differential count, based on Schilling's classification. Such an examination must be regarded as oversimplified and unsatisfactory from the haematological point of view.

The differential count according to Arneth's classification is also inadequate. Information concerning the number of nuclear lobes is of little value, unless supplemented by a description of chromatin distribution and of signs of pycnosis or of "immaturity" of the nuclear structure. It may happen that a granulocyte with a segmented or even multisegmented nucleus is erroneously classified as a mature cell, although close inspection of its nuclear structure reveals signs of immaturity. And, on the other hand, nuclei of "immature" shape (Pelger's anomaly) may be pycnotic, "old" in structure. Such examples demonstrate how unreliable are numerical data alone. Information pertaining to the morphological features

of granulocytic nuclei may in rare instances be found among the results of peripheral blood examination. Such information is usually overlooked although, for example, the presence of hyperpycnotic staff-nuclei, or of blurred nuclear structure would point to profound disturbances in the white-cell series.

Great importance should be attributed to qualitative changes in the cytoplasm of granulocytes. A number of deviations from normal are mentioned below.

#### PATHOLOGICAL GRANULATION

*("Toxic" granulation, immature granulation, persistent granulation)*

Coarse, dark violet or dark blue granulation may be encountered in the cytoplasm of neutrophilic granulocytes during the course of several infections, lobar pneumonia, tuberculosis, septicaemia, typhoid fever, erysipelas, certain poisonings and, in some instances, during the course of carcinoma of the stomach (Netoušek). Such granulation differs considerably from the minute granulation of normal granulocytes, and is, therefore, termed "pathological" or "toxic" granulation. The latter term originated from the opinion that pathological substances produced in inflammatory foci are stored in the cytoplasm of granulocytes. It was also assumed that certain substances may exert a direct toxic influence on granulocytes. These concepts have been abandoned. The view now prevalent (Stodtmeister, Klima, Netoušek, Hynek, Leitner, Thaddea) is that this granulation appears as the result of disturbances in the maturation of cytoplasm at the stage of promyelocyte or myelocyte. The azurophilia of this granulation supports the assumption that it represents persistent granula of the promyelocyte. It may be inferred that in cells demonstrating such granulation there exists a disproportion between the stage of maturity attained by the nucleus and that of the cytoplasm. The term "immature" or "persistent" granulation seems, in view of this opinion to be more appropriate.

This granulation is generally readily demonstrated in smears stained by the usual method at pH 6 to 7. It is, however, best visualised by staining in Mommson's buffer with Giemsa solution at pH 5.4.

Persistent granulation of granulocytes may be observed not only in the disorders mentioned above but also in several other conditions, such as after incompatible blood transfusion, intensive X-ray irradiation, in neoplastic disease and leukaemias.

The demonstration of immature granulation is, however, of major clinical importance in the course of infections. In these diseases (*e.g.* lobar pneumonia) the number of abnormal granulocytes indicates the severity of the morbid process. In such cases, serial blood examinations may be



of assistance in establishing the prognosis. A decrease in granulocytes containing immature granulation may be taken as a favourable sign.

Plate 48 illustrates neutrophils containing immature granulation in a case of severe lobar pneumonia.

Note should be taken of a rare familial anomaly — Alder's anomaly, which is characterised by the presence of coarse basophilic granulation in the cytoplasm of granulocytes, lymphocytes and monocytes. This granulation resembles the immature variety, but stains optimally at pH 5. This anomaly was described in healthy individuals.

#### DOEHLE'S BODIES

Basophilic, agranular areas may, in the course of certain infections, such as scarlet-fever or lobar pneumonia, be encountered in the cytoplasm of neutrophils. These areas are termed Doehle's bodies. Until recently, their presence was assumed to be specific for scarlet-fever. It has, however, been demonstrated that they may occur in the course of other infectious diseases.

The presence of limited basophilic areas point to disturbances in the maturation of cytoplasm. Such disturbances may manifest themselves in the persistence of immature granulation or the presence of focal basophilia in the cytoplasm.

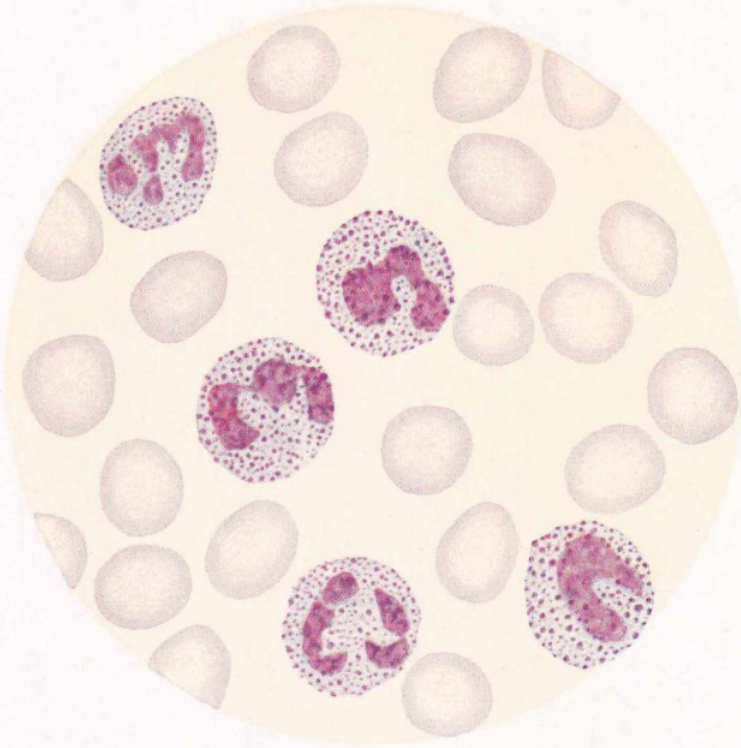
Plate 49 illustrates the presence of Doehle's bodies in two granulocytes (a case of diphtheria).

May and Hegglin described a rare familial anomaly consisting in the presence of focal basophilia in the cytoplasm of granulocytes and monocytes. This anomaly resembles Doehle's bodies. It has been demonstrated in healthy individuals and it is a constant trait.

#### VACUOLATION

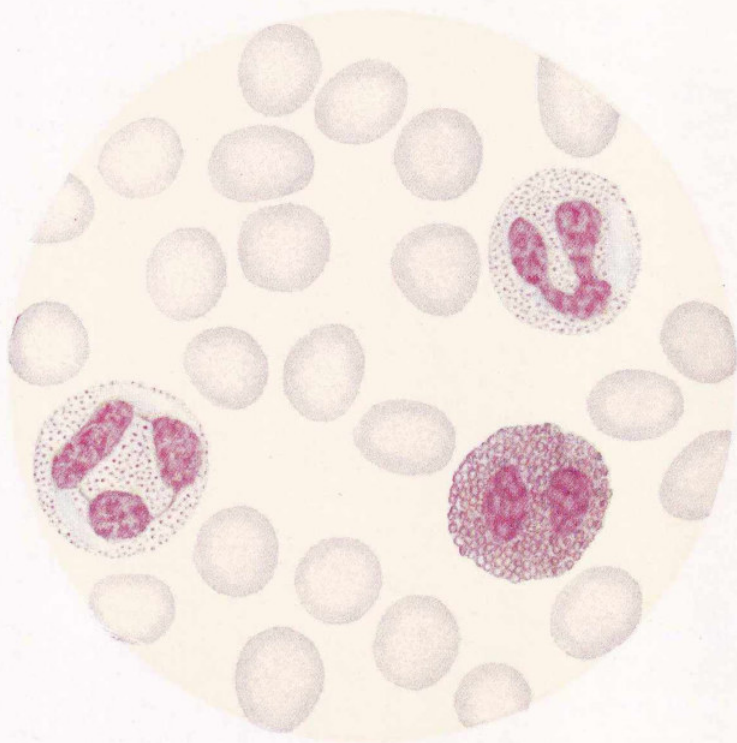
In granulocytes not only persistent granulation may be encountered, but also vacuolation of the cytoplasm and of the nucleus. The presence of vacuoles indicates degenerative changes and may indicate the severity of an infection. Vacuolar degeneration may be observed in rapidly progressing infections, in septicaemia, in severe diseases of the liver (particularly the course of yellow atrophy of the liver), in hepatic cirrhosis, in severe mushroom poisoning and in severe burns.

Opinions concerning the content of vacuoles differ widely. Undritz claims that they contain proteins; Netoušek maintains that the vacuoles originate artificially during the preparation of the smear. According to this author, the vacuoles contain substances readily dissolved in water. During staining, these substances are "washed out" leaving "empty space". It is also possible that the appearance of vacuoles may be caused

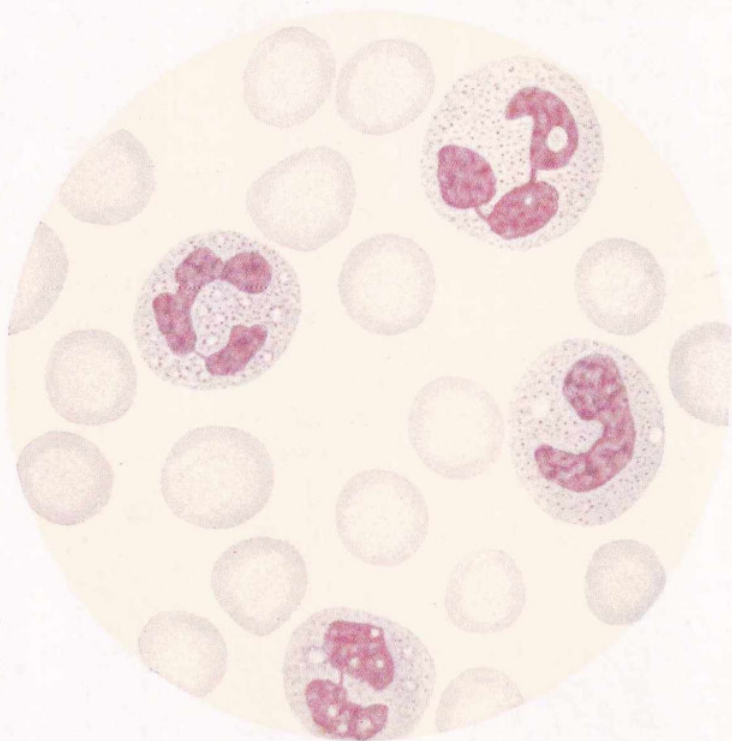


*Plate 48.* — “Toxic” granules — a coarse pathologic granulation in the cytoplasm of granulocytes.





*Plate 49. — Doehle's bodies.*



*Plate 50.* — Vacuolation of the cytoplasm of granulocytes.



by atrophy of the granules. It has been demonstrated by phase microscopy that vacuoles occur also in unfixed granulocytes suspended in their own plasma. Vacuolation appears in these cells as the first sign of ageing (Czerski, Łaukowicz, Porembińska).

### L. E. CELLS

Hargraves and coll. described the occurrence of white blood cells characterised by special morphological features. The nuclei of such cells are displaced to the periphery by a homogeneous, structureless, greyish-violet inclusion-body almost completely filling the whole of the cytoplasm. The positive result of Feulgen's reaction indicates that these cells contain phagocytised nuclear substance.

White cells of this variety were observed for the first time in cases of disseminated *lupus erythematosus* and termed therefore "L. E. cells". Many later reports demonstrated that the presence of these cells cannot be considered as a specific symptom for *lupus erythematosus*. They may be encountered in several disorders accompanied by dysproteinaemia. Among the first to note this lack of specificity were Tempka and Hanicki.

### THE NORMAL BONE MARROW

There are wide variations in the values of bone marrow differential count accepted by various authors as normal. Considerable variations may be caused by differences in the technique of obtaining samples for bone-marrow examination. The smears may be prepared from the liquid portion of the aspirate or from bone-marrow particles (units). The latter method is preferable because it minimises the admixture of peripheral blood to the bone marrow. The absolute counts of bone-marrow elements are, on the other hand, more readily performed on liquid material<sup>1</sup>.

It should, however, be borne in mind that the absolute count is subject to wide fluctuations and cannot be adequately standardised. Therefore, absolute counts are by many investigators considered of little or no value and such investigators base their conclusions on differential counts and qualitative evaluation of bone-marrow smears. The technique of bone marrow differential counts also varies greatly. Certain authors establish the percentage of individual bone marrow-cells counting 300 or 500 nucleated elements. Another group (Rohr) counts the leukocytes (granulocytes and lymphocytes) and establishes the percentage of erythroblasts and

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<sup>1</sup> The absolute count, together with a differential count, makes it possible to establish the absolute number of individual bone-marrow elements per cu.mm.

reticulum cells in relation to white blood cells. This method has its advantages because it makes possible the comparison of peripheral blood and bone marrow differential counts, as well as the evaluation of the rate of granulocyte release into the peripheral blood.

The present authors count 500—1,000 cells, and compute the percentage of individual bone-marrow elements. In addition maturation curves should be computed separately for the granulocytic and the erythroblastic series.

The performance of a bone marrow differential count is certainly more troublesome than a purely qualitative evaluation, but it yields more accurate results. It must be, however, stressed that considerable variations may be found even among several preparations of the same material. All values obtained should therefore be regarded as approximate and open to challenge and not as "rigid" and exact.

The composition of normal adult bone marrow according to Aleksandrowicz is the following:

|                                     |   |   |   |          |
|-------------------------------------|---|---|---|----------|
| cells of the erythropoietic series. | . | . | . | 8 — 20%  |
| cells of the lymphocytic series     | . | . | . | 1 — 10%  |
| cells of the granulopoietic series  | . | . | . | 60 — 80% |
| reticulum cells                     | . | . | . | 3 — 10%  |

The mean value for the cells of the megakaryocytic system amounts usually to from 0.2 to 4.0 %, according to Tempka, and to 0.4% according to Wintrobe.

Tempka and Braun were among the earliest investigators to record normal values for the myelogram. The results they obtained (expressed as percentages of the total number of nucleated elements) are tabulated below:

|                                 |   |   |   |   |   |              |
|---------------------------------|---|---|---|---|---|--------------|
| myeloblasts                     | . | . | . | . | . | 4.6 — 7.0%   |
| metamyeloblasts                 | . | . | . | . | . | 1.5 — 5.5%   |
| promyelocytes                   | . | . | . | . | . | 3.7 — 6.8%   |
| neutrophilic myelocytes         | . | . | . | . | . | 12.7 — 13.3% |
| eosinophilic myelocytes         | . | . | . | . | . | 1.5 — 2.6%   |
| basophilic myelocytes           | . | . | . | . | . | 0.0 — 0.3%   |
| neutrophilic metamyelocytes     | . | . | . | . | . | 14.3 — 16.7% |
| eosinophilic metamyelocytes     | . | . | . | . | . | 0.3 — 3.6%   |
| basophilic metamyelocytes       | . | . | . | . | . | 0.0 — 0.1%   |
| neutrophilic staff cells        | . | . | . | . | . | 17.7 — 22.5% |
| eosinophilic staff cells        | . | . | . | . | . | 0.5 — 1.0%   |
| basophilic staff cells          | . | . | . | . | . | 0.0 — 0.16%  |
| neutrophilic polymorphonuclears | . | . | . | . | . | 16.1 — 20.3% |
| eosinophilic polymorphonuclears | . | . | . | . | . | 0.6 — 2.5%   |
| basophilic polymorphonuclears   | . | . | . | . | . | 0.2 — 0.3%   |
| lymphocytes                     | . | . | . | . | . | 2.7 — 3.2%   |



|                         |           |            |
|-------------------------|-----------|------------|
| monocytes.              | . . . . . | 0.5 — 0.7% |
| reticulum cells .       | . . . . . | 1.3 — 3.7% |
| plasmocytes .           | . . . . . | 0.2 — 1.6% |
| undifferentiated cells. | . . . . . | 0.0 — 3.0% |

The number of erythroblasts was expressed by Tempka and Braun as a percentage of the number of red cells.

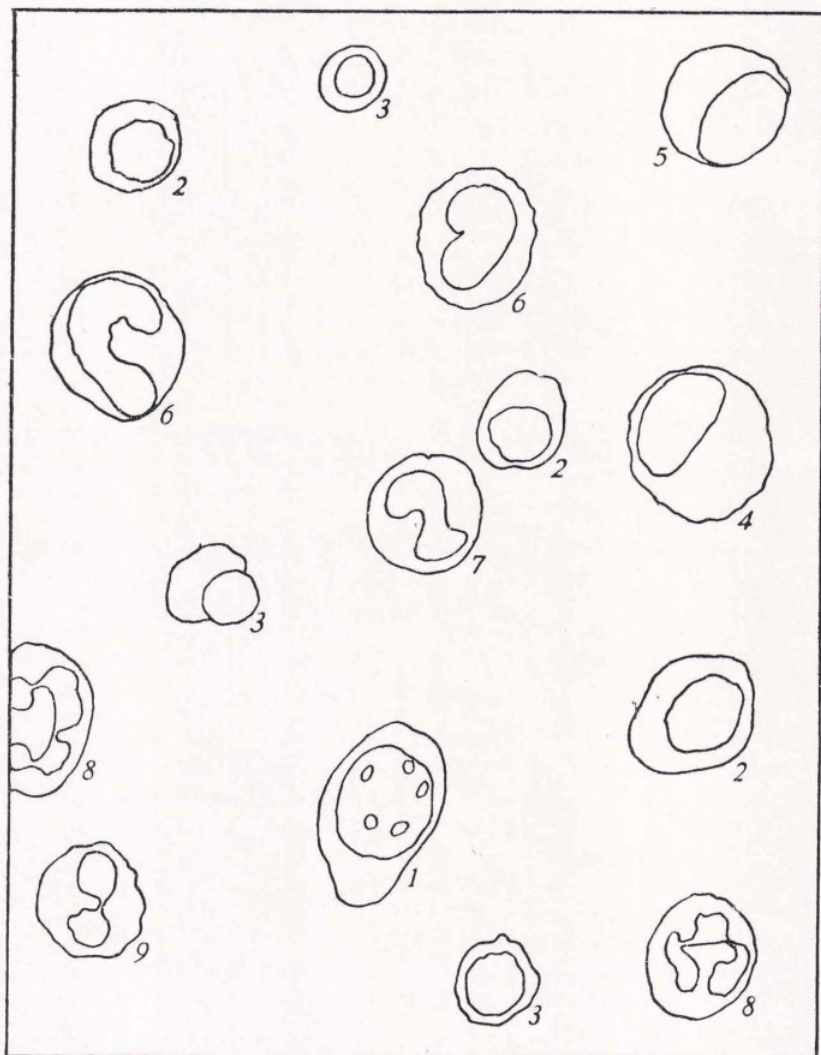
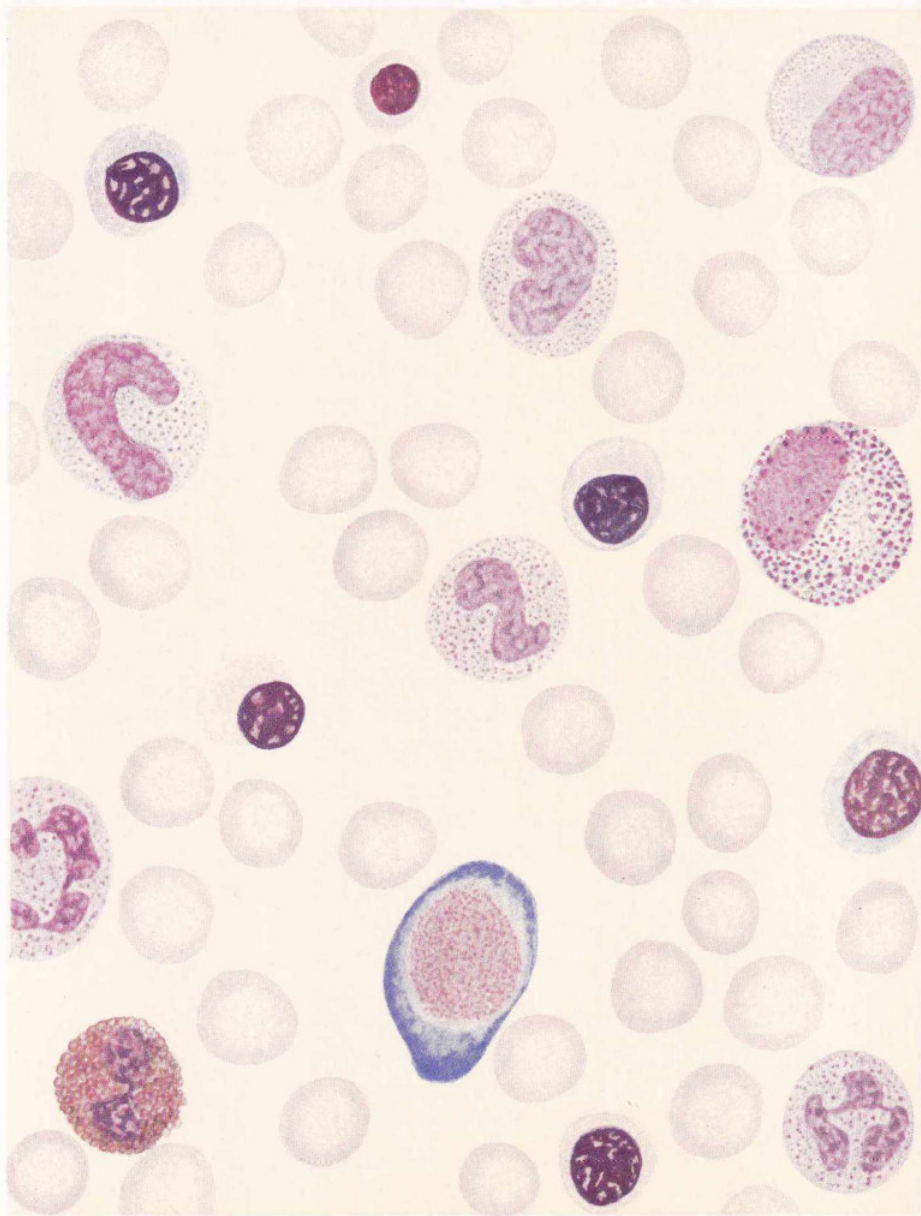


Plate 51. — Normal bone marrow: 1. Proerythroblast. 2. Polychromatic erythroblast. 3. Orthochromatic erythroblast. 4. Promyelocyte. 5. Neutrophilic myelocyte. 6. Neutrophilic metamyelocyte. 7. Neutrophilic staff cell. 8. Segmented neutrophil. 9. Segmented eosinophil.



*Plate 51.*—Normal bone marrow.



The following values of a normal adult bone-marrow count were determined by one of the present authors (together with Porembińska):

|                                                  |              |
|--------------------------------------------------|--------------|
| <i>The erythropoietic series:</i>                | 10.6 — 30.4% |
| mitotic forms . . . . .                          | 0.0 — 0.3%   |
| proerythroblasts . . . . .                       | 0.0 — 1.4%   |
| basophilic erythroblasts . . . . .               | 0.8 — 3.6%   |
| polychromatic erythroblasts . . . . .            | 4.8 — 15.0%  |
| orthochromatic erythroblasts . . . . .           | 2.2 — 12.4%  |
| <i>The granulopoietic series:</i>                | 62.2 — 77.2% |
| mitotic forms . . . . .                          | 0.0 — 0.2%   |
| myeloblasts . . . . .                            | 0.0 — 1.0%   |
| promyelocytes . . . . .                          | 0.0 — 4.8%   |
| neutrophilic myelocytes . . . . .                | 4.8 — 19.2%  |
| eosinophilic myelocytes . . . . .                | 0.8 — 2.6%   |
| basophilic myelocytes . . . . .                  | 0.0 — 0.4%   |
| neutrophilic metamyelocytes . . . . .            | 12.6 — 26.4% |
| eosinophilic metamyelocytes . . . . .            | 0.4 — 1.2%   |
| basophilic metamyelocytes . . . . .              | 0.0 — 0.2%   |
| neutrophilic staff cells . . . . .               | 9.6 — 18.0%  |
| eosinophilic staff cells . . . . .               | 0.0 — 0.8%   |
| basophilic staff cells . . . . .                 | 0.0%         |
| neutrophilic polymorphonuclears . . . . .        | 15.2 — 32.0% |
| eosinophilic polymorphonuclears . . . . .        | 0.4 — 2.4%   |
| basophilic polymorphonuclears . . . . .          | 0.0 — 0.2%   |
| macropolycytes . . . . .                         | 0.0 — 1.0%   |
| <i>Lymphocytes:</i>                              | 3.2 — 12.0%  |
| <i>Cells of the reticulo-endothelial system:</i> | 3.0 — 5.0%   |
| reticulum cells proper (mesenchymatic cells)     | 0.0 — 2.0%   |
| Ferrata cells . . . . .                          | 0.0 — 0.4%   |
| lymphoidal reticulum cells . . . . .             | 1.0 — 3.8%   |
| monocytes . . . . .                              | 0.0 — 1.2%   |
| plasmocytes . . . . .                            | 0.4 — 0.8%   |

Plate 51 illustrates the normal bone-marrow picture.

Granulocytes at various developmental stages are the most frequent forms, above all polymorphonuclears and staff cells. More immature cells, such as myelocytes and metamyelocytes are also encountered. The number of myeloblasts and promyelocytes amounts usually in normal adult bone marrow from 0.0 to 4.0% of granulocytes. Granulocytes are usually evenly distributed throughout the smears.

Erythroblasts are not always present in every field of vision, they are less numerous and show a tendency to occur in clusters ("erythropoietic nests"). Polychromatic and orthochromatic erythroblasts preponderate. Basophilic erythroblasts and proerythroblasts and proerythroblasts occur only sporadically. Mature normally stained red cells are abundant.

The preponderance of the granulopoietic series is also marked in normal bone marrow of infants. The number of myeloblasts and promyelocytes is, however, higher here than in adults. The lymphocytes are also more numerous, late erythroblasts are larger. Pioneer histological investigations of the foetal bone marrow were initiated by K. Horwitz-Kunciewiczowa in 1904.

## DECREASE IN GRANULOCYTE COUNT IN THE PERIPHERAL BLOOD

In healthy individuals, the leukocyte count ranges from 5,000 to 8,000 per cu.mm. Leukocytosis depends on various factors, such as: age, diurnal variations, physical exercise, nourishment etc. After meals, there appears "digestive leukocytosis".

Neuro-humoral regulation exerts a decisive influence on the level of leukocytes. Such generally known phenomena as emotional leukocytosis, or leukocytosis induced by conditioned reflexes, point to the importance of these mechanisms.

Variations in the leukocyte count in peripheral blood (leukopenia or leukocytosis) do not always depend solely on the activity of the bone marrow. The distribution of these cells throughout the body also plays an important rôle. For example they may be temporarily retained (stored) in the capillary bed of various organs, such as the spleen, lungs or liver. A temporary decrease in leukocytes after the administration of foreign protein may serve as an example of leukopenia of this type.

A decrease in the leukocyte count below 4,000 per cu.mm. is termed leukopenia. This symptom may, in addition to constitutional leukopenia, be encountered in various infectious diseases, poisoning, allergy and may accompany a number of haematopoietic disorders. In numerous instances leukopenia is of importance in both diagnosis and prognosis.

Leukopenia may appear as the result of increased destruction in the peripheral blood or as the consequence of disturbances in granulopoietic activity of the blood-forming tissues.

A wide range of different factors may cause an injury of the bone marrow and subsequent hypoplasia or aplasia of the granulopoietic tissue.

From the clinical point of view, acute or chronic forms of granulocytopenia are differentiated.

As in aplastic anaemia so in granulocytopenia, the pathogenetic mechanisms are extremely complex and only partly known. Certain injurious factors exert influence limited to the granulopoietic series only. Other factors, such as neoplastic proliferation originating in the bone marrow or neoplastic metastases, may depress the haematopoietic activity of the



entire blood-forming tissue. In recent years, much more attention has been devoted to the rôle of immuno-allergic mechanisms in the pathogenesis of granulocytopenia. It is important to remember in view of the above that granulocytopenia should be regarded as a symptom only, and not as a morbid entity.

Our knowledge concerning the aetiology of granulocytopenia is very scanty. It is, therefore, permissible so far to consider Werner-Schultz's syndrome of agranulocytosis as an idiopathic disorder. On the other hand, it should be borne in mind that in near future the gaps in our knowledge will be filled and the causes of "idiopathic" syndromes will be understood better.

Several drugs in common use may cause profound disturbances in granulopoiesis. The syndrome of agranulocytosis (aregeneratory agranulocytosis, *agranulocytosis asthenica*, *granulocytophthisis*, *granulocytopenia maligna*, *neutropenia maligna*, *agranulocythaemia*) is therefore of particular interest. Among drugs which may provoke agranulocytosis, the following should be mentioned: pyrimidon and related compounds, gold salts, arsene, mercury, quinine, barbiturates, streptomycin, chloramphenicol, sulphonamides (in particular sulphapyridine, sulphadiazine and sulphaguanidine), thiouracyl and its derivatives.

Individual sensitivity (idiosyncrasy) may be responsible for the development of granulocytopenia. On the other hand, it has been assumed that orally administered sulphonamides or thiouracyls may interfere with the normal growth of intestinal bacterial flora. These microorganisms synthesise folic acid or its conjugated compounds. It is generally known that folic acid is necessary for the normal maturation of granulocytes in the bone marrow.

Among other causes of leukopenia are infectious diseases: typhoid fever, diphtheria, miliary tuberculosis, syphilis, chronic rheumatoid arthritis, sepsis, malaria, kala-azar, exposure to X-rays or radium, and hypersplenism<sup>1</sup>.

Leukopenia is the most characteristic symptom of Werner-Schultz's syndrome. The leukocyte count ranges usually from 1,000 to 3,000 per cu.mm.; lower values — 50 to 150 leukocytes per cu.mm. — have, however, been recorded. The percentage of neutrophils is markedly diminished and ranges from 0.0 % to 20.0 %. The number of eosinophils and basophils

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<sup>1</sup> Kubiczek and Aleksandrowicz recently presented a new concept of "leukolytic-disease". They claim that among the causes of myeloblastic leukaemia may be the hyperactivity of leukolytic mechanisms. This is, according to these authors, accompanied by compensatory hyperplasia of granulocytoblastic tissues, which in certain conditions may assume a malignant character.

is also usually diminished being only in rare instances normal. In several personal cases, the present authors have observed a total lack of neutrophils, whilst from 3.0 to 5.0 % of eosinophils have been demonstrated. The eosinophilic granules of these cells were markedly abnormal — minute in size and of blurred outline; the cytoplasm was vacuolated. The present authors are of the opinion that the presence of eosinophils cannot be regarded as a favourable sign.

It should be added, that even in cases of total leukopenia it is only when the percentage of neutrophilic drops below 50.0 % that granulocytopenia can be diagnosed.

In peripheral blood smears, the lymphocytes constitute the majority of white cells. In some instances, single plasmocytes may be encountered. Monocytic reactions have also been noted. Degenerative changes of cytoplasm and nuclei may be observed in granulocytes (vacuolation, blurred nuclear structure, pycnosis, chromophobic granulation).

In typical cases, neither the erythrocytes nor the platelets demonstrate abnormalities. The reticulocyte count is normal.

The bone-marrow picture depends on the injurious agent, the regenerative capacity of the organism and the stage of the disorder.

Serial biopsies make it possible to evaluate the course of the disorder in spite of the manifold nature of the bone-marrow pictures. Bone-marrow biopsy also enables the ruling out of leukopenia caused by neoplastic proliferation, latent leukaemia or granulomatous changes.

In extreme cases, the bone-marrow changes consist in aplasia of granulopoietic tissue or in excessive proliferation of this tissue accompanied by arrested or abnormal maturation. The picture depends also on the degree of involvement of megakaryocytic and erythrocytic series. Although, as mentioned above, in typical cases both these myeloid systems remain unchanged, the transition from isolated granulocytopenia to pancytopenia is but gradual. Rohr classifies the bone-marrow pictures into the following categories:

1. Normal or nearly normal bone marrow; agranulocytosis is the result of disturbances in the release of granulocytes into the peripheral blood;
2. Bone marrow characterised by an increase in promyelocytes and myelocytes (arrested maturation);
3. Aplasia of granulopoietic tissue and reticulum-cells proliferation;
4. Aplasia of granulopoietic tissue and of the reticulum (a "dry" bone marrow);
5. Peripheral agranulocytosis caused by increased destruction of leukocytes in peripheral blood (leukolysis).

Plate 52 illustrates the bone-marrow picture in the course of granulocytosis (promyelocytic response — arrested maturation). Such a picture



may be difficult to differentiate from a latent leukaemia. Aleksandrowicz is of the opinion that "prolonged granulocytopenia accompanied by both a hyperplastic bone-marrow response with signs of arrested maturation should be classified as leukaemia, usually of the myeloblastic type". Clinical observation and serial bone-marrow biopsies are decisive in diagnosis.

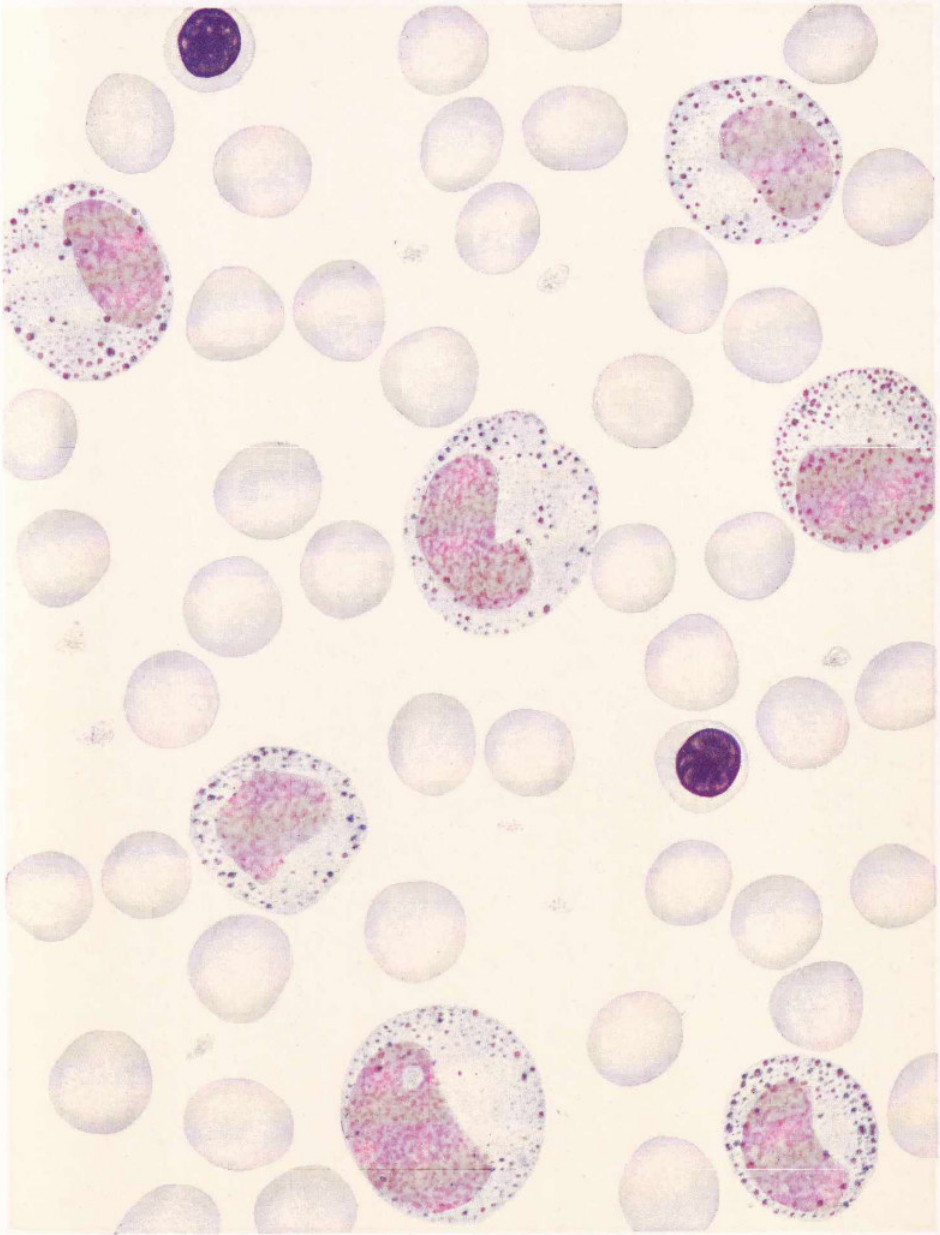
The utmost prudence is recommended in establishing the prognosis on the basis of a cellular or acellular bone marrow. Complete recovery in cases where total bone-marrow aplasia is present are known. Monocytosis in the peripheral blood is considered as a favourable sign (even in cases of maturation arrest).

When a diagnosis of agranulocytosis is made the following points should be taken into account:

1. Haematological findings: granulocytopenia and absence of marked changes in the erythrocytic and megakaryocytic system;
2. Meticulously gathered case history, in particular as to previous diseases and the treatment (drugs administered);
3. The disorder has been observed almost exclusively in adults between the ages of 25 and 60 years;
4. Complaints of marked weakness and rapid fatigue;
5. In acute cases; symptoms of a septic condition, necrotic tonsillar lesions, necrosis in the pharyngeal cavity, gums and mucosal membranes; in many instances, ulceration of rectal mucosa;
6. In acute cases, the spleen is not enlarged; in chronic granulocytopenia, splenomegaly may be frequently met;
7. No enlargement of lymph nodes; in certain instances, only the cervical and submaxillary lymph nodes are enlarged because of pharyngeal and oral lesions;
8. The absence of signs of a haemorrhagic diathesis;
9. Increased sedimentation rate of erythrocytes.

Latent acute leukaemia may be ruled out when there are no symptoms of anaemia, of haemorrhagic diathesis and single immature granulocytes do not occur in the peripheral blood.

In cases demonstrating a syndrome of granulocytopenia, anaemia and haemorrhagic diathesis aplastic anaemia should be suspected. In the course of this disorder, the presence of immature granulocytes in the peripheral blood is extremely rare.



*Plate 52.* — Agranulocytosis (bone marrow).

Maturation arrest of granulocytes at the stage of promyelocyte ("a promyelocytic reaction").

In addition to promyelocytes, two orthochromatic erythroblasts are present.

*Note:* mature red cells are normal; numerous platelets are present; the pathological process is limited to the granulocytic series only.





## CHAPTER IV

### THE MEGAKARYOCYTIC SYSTEM

#### THE DEVELOPMENTAL CYCLE OF THE MEGAKARYOCYTIC SYSTEM

In spite of extensive cytological research, the megakaryocytic system remains the least known among the three main myeloid cell series. Although comprehensive investigations have been carried out by numerous workers and despite the vast literature on the subject, megakaryocytes still present a poorly understood problem. It is known, however, that their most important function consists in the production of platelets. This theory, originally advanced by Wright (1906), has found ample confirmation in animal experiments, as well as in serological tests. Heilmeyer and Schlosshardt demonstrated that the peripheral parts of the cytoplasm of megakaryocytes and platelets both show identical properties of luminescence. Cytochemical methods also point to the megakaryocytic origin of platelets. It should, however, be emphasised that knowledge concerning the development and differentiation of these elements reveals considerable gaps. The lack of a uniform nomenclature for the individual developmental stages creates also additional difficulties.

Numerous observations indicate that megakaryocytes originate from a separate stem-cell, differing in many respects, particularly in size, from myeloblasts and proerythroblasts. This stem-cell is termed megakaryoblast.

This cell, from 20 to 40  $\mu$  in diameter, possesses a round or, in rare instances, slightly indented nucleus. It is poor in chromatin and reveals the presence of faint nucleoli. The cytoplasm is narrow, usually round and regular in outline, but may occasionally form pseudopodial processes. The cytoplasm is deeply basophilic agranular of dark blue colour. It seems that megakaryoblasts undergo a developmental cycle, during which the volume of the cell increases and the nucleus becomes slightly lobulated, or the cell may contain two nuclei.

Though the cytoplasm becomes more abundant, it remains distinctly basophilic. The recognition of a megakaryoblast offers considerable difficulties even to an experienced haematologist.



The next developmental form, termed promegakaryocyte, is from 30 to 50  $\mu$  in diameter. Basophilia gradually receding, the cytoplasm acquires polychromatic properties, whilst minute azurophilic granules appear in it. The nucleus is generally round, in other instances of irregular outline, or slightly indented. Its chromatin becomes more compact, the nucleoli are no longer visible.

The mature form — the megakaryocyte is from 50 to 100  $\mu$  in diameter. Its nucleus is irregular in shape, polymorphous, consisting of many lobes.

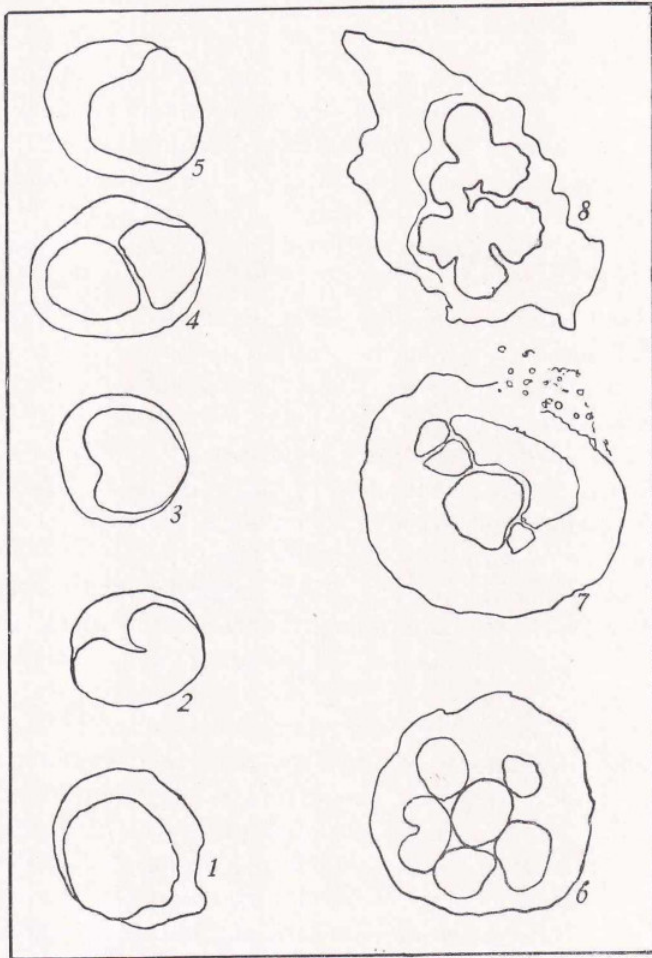
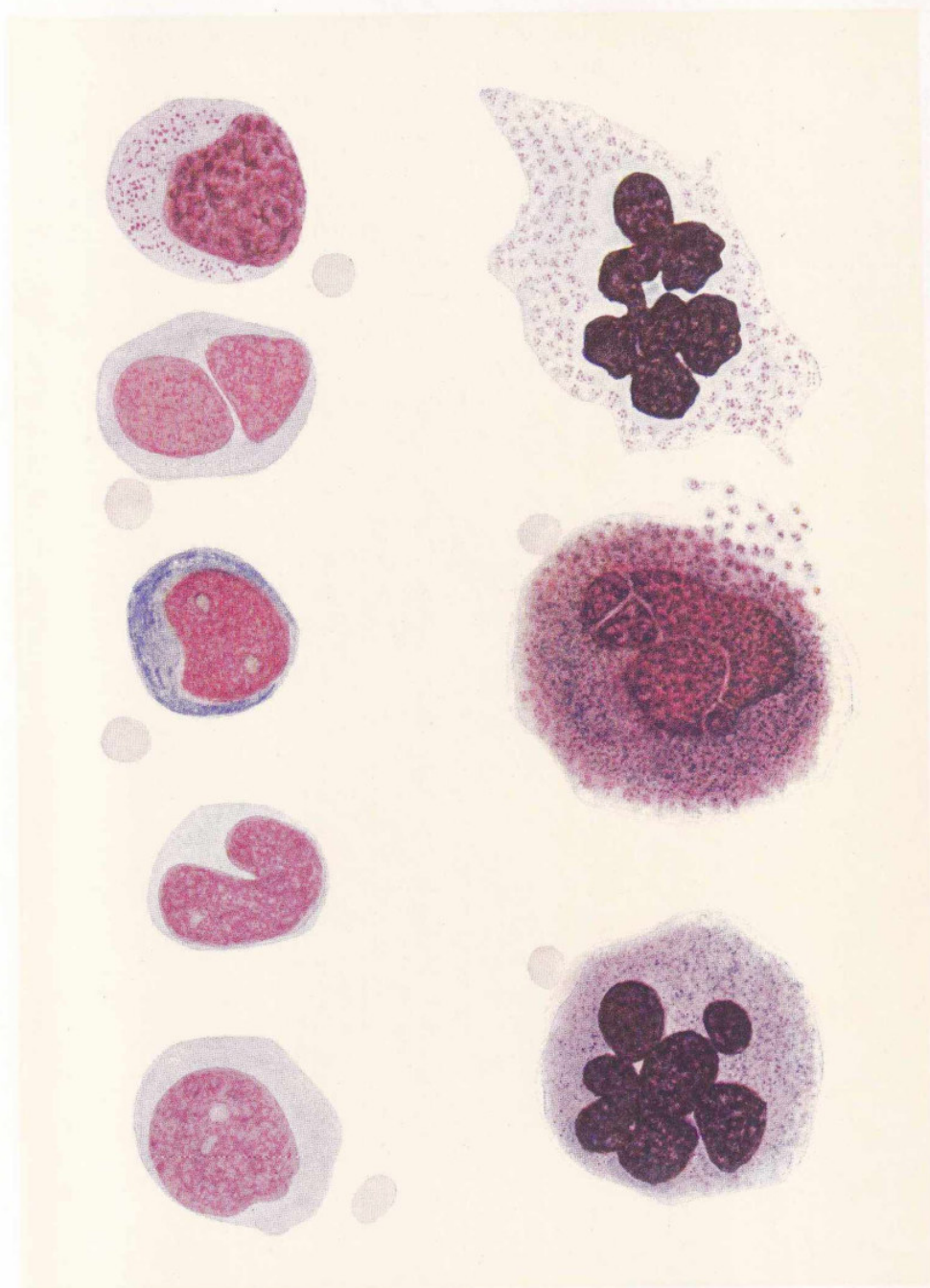


Plate 53. — The developmental cycle of megakaryocytes: 1, 2, 3, 4. Megakaryoblasts. 5. Promegakaryocyte. 6. Megakaryocyte with basophilic cytoplasm. 7. Megakaryocyte with acidophilic cytoplasm. 8. Involutive form of megakaryocyte.

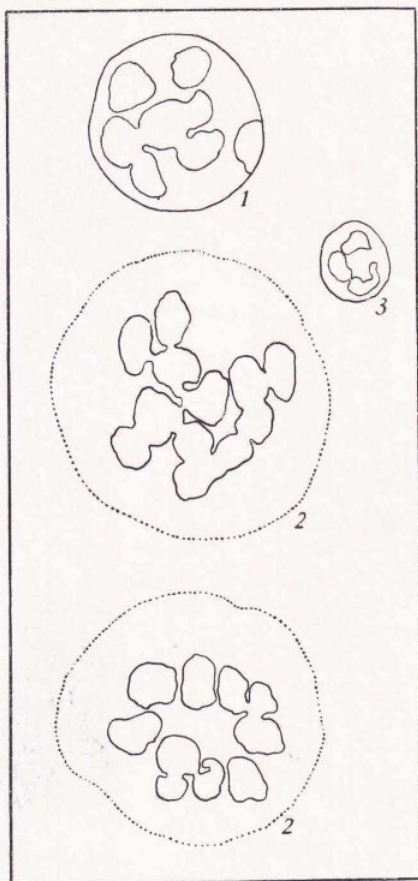
The size of the cells should be compared with that of the erythrocytes.



*Plate 53.* — The developmental cycle of megakaryocytes.

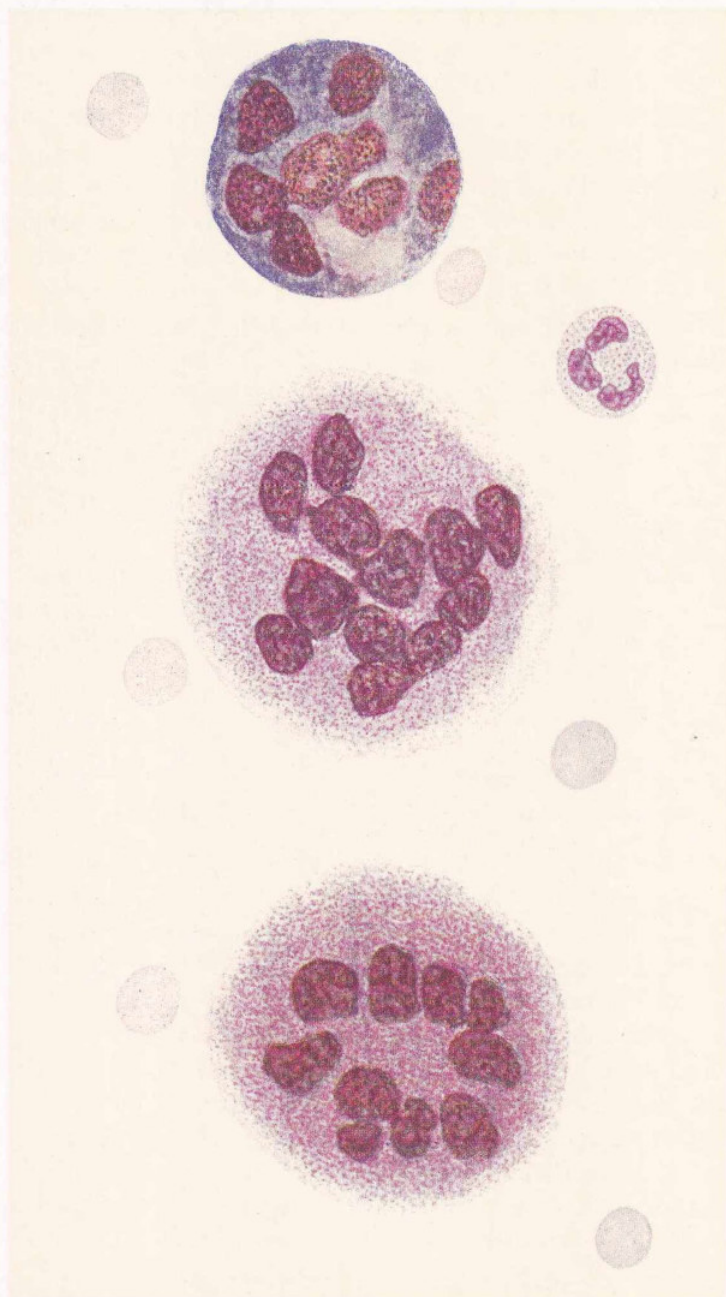


The nuclear membrane is sharply outlined. The nuclear structure is pycnotic, compact, revealing no distinctive features. In some instances, the nuclear lobes have a grape-like arrangement. They may also lack any orderly arrangement forming in irregular, twisted lumps extremely varied in shape. The cytoplasm is abundant and often of irregular outline. Immature forms retain a certain degree of basophilia; mature megakaryocytes possess a neutrophilic (amphophilic) pinkish cytoplasm, which contains a minute azurophilic granulation. In immature cells, the granules are evenly distributed. As differentiation progresses, they tend to mass, forming densely granular fields separated by strands of agranular cytoplasm.



*Plate 54.* — Megakaryocytes with hypersegmented nuclei: 1. Megakaryoblast. 2. Mature megakaryocytes. 3. Neutrophilic granulocyte.

Hypersegmented megakaryocytes should be differentiated from osteoclasts (polykaryocytes).  
cf. text.



*Plate 54.* — Megakaryocytes with hypersegmented nuclei.



This phenomenon is taken as a sign of incipient platelet formation. Cells at this stage form cytoplasmatic projections, which later become detached from the bulk of the megakaryocyte and give rise to platelets (Galinowski). Such projections demonstrate a distinct internal structure — a central granular and a peripheral hyaline zone, corresponding to the granulomere and hyalomere of the platelet (thrombocyte). In the light of more recent investigations, the formation of platelets is not restricted to mature megakaryocytes only. The early cells of this series may also demonstrate such an activity (V. de la Fuente).

It should be remembered that platelets, being markedly adhesive, may secondarily adhere to the surface of megakaryocytes. This phenomenon may cause serious difficulties in the evaluation of the platelet-forming activity.

Naked nuclei of megakaryocytes and involutive forms of these cells (characterised by total transformation of the cytoplasm into platelets) may be encountered in bone-marrow smears.

The number of megakaryocytes present in bone-marrow smears is given by different authors as from 0.03 % up to 4 % of all nucleated elements. According to Heilmeyer, various forms of megakaryocytes are represented in the following proportion:

|                             |          |
|-----------------------------|----------|
| megakaryoblasts . . . . .   | 0 — 5%   |
| promegakaryocytes . . . . . | 4 — 12%  |
| megakaryocytes . . . . .    | 46 — 76% |
| involutive forms . . . . .  | 28 — 30% |

In normal bone-marrow smears,  $1/4$  of all megakaryocytes are actively producing platelets,  $3/4$  are inactive.

The megakaryocytes are scarce and irregularly distributed in bone-marrow smears. The number of them as obtained by a bone marrow differential count (counting of 1,000 myeloid cells) is therefore unreliable and owes a great deal to chance.

Plate 53 illustrates the developmental cycle of megakaryocytes.

Plate 54. Megakaryocytes with hypersegmented nuclei. Such forms are often encountered in Addison-Biermer's disease. Their presence is one of the characteristic feature of this disorder and an aid to correct diagnosis. It should be emphasised that hypersegmentation of the nucleus may occur at an early stage — in megakaryoblasts.

An immature, basophilic giant bone marrow cell (probably a megakaryoblast) is shown at the upper part of Plate 54. The hypersegmentation of the nucleus institutes a pathological phenomenon. The remaining two cells are mature megakaryocytes with a marked nuclear hypersegmentation.

Serious difficulties are encountered in differentiation between megakaryocytes with hypersegmented nuclei and another variety of giant bone marrow cells — the osteoclasts (polykaryocytes). These do not belong to the haematopoietic system, but are bone destroying cells. They increase in number in the course of disturbances in the osseous tissue — such as *e.g.* bone fractures, *osteitis deformans*, *osteitis fibrosa cystica*, *rachitis* etc.

The cytoplasm of osteoclasts is basophilic, its peripheral parts may be azurophilic. These cells possess a considerable number of nuclei. The structure of oval or round nuclei constitutes the most characteristic feature of the polykaryocyte. Coarse chromatin strands arranged into a reticular network resemble the nuclei of reticulum elements. Distinct, large, blue single nucleoli can be seen in osteoclasts. Such a nuclear structure is entirely different from the fine threads of chromatin entwined in a delicate skein in megakaryocytic nuclei.

### THROMBOCYTOPENIA

Thrombocytopenia is the most commonly encountered disorder of the megakaryocytic system. Like anaemia, thrombocytopenia should be regarded as a symptom common to many different disorders and not as a morbid entity. Platelets are unstable in an extreme degree, they readily agglutinate and disintegrate. These properties cause serious difficulties in the enumeration of platelets. Numerous (over 100) methods have been devised for that purpose. Yet none of the results obtained by any of these methods can be considered as entirely reliable. Among these various procedures, Fonio's indirect method has been found by the present authors to be the most convenient in clinical practice. Normal values obtained by this method range from 200,000 to 350,000 of platelets per cu.mm. These values have recently been confirmed by direct counts performed with the use of phase-contrast microscopy (Discombe, Brecher and Cronkite).

A decrease in platelets (*e.g.* 70,000 per cu.mm.) may often be found accidentally. Such thrombopenia frequently gives rise to no clinical symptoms or haemorrhagic phenomena and remains undiscovered or is revealed by blood examination performed for other causes. Asymptomatic thrombopenia may occasionally persist for a long time; in other instances it may be transient and repeated examinations yield normal values. Nevertheless, the development of signs of haemorrhagic diathesis may (and should) be expected in cases of persistent asymptomatic thrombocytopenia (when the platelet count drops below 70,000 per cu.mm.). A decrease in platelets below critical values (30,000 to 50,000 per cu.mm.) is usually accompanied by prolonged bleeding time and impaired clot retraction.



The origin of thrombocytopenia may be extremely varied.

From the clinical point of view, the most important group of thrombocytopenias institute secondary or symptomatic forms.

These appear:

1. In the course of a number of infectious diseases (meningitis, miliary tuberculosis, sepsis, upper respiratory tract infections, typhoid fever, typhus, smallpox, subacute bacterial endocarditis);
2. In industrial and drug poisoning (organic arsenicals, gold salts, benzene, sulphonamides, chloromycetin, bismuth, iodine compounds, nitrogen mustards, sedormid, quinine);
3. In alimentary allergy;
4. In haematopoietic disorders (leukaemias, aplastic anaemia, Banti's syndrome, Gaucher's disease, Felty's syndrome and related syndromes; in some instances in sarcoidosis or tuberculosis of the spleen, lymphosarcoma of the spleen and other "hypersplenic" syndromes, Addison-Biermer's disease etc.);
5. In the course of neoplastic proliferation originating in the myeloid tissue or tumour metastases to the bone marrow;
6. In osteosclerosis;
7. During pregnancy (rather rare);
8. Exposure to ionising radiations;

### IDIOPATHIC THROMBOCYTOPENIA

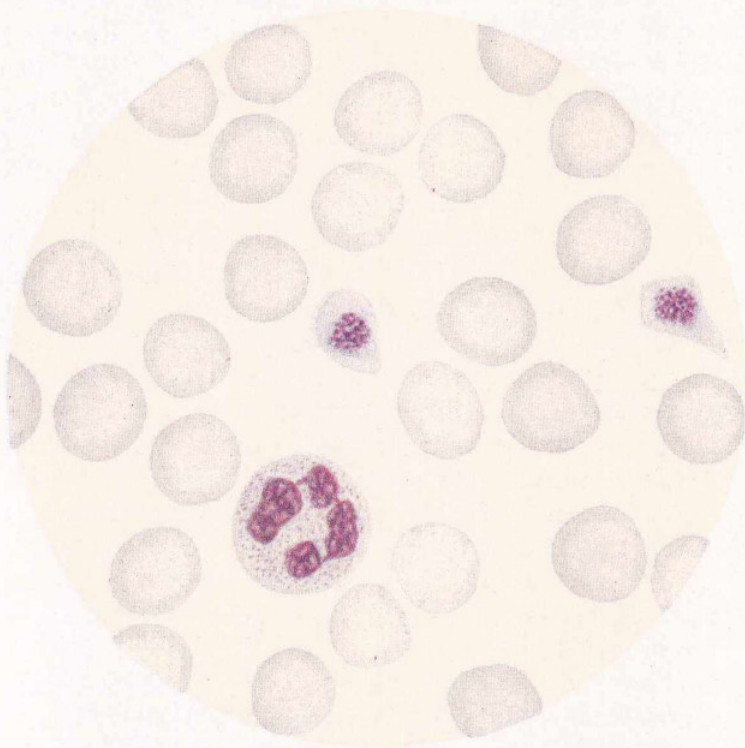
*Idiopathic Thrombocytopenic Purpura — Werlhof's Disease*  
*Essential Thrombocytopenia*

Idiopathic thrombocytopenia institutes a separate syndrome, occurring at times in several members of the same family.

Werlhof's disease is rarely encountered, it institutes, from 2 to 3% of all cases of haemorrhagic diseases. Correct diagnosis of this disorder is important from the therapeutic point of view. Examination of peripheral blood reveals:

1. Thrombocytopenia (decrease in platelets);
2. Prolonged bleeding time, the intensity of blood flow shows irregular fluctuations during the examination (diminishes and increases alternately);
3. Normal coagulation time;
4. Impaired clot retraction;
5. Deficient prothrombin consumption; although the prothrombin time determined by the one-stage method is usually normal, it may in certain cases be prolonged.

Among other symptoms, the tourniquet test (Konczalovsky, Rumpel-Leede) is frequently positive. The haemorrhagic symptoms may become



*Plate 55.* — Idiopathic thrombocytopenia (peripheral blood).  
Giant platelets are represented on the drawing.



evident as purpuric lesions of the skin (minute, red foci of haemorrhage, pinhead-size), as large suffusions (large, purple or violet areas) or as ready bruising. In women, persistent uterine bleedings (menorrhagia) may be sometimes the sole symptom. Such patients often seek advice of gynaecologists. In other instances the clinical picture may be dominated by epistaxis, gum bleedings or haemorrhages from the oral cavity.

The symptoms mentioned above are common in thrombocytopenia irrespective of its origin. They are not pathognomonic of Werlhof's disease. It should be emphasised that the presence of splenomegaly is more frequently encountered in secondary forms of thrombocytopenia (as, *e.g.*, in the course of such a systemic disease as Hodgkin's granuloma). Enlargement of the spleen is rather uncommon in idiopathic thrombocytopenia.

In cases of Werlhof's disease, the number of platelets is greatly diminished, and the apparent absence of these elements institutes one of the outstanding traits of peripheral blood smears. Platelet agglomerates are nearly always absent. The morphology of platelets is abnormal. They are irregular in shape and vary greatly in size. In some instances, sausage-shaped or sickle-shaped giant platelets are encountered. They stain inhomogeneously. Certain platelets may be basophilic and may contain coarse granules. A few microthrombocytes may also be present.

Two giant platelets are represented in the drawing (Plate 55). The red-cell picture is characterised by slight anisocytosis and poikilocytosis.

The leukocyte count is usually normal. Copious skin bleeding may be accompanied by eosinophilia. In several cases, the authors observed an increase in basophils.

Bone-marrow examination is helpful in the diagnosis of Werlhof's disease. It helps, moreover, in ruling out latent disorders of the haematopoietic system, such as acute aleukaemic leukaemia or neoplastic metastases, accompanied by thrombocytopenia.

It is generally held that among the characteristic features of the bone marrow in Werlhof's disease the foremost is an increase in megakaryocytes. Under low power, several megakaryocytes may often be present in a single field of vision. Such an increase indicates a hyperplasia of the megakaryocytic system. A more detailed examination reveals, in addition to quantitative changes, qualitative abnormalities of the megakaryocytic system. These abnormalities consist in disturbed maturation and degeneration of the megakaryocytes. Cells with a deeply basophilic, dark cytoplasm are numerous. The cytoplasm is often hyaline and almost completely agranular. In certain cells, however, a few azurophilic granules may be discerned. Vacuolation of the cytoplasm is very often encountered. The vacuoles are located on the periphery of the cell, giving the impression of a frilled, irregular border.



The nucleus is "primitive" in shape, and hyposegmented — only few nuclear lobes being present. Sometimes, the outline of the nucleus is regular and lobulation is entirely lacking. Cells with a granular, "mature" cytoplasm and "immature" nuclei (fine nuclear structure, the presence of nucleoli) may be encountered. Certain nuclei have blurred, irregular outlines. Platelet producing megakaryocytes are very scanty or even totally absent. The megakaryocytes, often of diminished dimensions, show a high degree of anisocytosis.

The presence of dwarf, deeply basophilic megakaryocytes is among the main features of Werlhof's disease.

The increase in megakaryocytes was, until recently, considered as one of the most reliable bone-marrow features in Werlhof's disease. Numerous observations recorded in the literature, as well as personal experiences, indicate however, that such a view is no longer tenable. The composition of the bone marrow obtained by puncture is greatly variable, the samples being taken at random, and the number of aspirated megakaryocytes depends largely on chance. On the other hand, a large group of clinicians base their indications for splenectomy on the increase in megakaryocytes. This standpoint should, however, be revised. Typical cases of Werlhof's disease in which the number of megakaryocytes is normal or even decreased are encountered.

It may therefore be considered that hyperplasia of the megakaryocytic system is not a constant symptom of Werlhof's disease.

These considerations lead to the conclusion that the diagnosis of Werlhof's disease should be based on qualitative changes in megakaryocytes, rather than on the number of in bone-marrow smears. With the object of gaining more insight into qualitative changes of megakaryocytes, cytochemical investigations were undertaken by Ławkowicz, Czerski and Porembińska. These authors performed several histochemical reactions on platelets and megakaryocytes of normal individuals and from patients with Werlhof's diseases, as well as cases of symptomatic thrombocytopenias of various origin. Findings of particular interest were obtained by means of Feulgen's reaction (test for the presence of desoxyribonucleic acid). It should be emphasised that the results of Feulgen's reaction depend on the mode of fixation and the time of hydrolysis employed. The above mentioned authors fixed the smears by heat, and hydrolysed for 3 to 4 minutes in 1 N HCl at 60°C ( $\pm 1^\circ$ ). Feulgen's reaction was negative in platelets and the cytoplasm of megakaryocytes of normal subjects and in cases of symptomatic thrombocytopenia. On the other hand, Feulgen's reaction was positive in the cytoplasm of megakaryocytes and in platelets in an appreciably large group of cases of Werlhof's disease (idiopathic thrombocytopenic purpura). Moreover, the number of Feulgen-positive



platelets varied from case to case and appeared usually in proportion to the severity of haemorrhagic symptoms. Among other cases studied, the platelets and the cytoplasm of megakaryocytes contained Feulgen-positive granula or was diffusely Feulgen-positive in several cases of bone-marrow aplasia (pancytopenia).

In Werlhof's disease, the erythropoietic and leukopoietic systems usually remain unchanged. In cases, accompanied by severe haemorrhages, these two systems present a picture corresponding to that described in the chapter on anaemia due to blood loss.

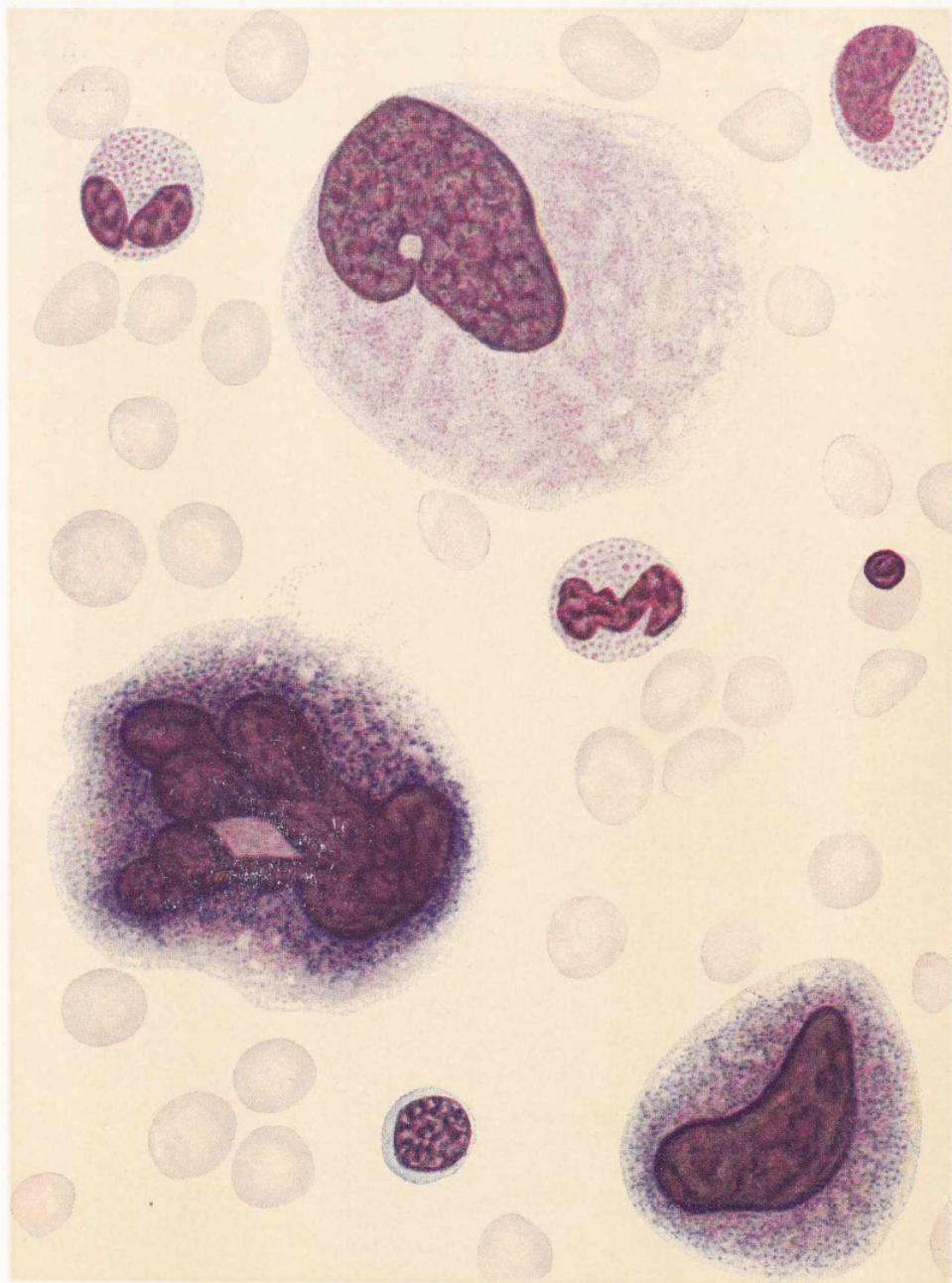
Plate 56 presents three megakaryocytes, which are distinctly abnormal. Two of these cells possess nuclei "primitive" in shape, one — a hypersegmented nucleus. The cytoplasm of two megakaryocytes is vacuolated. The granulation is blurred, unequal in size and irregularly distributed. The cytoplasm demonstrates a varying degree of basophilia. None of the megakaryocytes demonstrates platelet production.

The following points are essential in the diagnosis of Werlhof's disease:

1. Carefully collected history: previous diseases, drugs administered; toxic influences should be ruled out;
2. The disease develops most frequently in children or young adults;
3. The presence of haemorrhagic episodes, skin and mucous membranes without any inflammatory symptoms or febrile reactions;
4. Haematological findings;
5. Normal coagulation time and, usually, normal prothrombine time;
6. Absence of splenomegaly or lymph-node enlargement;
7. Absence of pathological cell elements in peripheral blood or bone marrow (except qualitative changes in megakaryocytes described above).

The bone-marrow picture in symptomatic thrombocytopenias is variable. The number of megakaryocytes is usually diminished, normal or increased in rare instances. Aplasia of the megakaryocytic system may be also encountered. Degenerative changes of megakaryocytes or maturation disturbances may be present. This depends on the degree of injury by the causative factor. In allergic thrombocytopenia, bone-marrow eosinophilia may be helpful in diagnosis. In cases of severe injury, morbid changes may be observed in all myeloid cell series.

Numerous new clinical and experimental investigations have contributed in recent years to a better understanding of the pathogenesis of thrombocytopenia. It now seems beyond doubt that in a group of cases humoral mechanisms are operative. This group, characterised by the presence of specific platelet antibodies causing thrombocytopenia, may be termed immuno-thrombocytopenia. Such cases, being secondary to abnormal immunologic phenomena should be considered separately from idiopathic thrombocytopenia. Thrombocytopenia may accompany acquired



*Plate 56.* — Idiopathic thrombocytopenia (bone marrow).



haemolytic anaemia (Evans and coll). It has also been possible to demonstrate immunologic mechanisms in thrombocytopenia due to sensitivity to certain drugs, such as for example sedormid, quinine or quinidine and others. There is a growing awareness that immunologic mechanisms may be responsible for various cytopenias of hitherto undetermined origin.

The investigations of the Russian haematological school have cast additional light on several pathogenetic aspects of thrombocytopenia.

According to B. P. Shvedsky, former pathogenetic concepts are one-sided, pertaining only to disturbances in the coagulation mechanisms. Coagulation phenomena were hitherto considered apart from the organism as a whole. This approach limited research in this field to phenomena occurring in the blood. Investigations by the Russian school followed an entirely different course. The haemorrhagic diseases are considered as the result of interaction between numerous endo- and exogeneous factors influencing the nervous system. Cases are known not only of haemorrhagic diseases (thrombocytopenia, vascular disorders), but even of pancytopenia, following neuro-psychical trauma. Numerous clinical observations demonstrate that this approach to the pathogenesis of haemorrhagic diseases deserves attention. Several problems, however, remain obscure, and elucidation of the rôle of the individual nervous receptors is needed before complete understanding of the reciprocal relationships is achieved.

Werlhof's disease may be taken as an example. It should be emphasised that thrombocytopenia does not constitute the sole or the most significant symptom of this disease. The pathological changes in the megakaryocytic system should not overshadow the complex disturbances in the entire organism.

In untreated cases, capillaroscopy demonstrates profound disturbances in the vascular system. It has been established that in cases of Werlhof's disease accompanied by severe haemorrhagic phenomena, the bleeding stops at the moment when the spleen vessels are clamped (viz. before splenectomy), while the platelet count still remains low.

Cases of persistent thrombocytopenia in spite of splenectomy are generally known.

Extensive modern clinical examination reveals in certain cases of Werlhof's disease deep trophic disturbances in the skeletal system (osteoporosis of dental alveoli), in the skin and subcutaneous tissue (B. P. Shvedsky). The trophic lesions in the vascular system depend on the degree of disturbances in the nervous system. These observations open new ways of approach to the problem of haemorrhagic diseases and offer new opportunities for research.

## CHAPTER V

# LYMPHOCYTES

### THE DEVELOPMENTAL CYCLE OF LYMPHOCYTES

In normal conditions, the lymphocytes originate in lymphoid tissue dispersed throughout the tissues of the entire body. In the course of the development and maturation of lymphocytes, no distinct stages can be discerned, which makes investigations over the course of these phenomena difficult. Additional difficulties arise from the fact that the morphological features of lymphocytes vary with their functional activity. Immature lymphocytes are rarely encountered in peripheral blood; usually, only in the course of acute or chronic lymphocytic leukaemia. The recognition of lymphocytes is not always easy. In spite of their apparently simple structure, they may resemble numerous cells of an entirely different origin.

Our knowledge about the morphology of the lymphoid system is derived chiefly from lymph-node biopsy. Among bioptic procedures, the ingenious method of two Polish authors (Kubacki and Wolański) deserves special mention. By puncturing the tonsils these authors could obtain material rich in lymphoid tissue for cytological examination, in cases where no superficial lymph nodes are enlarged.

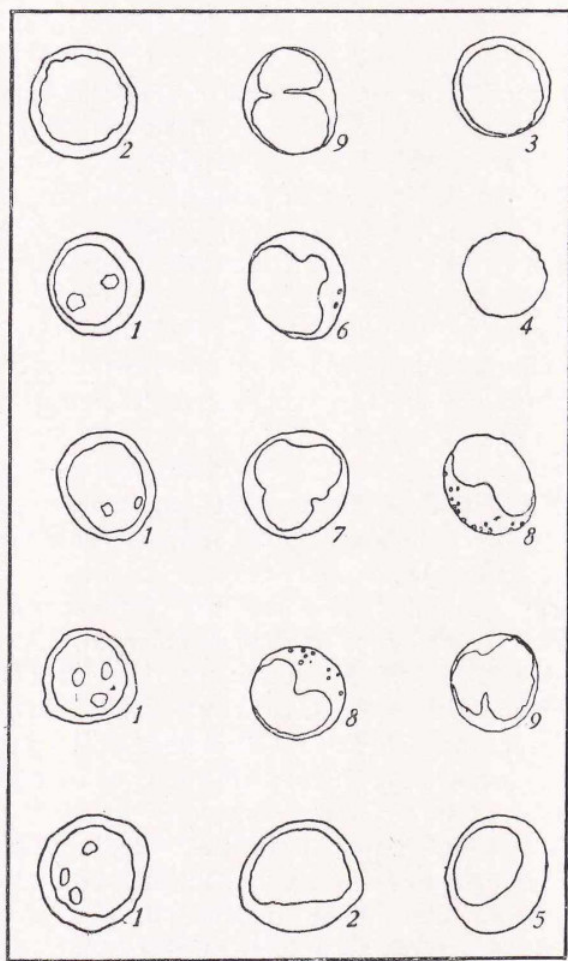
Bioptic procedures have contributed greatly to the elucidation of lymphocytogenesis. The lymphocytes originate from lymphoblasts.

Lymphoblasts are from 10 to 15  $\mu$  in diameter. The nucleus is round, poor in chromatin, stains lightly and contains one or two nucleoli. Although the number of nucleoli is generally considered of use in differentiation between lymphoblasts and myeloblasts, this feature may, nevertheless, be misleading. The cytoplasm is basophilic and contains no granules. Its colour ranges from greyish-blue to dark blue.

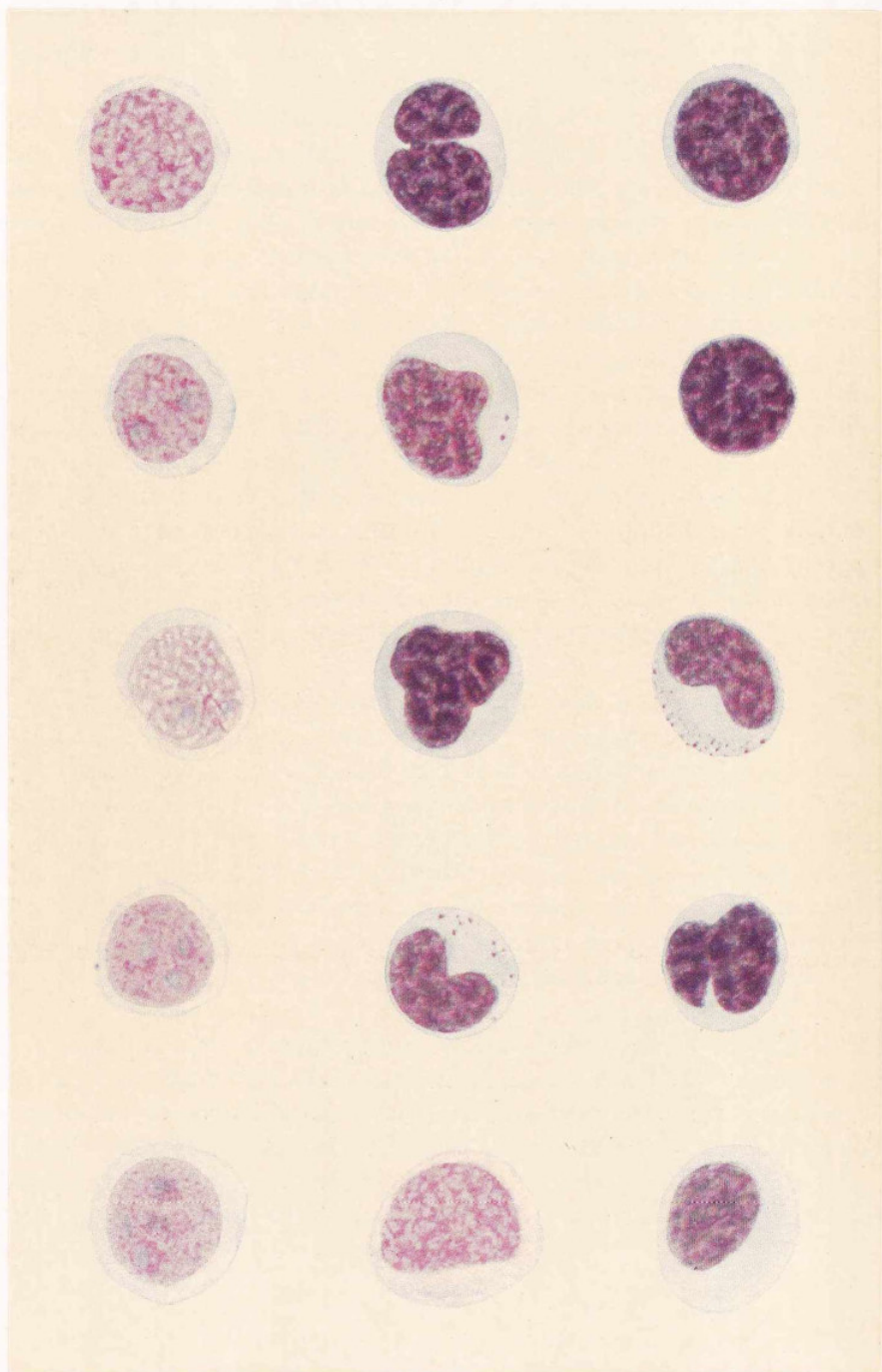
The prolymphocyte represents a more mature stage. The nucleus is round and because of a denser structure than that of the nuclei of lymphoblasts, stains more deeply. Indistinct nucleoli may be present. The cytoplasm is narrow, stains greyish-blue and may contain single azurophilic granules. The size of prolymphocytes is variable.



The most frequently encountered form of mature lymphocytes in peripheral blood is the "small lymphocyte". In size it corresponds to that of a red cell, or is only slightly larger. The darkly stained nucleus is usually round, in some instances oval or slightly indented. The nuclear structure is compact, pycnotic of dark violet colour. The chromatin arrangement is not always discernible, light irregular zones or a wheel-spoke pattern — being occasionally seen. The nucleus is excentric, showing a narrow, semilunar rim of light cytoplasm.



*Plate 57.* — Developmental cycle of lymphocytes: 1. Lymphoblast. 2. Prolymphocyte. 3. Typical mature lymphocyte. 4. Naked lymphocytic nucleus. 5. Large lymphocyte. 6. Prolymphocyte with an atypical nucleus (Rieder-form). 7. Mature lymphocytes with atypical nuclei (Rieder's cells). 8. Rieder's cells with azurophilic granulation in the cytoplasm. 9. "Amitotic" lymphocyte or "haematogonium".



*Plate 57.* — Developmental cycle of lymphocytes.



The cytoplasm is agranular or may contain a few azurophilic granules. These may serve as an aid to recognising lymphocytes. Abundant azurophilic granulation is but very rarely encountered in lymphocytes.

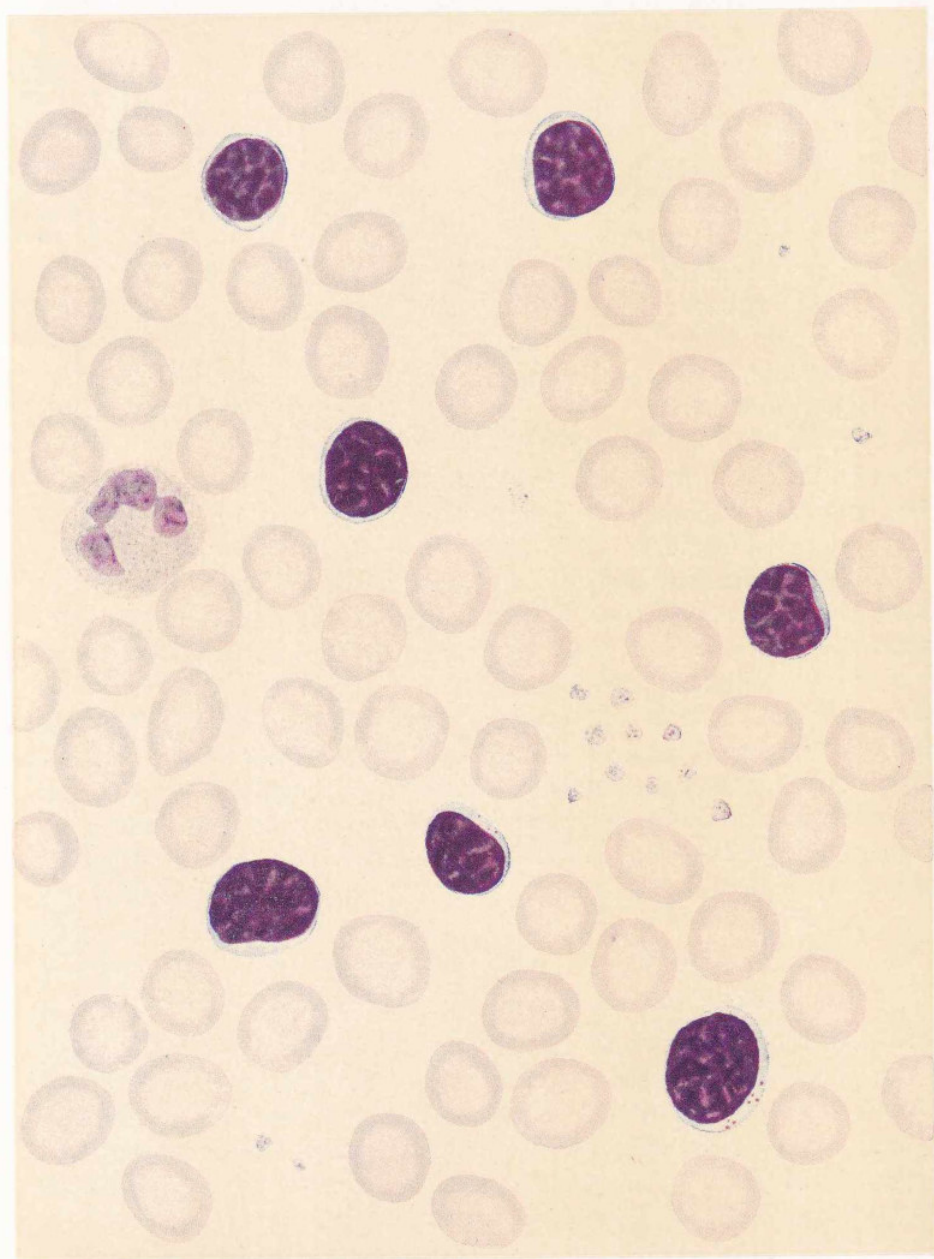
The large lymphocyte is another form present in peripheral blood. This cell is 10—12  $\mu$  in diameter. The nucleus has a loose structure and stains lightly. The zone of cytoplasm is much broader than that of small lymphocytes. It stains light blue and may contain azurophilic granules. Sometimes difficulties occur in differentiation between large lymphocytes and monocytes. In normal conditions, large lymphocytes are present in markedly smaller quantities than small lymphocytes. Opinions as to the functional significance of these lymphocyte varieties differ widely. Certain investigators maintain that the leptochromatic nucleus of large lymphocytes should be considered as a sign of immaturity. Another school of thought claims that large lymphocytes derive from a stem-cell common to them and to monocytes, while small lymphocytes originate from the lymphoid system. A third group of authors determines the degree of maturity of lymphocytes only by the basophilia of their cytoplasm. Observation of cells surviving *in vitro* support the belief that the large form, the small form and naked lymphocytic nuclei represent successive stages of ageing of the lymphocytes (Czerski, Ławkowicz and Porembińska).

Atypical lymphocytes may be encountered in peripheral blood. Among them are naked lymphocytic nuclei (round, markedly pycnotic nuclei with no visible cytoplasmatic rim) and Rieder's cells. These possess kidney-shaped or bizarre indented nuclei. A Rieder's cell with a bilobed nucleus may be encountered, though rarely. Such a cell is sometimes termed "amitotic lymphocyte" or haematogonium (N. Rosenthal). The cytoplasm of such cells may be vacuolated. The number of Rieder's cells becomes increased in pathological conditions.

Lymphocytes are peroxidase-negative and stain very faintly with Sudan black B.

Little is known about the rôle of lymphocytes in the organism. They may be encountered in mucous membranes and inflammatory foci, where they are presumably concerned in defence mechanisms of the organism.

Several authors have ascribed to the lymphocytes the synthesis of gamma-globulins and antibodies. Recent investigations, however, point to the plasmocytic series, as the source of antibodies and gamma-globulins.



*Plate 58.* — Lymphocytosis in peripheral blood. Case of whooping-cough.



## LYMPHOCYTOSIS IN PERIPHERAL BLOOD

Lymphocytosis may develop in certain physiological states and in a wide range of pathologic conditions, the clinically most important of which are:

1. Infancy;
2. Following ingestion of fats;
3. Certain infectious diseases, such as whooping-cough, rubeola, mumps (epidemic parotitis), infectious mononucleosis and undulant fever;
4. Reconvalescence following infectious diseases;
5. Lymphocytic reactions in children during infection with pyogenic microorganism;
6. Tuberculosis during periods of increased immunity;
7. Hyperthyroidism;
8. Lymphocytic leukaemia;
9. Acute and chronic infectious lymphocytosis;
10. Following exposure to X-rays or radium;
11. Congenital syphilis;
12. Relative lymphocytosis accompanying granulocytopenia.

The total leukocyte count in the course of whooping-cough may be high (from 15,000 to 200,000 per cu.mm.), the lymphocytes instituting up to 70 % of white blood cells.

On the drawing (Plate 58) are to be seen mature small lymphocytes, the cytoplasm of several cells contains azurophilic granules. Since in lymphatic leukaemia granular lymphocytes occur rarely, the presence of granules may aid in differential diagnosis of lymphocytosis. Moreover, chronic lymphadenosis is characterised by the presence of naked lymphocytic nuclei and Gumprecht's cells (cf Plate 78).

## INFECTIOUS MONONUCLEOSIS

*Filatov-Pfeiffer Glandular Fever — Angina Monocytica*

The haematological picture of infectious mononucleosis is characterised by the presence of markedly atypical cells. This disease is usually accompanied by leukocytosis ranging from 10,000 to 20,000 per cu.mm. From 50 up to 90 % of leukocytes institute mononuclear cells of varied morphological features. Typical small lymphocytes, large lymphocytes, "monocytoidal lymphocytes" and intermediate forms are encountered. The origin of these cells is not clear and opinions of individual haematologists concerning it differ widely. It has been suggested that the haematological picture of infectious mononucleosis comprises two reactions — a lymphocytic and a monocytic response, the monocytes disappearing from the peripheral blood at an earlier stage of the disease. Lymphocytosis may persist for a period of many months after recovery.

The white-cell picture of infectious mononucleosis is markedly variable. In certain instances, typical lymphocytes or Rieder's cells may preponderate. In addition, there are encountered large cells with indented monocytoidal nuclei and a varying degree of basophilia of the cytoplasm. The cytoplasm is agranular or may contain large azurophilic granules, such as are found in lymphocytes. Only rarely are minute azurophilic granules characteristic of monocytes present. Such cells are termed "monocytoidal lymphocytes", "monocytoids" or "lymphomonocytes". The fact that they are peroxidase-negative may be regarded as standing in opposition to their monocytic origin, and supporting the hypothesis that they are pathologically changed lymphocytes.

The cytoplasm of certain such cells is distinctly basophilic and often vacuolated. In some instances they resemble plasmocytes though their nuclei are abnormal in shape — kidney-shaped or indented. Kassirsky distinguishes three varieties of the haematological picture of infectious mononucleosis: lymphocytic, plasmocytic and monocytic.

Downey and MacKinley divided the cells characteristic of infectious mononucleosis into three categories. Type I is a cell with an excentric nucleus, indented, of irregular outlines, in some instances lobulated. The cytoplasm is basophilic and vacuolated. Type II resembles large lymphocytes differing from them chiefly in chromatin arrangement. The cytoplasm demonstrates alternating chromophobic and basophilic stripes. Type III, rarely encountered, resembles type I, but its nucleus contains 1 or 2 vesicular, blue nucleoli. The latter type is extremely difficult to differentiate from cells occurring in acute stem-cell leukaemias.

In diagnosis of infectious mononucleosis, the following points should be taken into account:

1. The disease develops usually in children or juveniles;
2. The onset is acute, accompanied by fever and malaise;
3. Lymph-node enlargement is among the most frequent symptoms (usually cervical lymph nodes posterior to the sterno-mastoid muscle); in contradistinction from leukaemia, the lymph nodes are usually tender;
4. Slight spleen enlargement — in cases where the spleen is not palpable, it is enlarged on percussion (Kassirsky);
5. The clinical symptoms may be extremely varied (the classic glandular, the anginose or the febrile form, the type, characterised by indefinite general symptoms, and other clinical variants less frequently encountered);
6. The haematological picture is usually typical; the specific mononuclear cells may be readily differentiated from leukaemic elements. The course of the disease is usually mild, recurrences being sometimes observed;



7. In the great majority of cases, Hanganatzu-Deicher's or Paul-Bunnell's test is positive. This test is considered positive when the titer of heterophil antibodies is above 1:64. Unspecific positive results may be obtained in individuals treated previously with horse serum. A negative result does not rule out infectious mononucleosis. In from 6 to 10%, and according to certain authors (Kleomota after Heilmeyer) in as much as 20% of typical cases, the serological tests are negative;

8. The lymph node aspiration smear is characteristic (Czerski and Roszkowski, Hoerster). Typical endothelial "monocytoidal" and large reti-

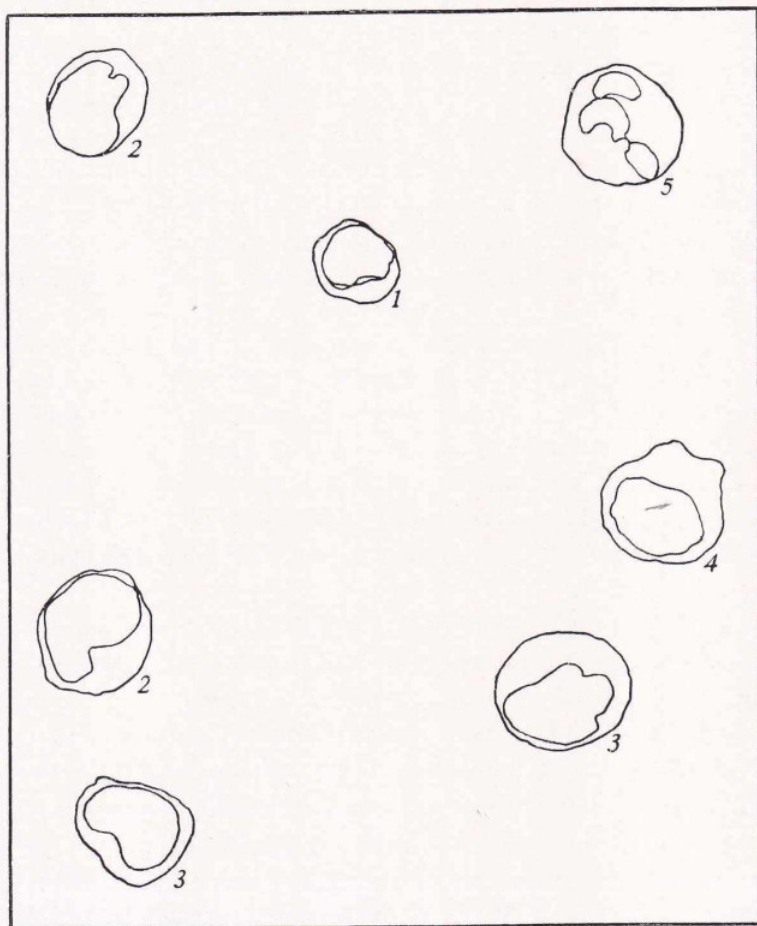
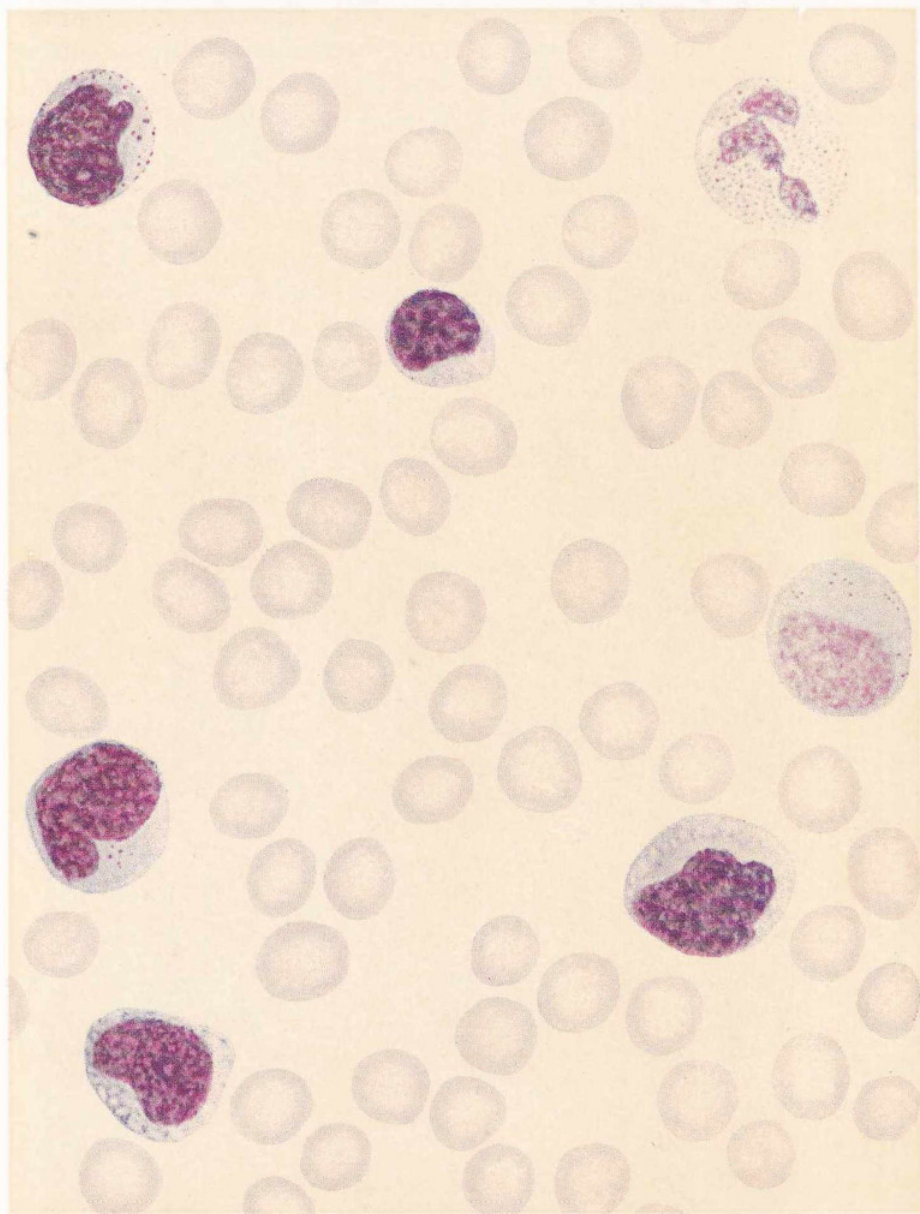


Plate 59. — Infectious mononucleosis (peripheral blood): 1. Small lymphocyte. 2. "Lymphomonocyte", cells of morphological features intermediate between a lymphocyte and a monocyte, the cytoplasm contains azurophilic granulations. 3. Similar cells with a distinct vacuolation of the cytoplasm; such cells are among the most characteristic features of the peripheral blood picture in this disease. 4. Lymphoid reticulum cell. 5. Neutrophilic granulocyte.



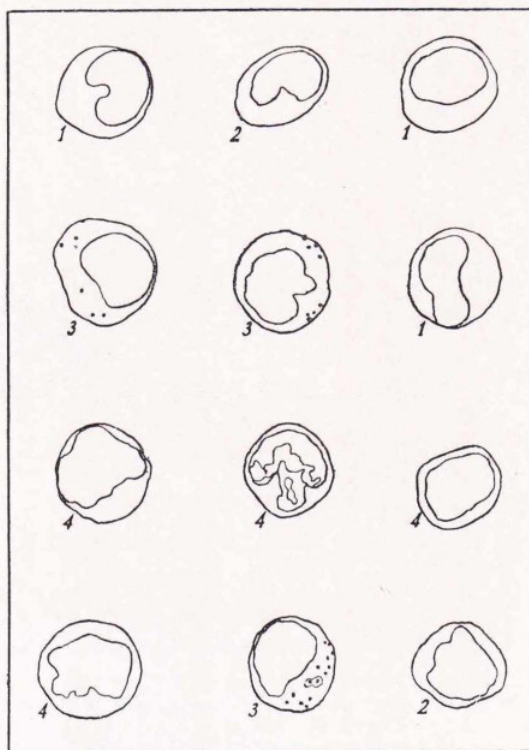
*Plate 59.* — Infectious mononucleosis (peripheral blood).



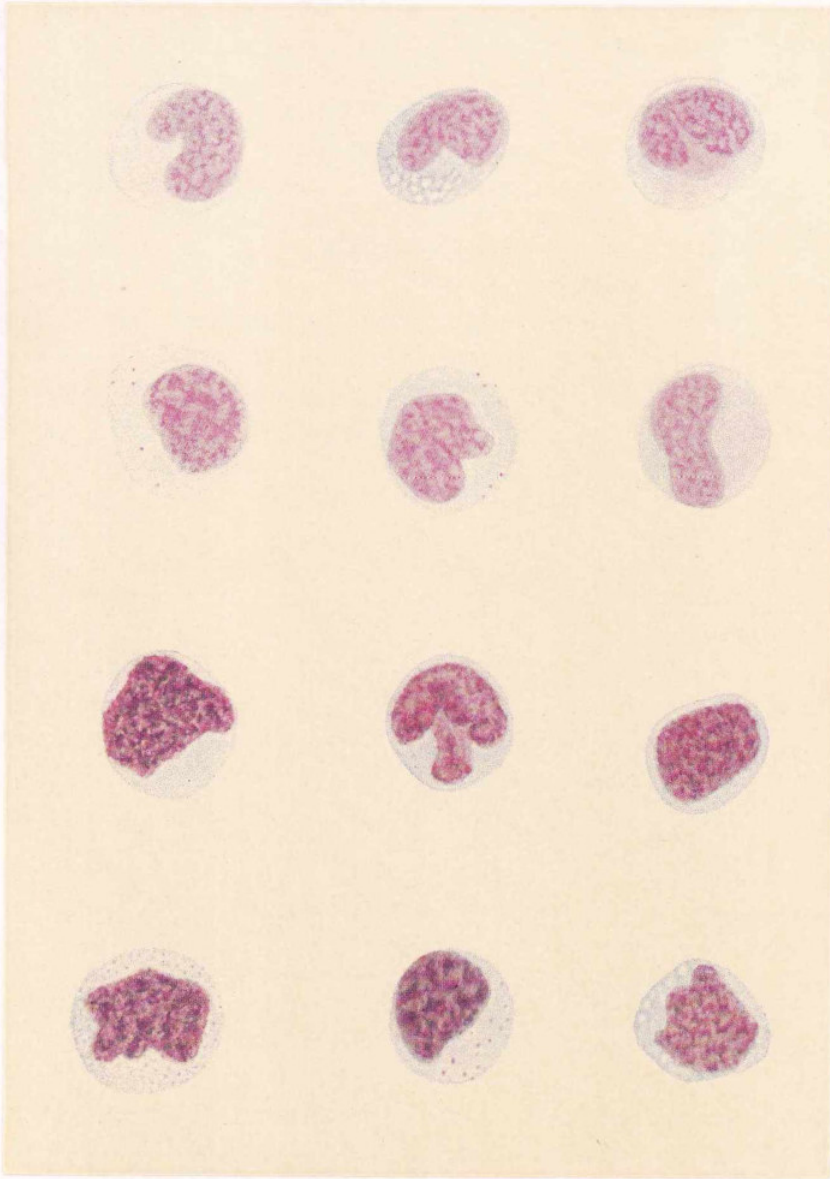
culum cells dominate the picture. Moreover, numerous developmental forms of the lymphocytic system are encountered. Because of the diversity of cell types, the smears present an extremely variegated picture.

A similar syndrome, probably also of viral origin, is infectious lymphocytosis. This may, however, in contradistinction from infectious mononucleosis, remain entirely without clinical symptoms. Infectious lymphocytosis is occasionally diagnosed on chance blood examination. The blood picture is characterised by leukocytosis up to as much as 100,000, small lymphocytes (70—90 %) predominating. Slight eosinophilia is also frequently present. This syndrome, known as Carl Smith disease (1941), was first observed by Kassirsky in 1938, and described by E. S. Karpienko (1940) as "leukocytosis with lymphocytosis in healthy children".

The course of the disease is mild. Usually there is no lymph node or spleen enlargement. This syndrome, occurring almost exclusively in children appears in adults only in exceptional cases.



*Plate 60.* — Several varieties of blood cells occurring in peripheral blood in infectious mononucleosis: 1. "Monocytoidal" cell. 2. Similar vacuolated cells. 3. "Lymphomonocytes" demonstrating distinct azurophilic granulation. 4. Cells, characterised by an abnormal structure and bizarre shape of the nucleus.



*Plate 60.* — Types of mononuclear cells encountered in infectious mononucleosis.





## CHAPTER VI

# MONOCYTES

## THE DEVELOPMENTAL CYCLE OF MONOCYTES

The development of monocytes has been one of the most disputed problems in haematology. Arguments about their myeloid or lymphoid origin are a thing of the past, but even now the theories concerning these cells are being constantly modified. It seems to be established beyond doubt that monocytes, formerly regarded as transitional forms between granulocytes and lymphocytes, are not directly connected with either of these series and institute a separate blood-cell type. Their morphological features — basophilia of the cytoplasm and fine nuclear structure — may be regarded as a sign of a near relationship to undifferentiated reticulum or endothelial cells.

In pathological conditions, the monocytes may undergo profound changes to such an extent as to make recognition extremely difficult. Numerous observations indicate that monocytes may be transformed into tissue histiocytes. It is held that these two morphologically distinct cell types are only different functional stages of the same cell. The theory that monocytes originate from reticulum and endothelial cells is amply documented by experimental studies, as well as by bioptic investigations.

A certain group of authors considers that monocytes possess a developmental cycle, describing the monoblast as their stem-cell. The cell is from 10 to 22  $\mu$  in diameter with a large nucleus, poor in chromatin and containing several nucleoli. The basophilic agranular cytoplasm surrounds the nucleus with a narrow zone. The monoblasts are peroxidase-negative and it is frequently impossible to differentiate them from myeloblasts. The presence of more mature stages and of typical monocytes may serve as an aid in recognition of monoblasts.

A cell, termed promonocyte, is described as the next developmental stage. This is similar in size to the monoblast; the nucleus is indented, of fine reticular structure, and nucleoli are invisible. The cytoplasm is basophilic, contains minute azurophilic granules and is much broader than in stem-cells. Promonocytes are also peroxidase-negative.



Mature monocytes are the largest among the normal blood cells. They range in size from 15 to 20  $\mu$ . The nucleus is leptochromatic, of finer structure than in granulocytes or lymphocytes. It is kidney- or horse-shoe shaped. The nucleus often has several indentations; it may even be lobulated or present an appearance of a complicated contorted mass. It is, however, always fine in structure. The cytoplasm is abundant, greyish-blue, opaque (by contrast to the clear blue of the cytoplasm of lymphocytes). It contains numerous dust-like azurophilic granules. No whitish perinuclear zone (the region near the indentation — “Kernhof” excepted) may be observed. A few vacuoles may in some instances be present. The outlines of the cell are irregular (Plate 61). The monocytes are usually faintly peroxi-

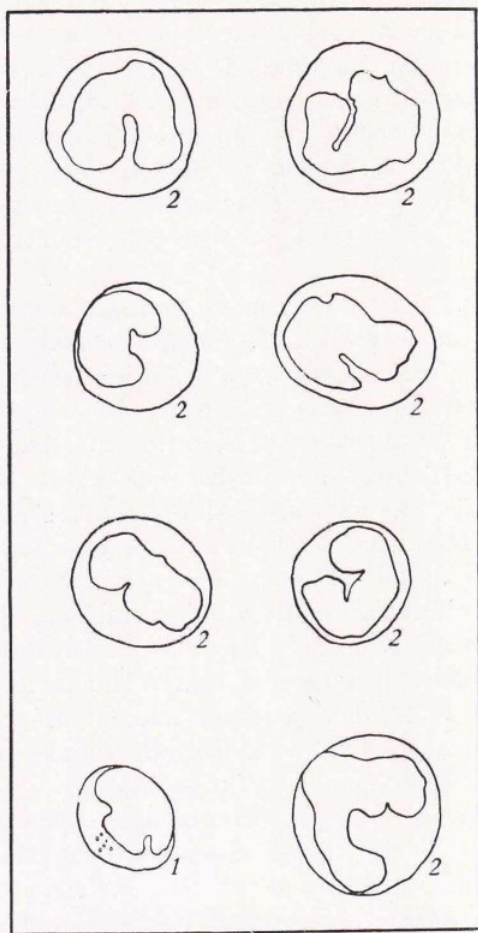
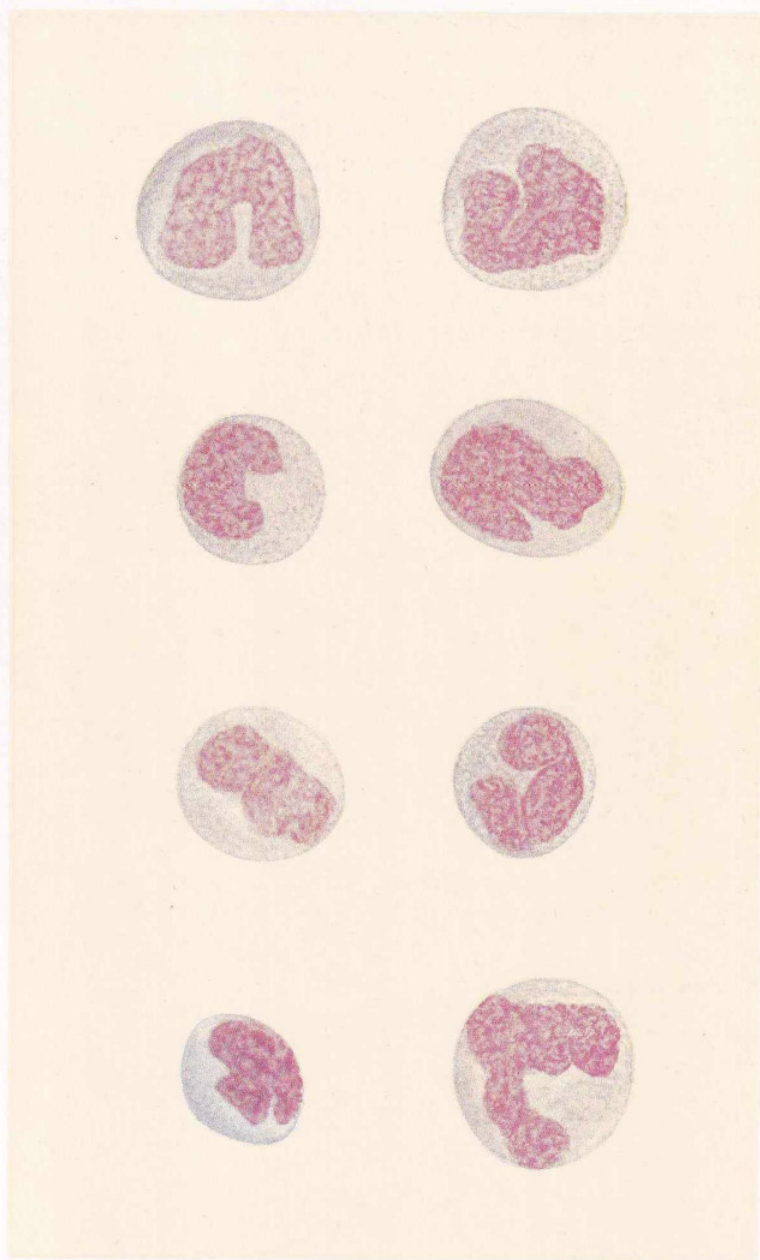


Plate 61. — Monocytes: 1. Monocytoidal cell (promonocyte ?). 2. Mature monocytes.



*Plate 61. — Monocytes.*



dase-positive. From 1 to 4 % of monocytes are peroxidase-negative (Undritz). In old blood smears stored for 30 to 40 days, all monocytes are peroxidase-negative, whilst peroxidases of promyelocytes, myelocytes and more mature granulocytes retain their activity. This property of monocytes may be used as a differential diagnostic test.

Intravital staining reveals the presence of a characteristic rosette of neutral red vacuoles. Such staining is seldom used in routine practice, except at a few haematological centres (Doan, Sabin, Cunningham). This method was introduced to haematology in Poland by J. Zeyland and is used in the Institute of Haematology in Warsaw as supplementary to phase-contrast microscopy.

The monocytes play a rôle in the defence mechanisms of the organism, monocytic reactions indicating the functional state of the reticulo-endothelial system. The monocytes demonstrate phagocytic and storing properties.

### MONOCYTOSIS IN PERIPHERAL BLOOD

Monocytosis may develop in various diseases of which the clinically most important are:

1. Certain infections, as for example: tuberculosis, late syphilis, subacute bacterial endocarditis, typhus fever, Bang's disease;
2. Infectious diseases, when acute symptoms are subsiding;
3. Certain forms of agranulocytosis;
4. Malaria, kala-azar and other diseases caused by *Protozoa*;
5. Hodgkin's disease;
6. Reticulo-endothelial with storage of pathological lipids (so-called "storage-diseases" — "tezauroses"), such as Gaucher's disease, Niemann-Pick disease etc.
7. Monocytic leukaemia.

## CHAPTER VII

### RETICULO-ENDOTHELIAL CELLS OF THE BONE MARROW

The reticulo-endothelial cells of the bone marrow are derived during foetal life from primitive mesenchyme and give rise to the matrix of blood-forming tissue. The cells of the reticulo-endothelial system form the stroma of the bone marrow and line the inner walls of sinuses and vessels. These cells are endowed with ability to phagocytise, store and metabolise colloids, colloid stains, compounds of various origins, decomposition products of body proteins etc. These properties enable the cells of the RES to play an important rôle in the defence mechanisms of the organism, in immunological phenomena and reparative processes. The repartition of the RES along the blood and lymph vessels is particularly adapted to its defensive functions — the destruction and neutralisation of bacterial and other toxic agents.

It is difficult to ascertain to what extent the cells of the RES retain in adult life their blood-forming properties. It seems beyond doubt, however, that in special conditions the primitive reticulum cells may give rise to highly differentiated blood cells.

In spite of the importance of the RES, knowledge concerning its cells is scanty and not so long ago many haematologists simply did not take it into consideration. Many valuable facts have been obtained by tissue culture methods (Maximov). The development and general use of bioptic procedures make possible new investigations on fresh, unchanged cells.

Reticulum cells are readily destroyed during the preparation of bone-marrow smears. Their fragility makes it difficult to ascertain the exact morphology of these cells. The fact that reticulum cells of the bone-marrow stroma are syncytial, creates additional difficulties, since in such cases only clusters of nuclei may be seen.

Rohr, whose extensive researches contributed a great deal to our knowledge concerning reticulum cells, classifies them into the following types:



1. Undifferentiated, small lymphoidal cells, which are probably functionally inactive ("resting") stages of repose (Tempka and Aleksandrowicz); the Russian school (Alekscev) maintains that these cells are identical with haemocytoblasts;

2. Large lymphoidal cells, which occur only rarely in normal bone marrow; these cells are, on the other hand, frequently encountered in hyperactive states, such as pancytopenia, agranulocytosis, Addison-Biermer's disease and related disorders, haemolytic and other anaemias, acute and chronic infections; these cells are variously termed by different investigators (primitive connective tissue cells, histiocytes, reticulocytes, undifferentiated reticulum cells, reticulum cells proper);

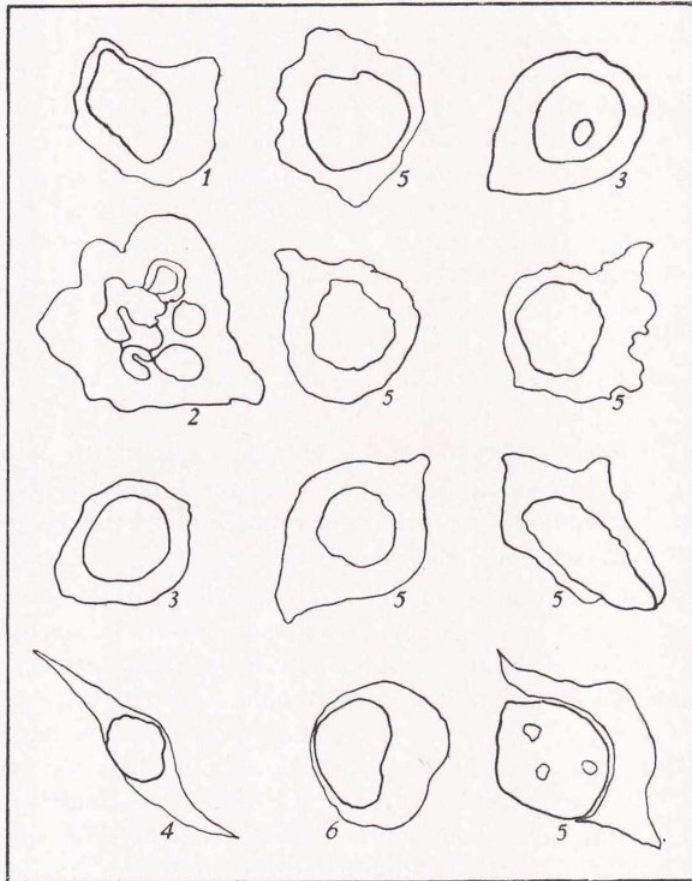


Plate 62. — Bone marrow reticulum cells: 1. Reticulum cell differentiating into a macrophage. 2. Typical macrophage (tissue macrophage). 3. Proper reticulum cell. 4. Reticulum cell — reticulocyte forming the stroma of the bone marrow. 5. Granular reticulum cells (basophilic and acidophilic reticulum cells — histiocytes). 6. Large lymphoidal reticulum cell.

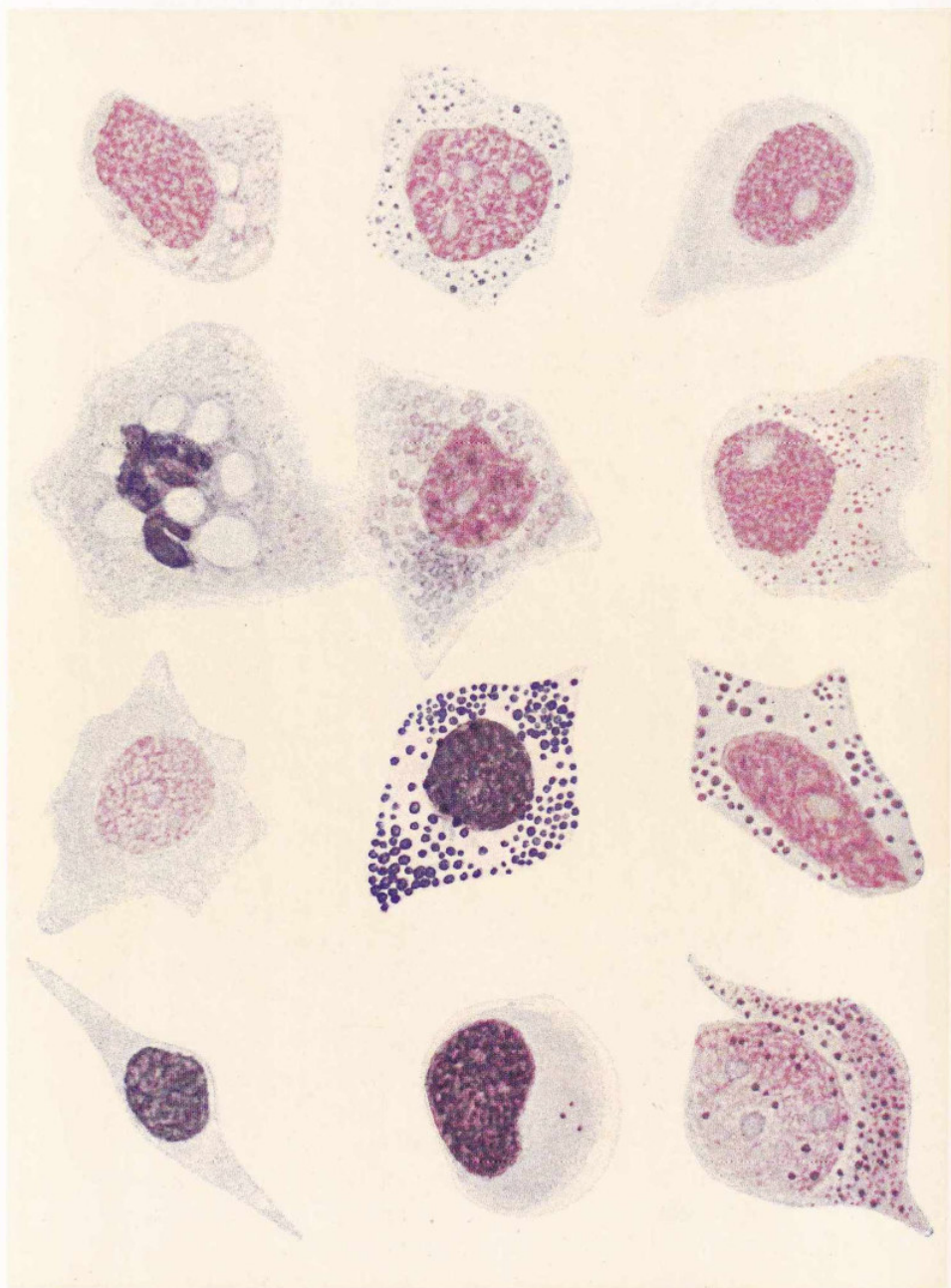


Plate 62. — Bone marrow reticulum cells.



3. Reticulum cells endowed with the property of phagocytosis (macrophages); in opposition to Rohr's opinion, Aleksandrowicz claims that not only undifferentiated, but also mature reticulum cells may demonstrate phagocytic activity;

4. Fat cells;

5. Plasmocytes.

Forms intermediate between these cell types are frequently encountered in bone-marrow smears.

Single small lymphoidal cells closely resemble lymphocytes, and it is not always possible to differentiate between these two cell types. The nuclei of lymphoidal reticulum cells stain less intensively than those of lymphocytes, and often contain minute clear blue nucleoli. The nucleoli are, however, not a constant feature. The recognition of these cells is less difficult when they form syncytia.

The primitive connective tissue cell (large lymphoidal reticulum cell) varies as to size, ranging, in diameter from 10 to 30  $\mu$ . The nucleus is round or oval, of fine, reticular structure and contains several light blue nucleoli. The cytoplasm is abundant, agranular and faintly basophilic — light blue or greyish-blue. The outlines of the cell are indistinct and irregular. In some instances, phagocytised substances may be present in the cytoplasm. These cells are frequently damaged during the preparation of smears and give rise to basket-cells (blurred nuclear remnants).

The cytoplasm of other reticulum cell types may be granular. The granules may be coarse, or, in other instances, minute. The granulation may be azurophilic, neutrophilic, acidophilic, basophilic or mixed. The nucleus contains distinct nucleoli and is of reticular structure. These cells are termed Ferrata cells. Some authors prefer the term histiocyte, adding (according to granulation) neutrophilic, acidophilic, basophilic or mixed granule type (Tempka and Aleksandrowicz). The origin of these cells is disputed. In some instances they resemble damaged promyelocytes or myelocytes. The nuclear structure and the presence of nucleoli seems, however, to indicate that the cells are reticulum elements.

The question remains undecided as to whether these cells constitute separate strains, such as neutrophilic, eosinophilic and basophilic granulocytes, or whether their granulations are accidental inclusions only. Aleksandrowicz claims that eosinophilic histiocytes play in allergic phenomena a rôle similar to that of eosinophilic granulocytes.

The nuclei of certain reticulum-cell types become during differentiation more pycnotic and resemble nuclei of lymphocytes. The term "large lymphoidal reticulum cell" seems adequate for this type of histiocyte.

The transition between a reticulum cell proper and a macrophage is gradual. The distinction between these two cell types is somewhat artif-

icial. Phagocytic properties are common to all reticulum cells. A histiocyte is considered as a macrophage when the presence of ingested substances, such as nuclei, microorganism, haemosiderine etc., is demonstrable in the cytoplasm. On Plate 62 a histiocyte is shown containing in the cytoplasm phagocytised nuclei and droplets of fat.

Fat cells are reticulum cells which specifically store droplets of fatty substances in the cytoplasm. They may be demonstrated in the bone-marrow stroma. Their number increases when the bone marrow undergoes fatty transformation. These cells are large in size, the nuclei are displaced by accumulating fat towards the periphery of the cytoplasm. Their appearance has suggested the term ring-cell.

Another variety of this group of cells is represented by the spindle-like endothelial cells lining the walls of sinuses and vessels.

### PLASMOCYTES

The plasmocytes are in normal conditions encountered among other reticulo-endothelial cells in the bone marrow, the spleen and lymph nodes. Mature plasmocytes constitute a clearly defined cell type. Immature plasmocytes may be less readily differentiated from other reticulum cells and numerous intermediate forms are encountered. These may be conveniently observed in cases of pathological hyperplasia of the plasmocytic system. It seems reasonable, therefore, to assume that plasmocytes originate from histiocytes, corresponding closely to reticulum cells proper (see description above). The nucleus is round, of fine structure, poor in chromatin and contains one or more blue nucleoli. The nucleus lies in the centre of the cell or may be slightly displaced towards the periphery. The agranular, irregular cytoplasm is light blue, with a greyish tinge.

As differentiation progresses, the nuclear structure becomes more dense and loses its reticular arrangement. At this stage, the nucleoli are usually visible. The cytoplasm may be of irregular or circular outline. It stains more deeply and is violet-blue (plasmoblasts).

The next stage in the developmental cycle is termed proplasmocyte (Türk's cell). It may be larger than the stem-cell. In shape it is more regular, oval or polygonal, and sharply outlined. The nucleus is round or oval and contains nucleoli. The nuclear structure is indistinct, but coarser than in plasmoblasts. The nuclei of more mature proplasmocytes are often excentric, the chromatin being arranged in strands. Among the characteristic features of this cell is its specific deep basophilia of the cytoplasm, which stains dark blue with a violet tinge. A whitish clear area may often be seen in the perinuclear region.



Mature plasmocytes (Unna's cells) are extremely varied in shape and size. Small plasmocytes,  $8\ \mu$  in diameter, forms intermediate in size, and large cells over  $20\ \mu$  in diameter are encountered in bone-marrow smears. The nucleus is usually oval and excentrically located. Its structure is characteristic — the large dark polygonal blocks of basochromatin are separated by clear oxychromatin. The chromatin arrangement gives the nucleus a wheel-spoke or tortoise-shell appearance. The cytoplasm is deeply basophilic and appears to be granular. It is often vacuolated. The vacuoles are frequently minute and numerous. The clear perinuclear zone (archoplasm) is distinct in mature cells. The shape of the cell may be round, oval or polygonal. Plasmocytes often occur in clusters. Binucleated cells or cells containing three, or less frequently four, nuclei may be encountered.

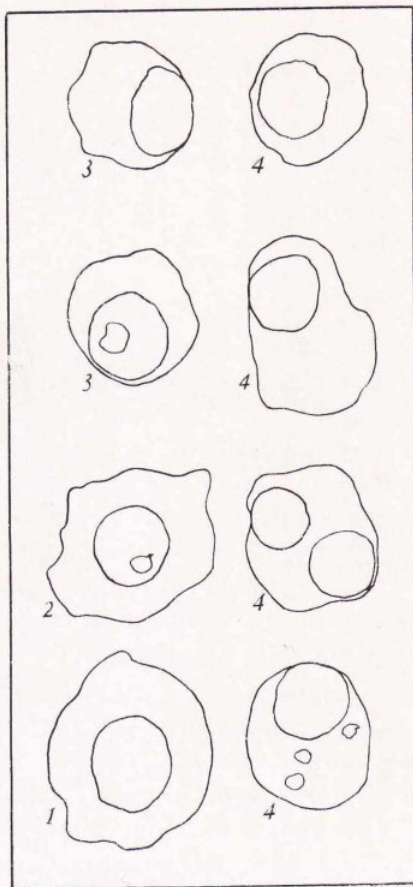
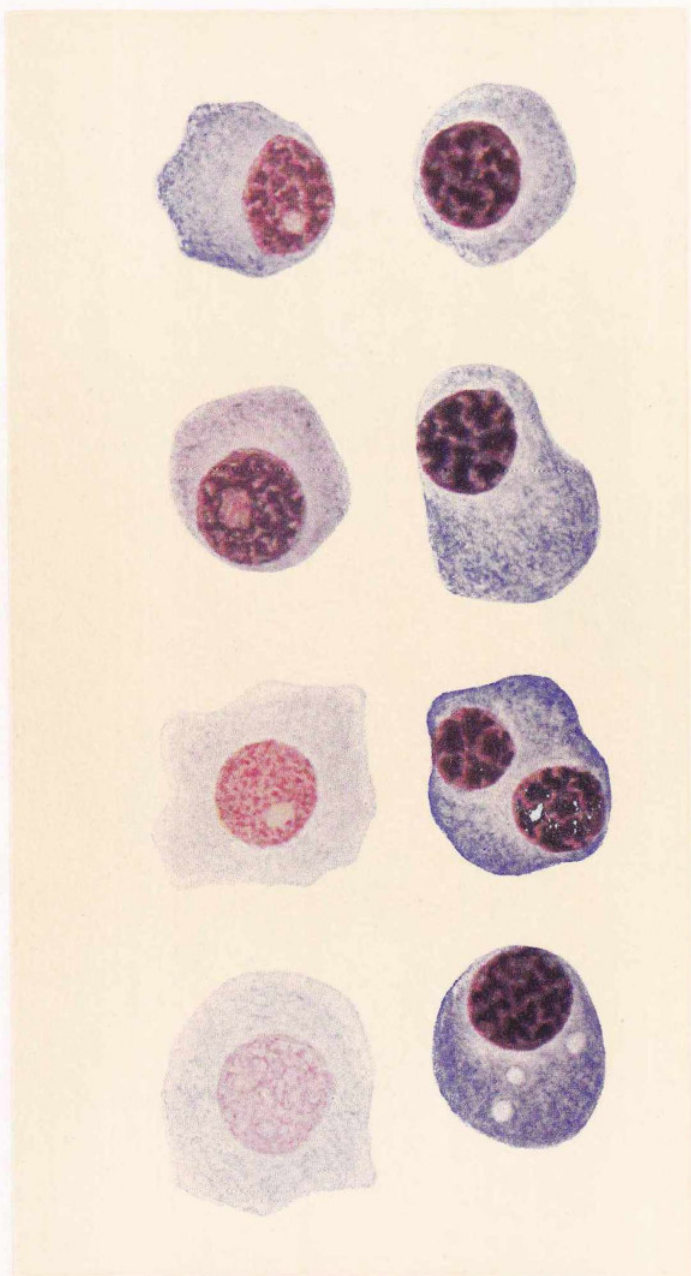
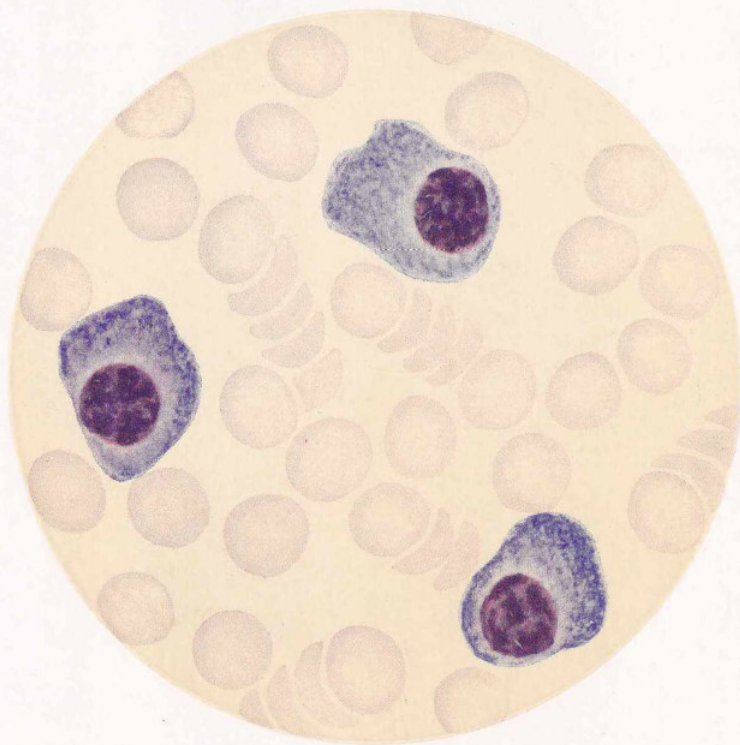


Plate 63. — Plasmocytes: 1. Reticulum cell. 2. Plasmoblast. 3. Proplasmocytes. 4. Plasmocytes.



*Plate 63. — Plasmocytes.*





*Plate 64.* — Plasmocytosis in peripheral blood.  
*Note:* the rouleaux formation in erythrocytes.

## PLASMOCYTOSIS IN THE PERIPHERAL BLOOD

The plasmocytes are normal bone-marrow constituents, encountered in the peripheral blood only in rare instances. They appear frequently in chronic inflammatory foci.

Plasmocytosis in peripheral blood may be observed in rubeola, measles, chickenpox, serum reactions, certain skin diseases, infectious mononucleosis, plasmocytic leukaemia and plasmocytoma.

Increase in bone-marrow plasmocytes is observed in the course of acute and chronic infections (tuberculosis), hepatic cirrhosis, rheumatism, Hodgkin's disease, anaphylactoid reactions, immuno-agranulocytosis and neoplastic proliferation of the plasmocytes (plasmocytoma). Plasmocytic reactions are usually accompanied by bone-marrow eosinophilia (Tempka).

Plate 64 illustrates plasmocytosis in peripheral blood. Three mature plasmocytes are represented on the drawing. Note the erythrocyte-rouleaux formation caused by hyperglobulinaemia.





## CHAPTER VIII

# NEOPLASTIC AND PROLIFERATIVE DISEASES OF THE LEUKOCYTIC AND RETICULO-ENDOTHELIAL SYSTEMS

### CHRONIC LEUKAEMIC MYELOID LEUKAEMIA

*Chronic Leukaemic Myelosis — Myelosis Leukaemica Chronica Manifesta  
Leukosis Chronica*

A fully developed myeloid leukaemia presents no difficulties in diagnosis. In peripheral blood, high leukocytosis with a shift to the left in granulocytes becomes manifest. The percentage of immature granulocytes varies at different periods of typical cases, all developmental stages of granulocytes are represented. As the morbid process becomes advanced, the preponderance of immature forms becomes marked (promyelocytes and myeloblasts), in contradistinction to the initial period, when staff cells and polymorphonuclears are more numerous. These cells may be atypical; they are frequent to some extent chromophobic. Large polymorphonuclears, with a loose "immature" nuclear structure and poorly staining granulation are often encountered. In other cells, the granules are unequal in size, small and large granules being present.

The large granules resemble coarse, immature granulation. Moreover, giant staff cells and giant metamyelocytes may occur in blood smears. Among the characteristic features of chronic myelosis is the increase in basophils. In certain cases, the basophils are so numerous that they may be seen in almost every field of vision. There is usually an increase also in eosinophils.

The increase in relative numbers of promyelocytes and myelocytes is interpreted as a sign of exacerbation of the disease. The red cell and platelet picture may for a long time remain unchanged but varies, at different periods of the disease. A decrease in platelets is considered as an unfavourable sign (Ch. Vlassos). In spite of absence of anaemia, erythroblasts may be found in peripheral blood smears (Plate 65).

In typical cases, bone-marrow examination yields no additional diagnostic findings. The bone-marrow picture may closely resemble that of the peri-



pheral blood. The bone marrow is markedly hypercellular. The granulocytes by far outnumber all other bone-marrow cells. Signs of arrested maturation, and maturation trouble, are frequently encountered. Giant metamyelocytes and granulocytes with atypical nuclei are more numerous than in peripheral blood. The numbers of basophils and eosinophils may be increased.

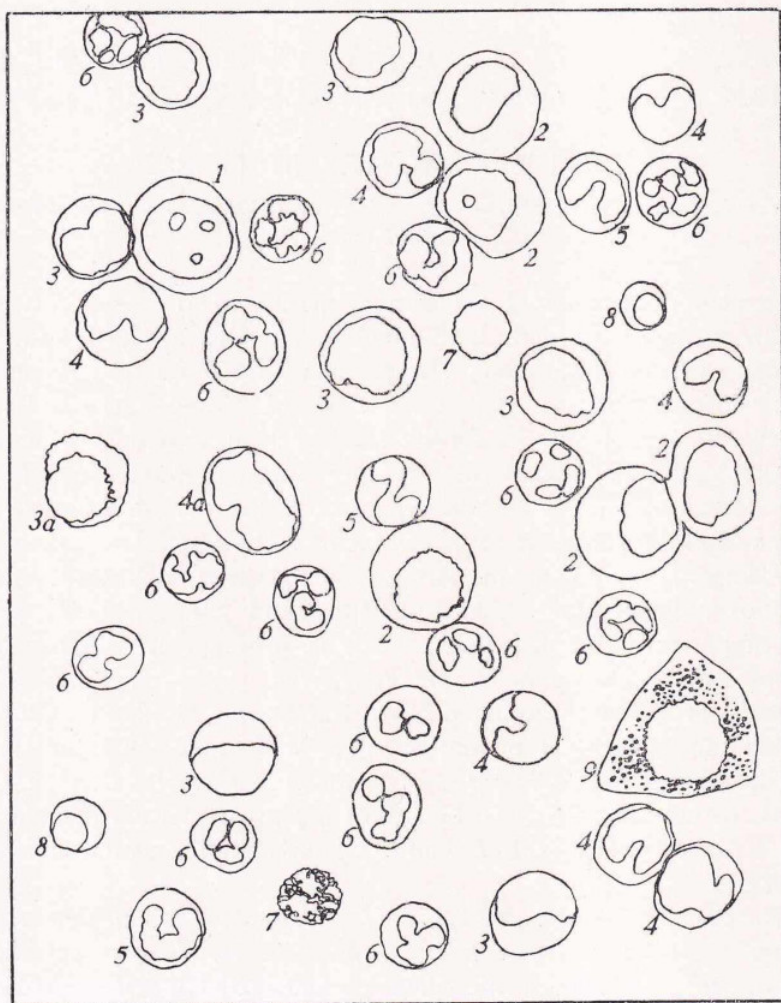
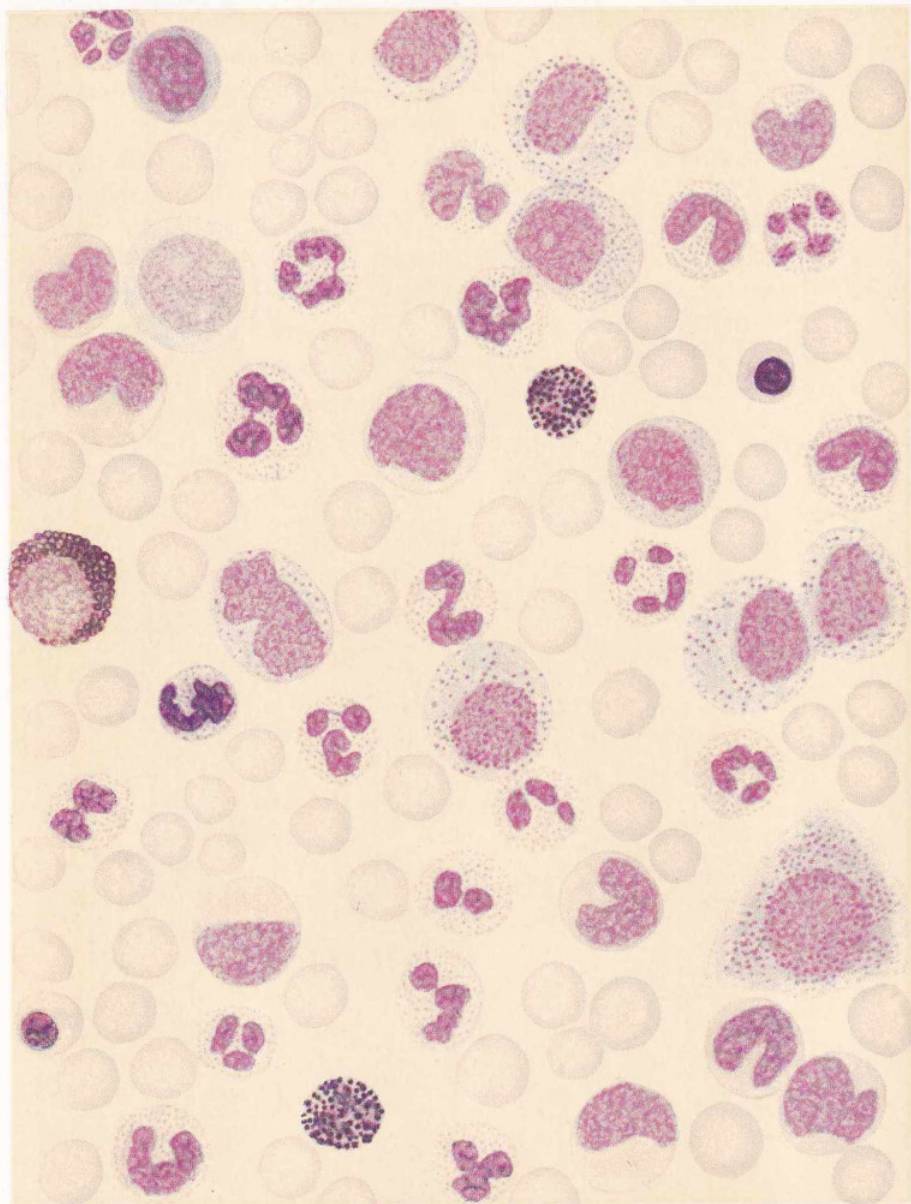


Plate 65. — Chronic myeloid leukaemia (peripheral blood): 1. Myeloblast. 2. Promyelocyte. 3. Neutrophil myelocyte. 3a. — eosinophil myelocyte. 4. Neutrophil metamyelocyte. 4a. — giant metamyelocyte. 5. Neutrophilic staff cell. 6. Mature, segmented neutrophils. 7. Basophils. 8. Orthochromatic erythroblast. 9. Reticulum cell.

*Note:* diversity of nuclear shape and structure; the specific granulation is blurred in numerous neutrophils, in several cells the granulation is even totally invisible.

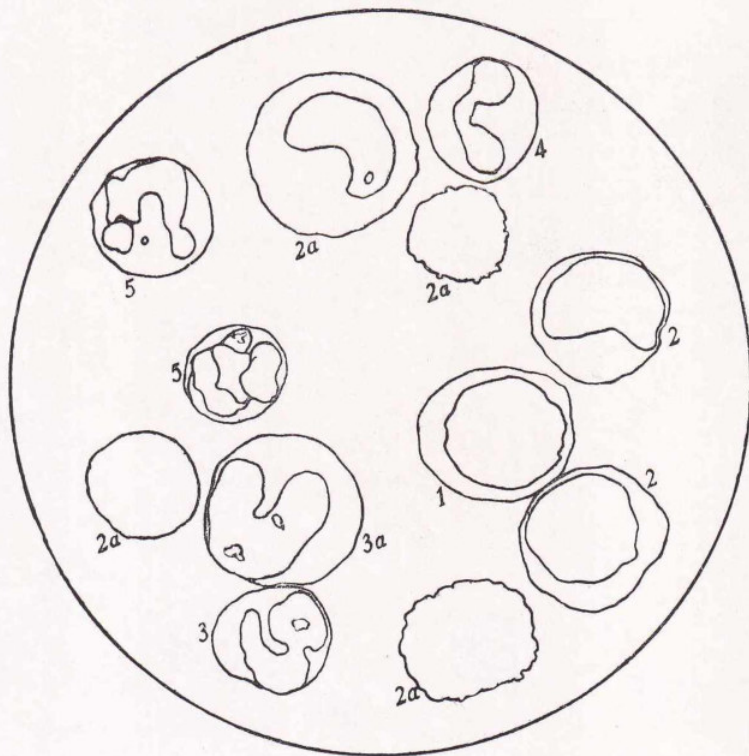


*Plate 65.* — Chronic myeloid leukaemia (peripheral blood).

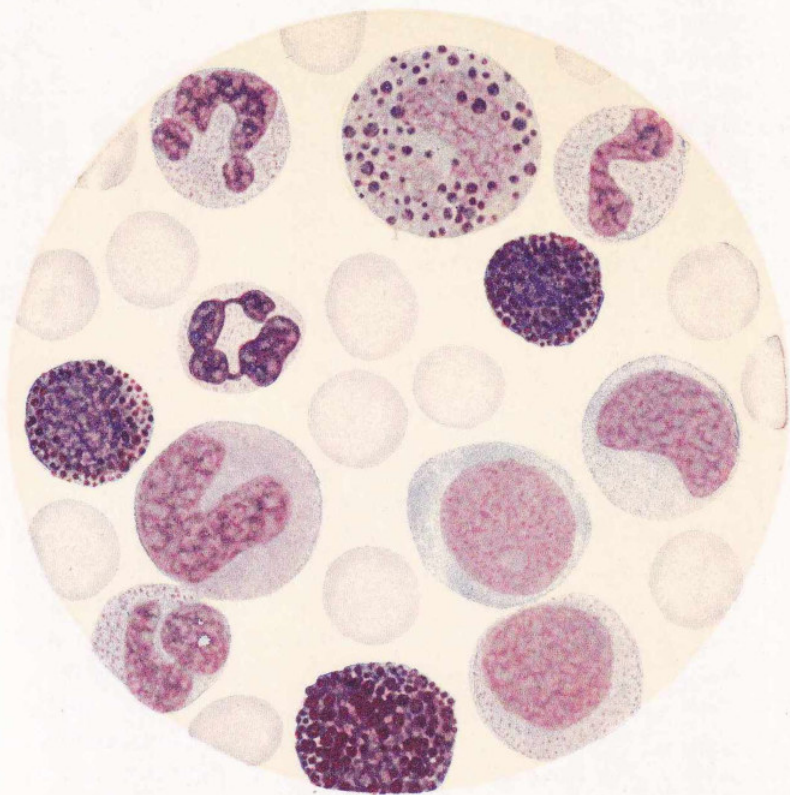


The shift to the left in granulocytes is frequently more pronounced in the bone marrow than in peripheral blood. This phenomenon is rarely observed in leukaemoid reactions during the course of an infectious disease. The differentiation between these two conditions may present considerable difficulty and is not always feasible.

The presence of immature ("toxic") granulation in the cytoplasm of granulocytes may be helpful, because it is more frequent in leukaemoid reactions than in leukaemia. The Russian haematologists (Vlados) emphasise the necessity of ruling out infectious diseases (first and foremost tuberculosis) before a diagnosis of leukaemia is made. Reversible leukaemoid reactions are generally known to occur in tuberculosis.



*Plate 66.* — Chronic myeloid leukaemia (bone marrow): 1. Mycloblast. 2. Neutrophilic myelocyte. 2a. — basophilic myelocyte. 3. Neutrophilic metamyelocyte. 3a. — Giant neutrophilic metamyelocyte. 4. Neutrophilic staff cell. 5. Polymorphonuclear neutrophil. Various forms of basophilic granulocytes encountered in chronic myeloid leukaemia are represented on the drawing. It is unusual to find them in such numbers in a single field of vision.



*Plate 66.* — Chronic myeloid leukaemia (bone marrow).



The diagnostic value of bone-marrow examination is greater in aleukaemic chronic myeloid leukaemia, although in such cases the bone-marrow picture is not sufficiently characteristic.

In diagnosis of chronic myeloid leukaemia the following should be taken into account:

1. Typical haematological findings;
2. Enlargement of the spleen;
3. Tenderness of the sternum on pressure;
4. Symptoms of haemorrhagic diathesis (inconstant symptoms);
5. Examination of the eyegrounds;
6. Increase in basal metabolic rate; this symptom develops in the majority of chronic cases and in acute leukaemia;
7. Increase in uric acid level in peripheral blood.

Additionally valuable diagnostic findings are increase in serum organic phosphorus and in alkaline phosphatase.

### ACUTE MYELOID LEUKAEMIA

#### *Myelosis Leukaemica Acuta*

By contrast with chronic leukaemia, all cellular series are concerned in the morbid process at an early stage. This becomes manifest by the development of anaemia, thrombocytopenia and the appearance of immature granulocytes in peripheral blood. The percentage of immature cells, as well as leukocytosis, is extremely variable. The leukocyte count is frequently low (from 2,000 to 3,000 per cu.mm.), stem-cells being present. The number of mature granulocytes gradually diminishes, while that of myeloblasts increases. Deep disturbances in maturation at the stage of the stem-cell are revealed by the lack of intermediate developmental stages. This "*hiatus leukaemicus*" strengthens the suspicion of a leukaemic process and constitutes an unfavourable prognostic sign. The finding of a few myelocytes or metamyelocytes in blood smears may facilitate the correct recognition of myeloblasts, which may be greatly changed.

In certain cases, the myeloblasts may demonstrate no morphological abnormalities and be identical with those encountered in normal bone marrow. Such cases offer no cytological diagnostic difficulties and are considered as typical myeloblastic leukaemia (Plate 67).

In other instances the myeloblasts show striking abnormalities, such as for example marked anisocytosis. The nuclei may contain exceptionally large nucleoli exceeding  $1/4$  of the diameter of nucleus in size. Such large nucleoli are characteristic of neoplastic cells.

Leukaemic cells may be so changed morphologically as to make recognition extremely difficult.

Among the more frequent varieties of myeloblastic leukaemia, is what is called the micromyeloblastic form, where myeloblasts resemble small and intermediate lymphocytes. The cytoplasm of these cells is no longer typically basophilic; the nucleus acquires a dense, pycnotic, "lymphocytoid" structure. The presence in some nuclei, however, of round or irregular nucleoli reveals immaturity. The presence of numerous nucleoli indicates the myeloid origin of the cell, single nucleoli being more characteristic of lymphoblasts. The nuclei of "lymphocytoid" myeloblasts may be irregular in shape, as seen in Rieder's forms of lymphocytes.

Plate 68 illustrates micromyeloblasts and lymphocytoid myeloblasts with irregular nuclei.

Is not always possible to differentiate micromyeloblasts from lymphocytes or lymphoblasts in peripheral blood smears. In bone-marrow smears, the presence of intermediate stages between micromyeloblasts and typical myeloblasts often makes correct recognition less difficult. The number of mitotic forms may also be helpful. These are numerous in acute myelosis, whereas karyokinesis is less frequently met in acute lymphadenoses and shows characteristic abnormalities.

Micromyeloblasts may contain in the cytoplasm slender azurophilic rods, stained reddish-violet (Auer's bodies, Auer's rods). These rods occur in lymphocytes only in exceptional cases and may serve as additional means of differentiation.

Plate 69 illustrates atypical myeloblasts containing Auer's rods in the cytoplasm.

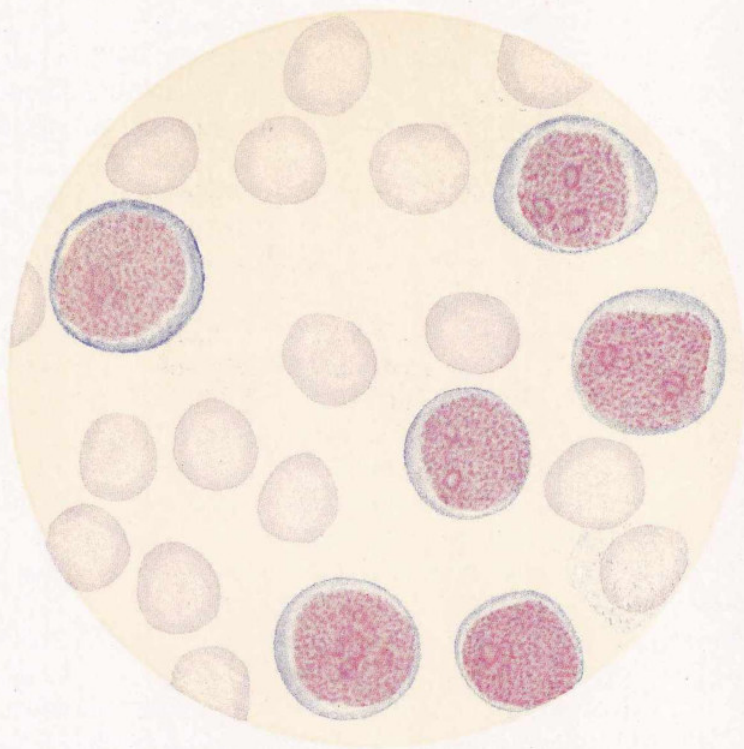
Another variety of this disease is usually termed paramyeloblastic leukaemia.

The nuclei of myeloblasts are indented, and, in some instances, even lobulated. These cells may suggest monocytes. Single paramyeloblasts are often encountered in typical myeloblastic leukaemia. In cases in which such forms are numerous the disease is termed acute monocytoid (not monocytic) myeloid leukaemia.

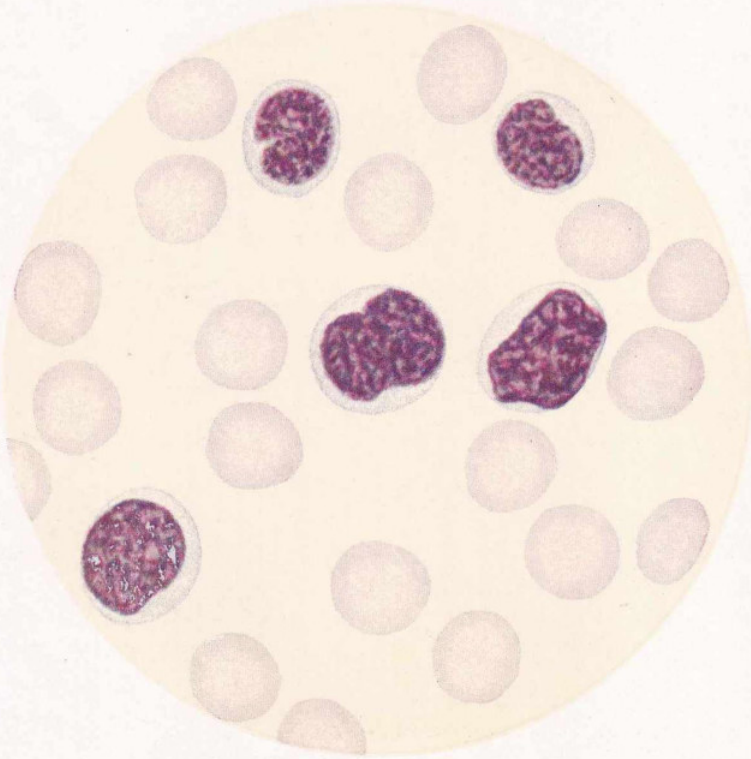
Plate 70 illustrates monocytoid myeloblasts.

To differentiate between monocytoid paramyeloblasts and monocytes in blood smears may be extremely difficult, if not altogether impossible. Such is the case when the cytoplasm of paramyeloblasts contains minute azurophilic granules and closely resembles the cytoplasm of monocytes. The nuclear structure and the degree of segmentation may be helpful. The nuclei of monocytes are kidney-shaped or only slightly lobulated. On the other hand, the nuclei of monocytoid paramyeloblasts are of bizarre shape, hypersegmented, the nuclear lobes fantastically contorted etc. Bone-marrow examination usually clears up any doubts, in view of the presence of typical myeloblasts and intermediate forms.





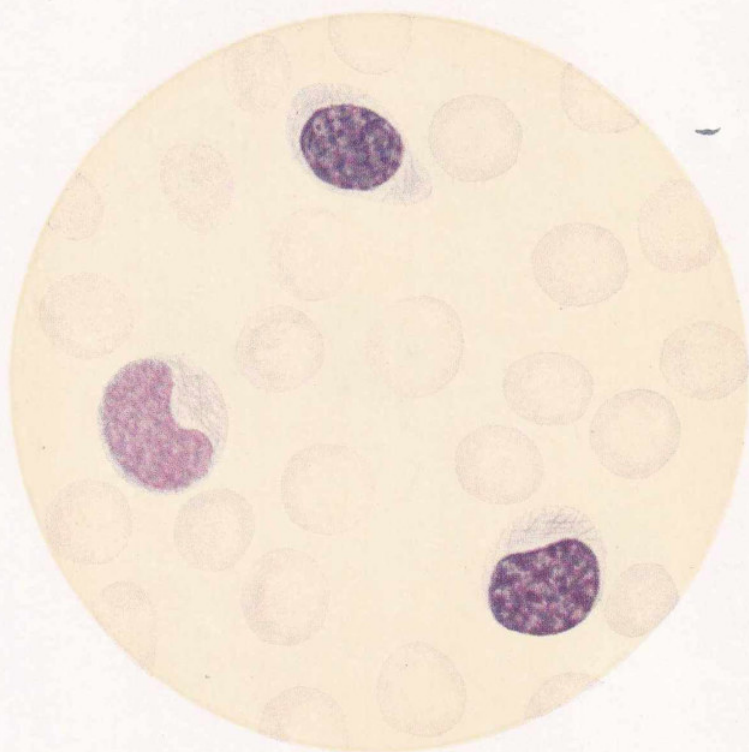
*Plate 67.* — Acute myeloid leukaemia (peripheral blood).  
Numerous myeloblasts are present. The cells do not demonstrate any marked abnormality.



*Plate 68.* — Acute myeloid leukaemia. Micromyeloblasts and “lymphocytoid” myeloblasts.

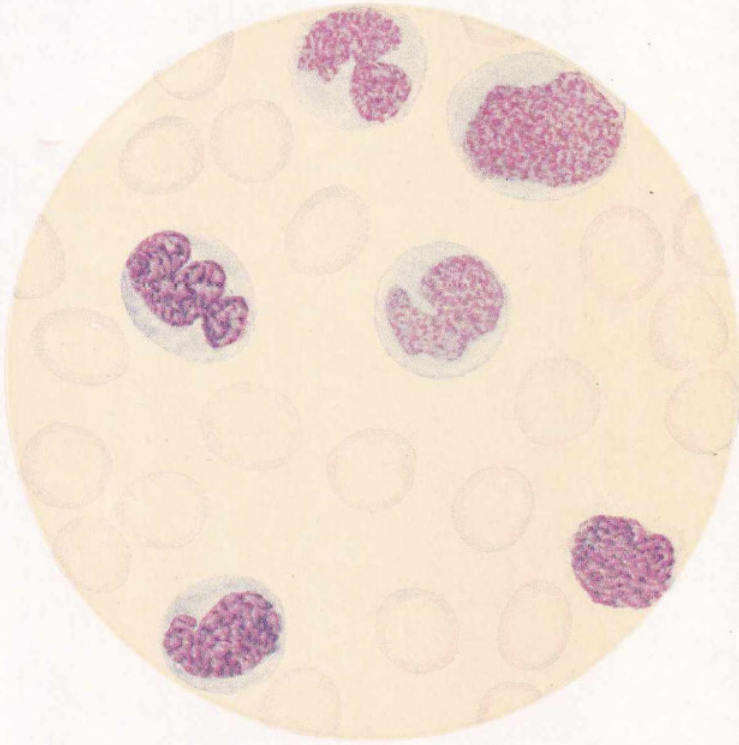
Such cells offer considerable difficulties in differentiation from the lymphocytic series. Special methods are in many cases required for correct diagnosis. The cells on the drawing give rise to doubts as to their lymphoid origin by their characteristically atypical nuclear structure (cf Plate 57).





*Plate 69.* — Acute myeloid leukaemia (peripheral blood).

Atypical myeloblasts with Auer's rods (slender, azurophilic inclusions of variable length) in the cytoplasm. The nuclear structure in one of the cells is similar to that of a monocyte ("monocytoidal" myeloblasts); in the other two — to lymphocytes ("lymphocytoidal" myeloblasts).



*Plate 70.* — Acute myeloid leukaemia. Monocytoidal paramyeloblasts (peripheral blood).

The nuclear structure is similar to that of monocytes, although the cytoplasm demonstrates differences (as for example lack of a dust-like azurophilic granulation; cf Plates 61 and 76).



In certain cases of acute myelosis, the myeloid stem-cells show signs of marked immaturity and possess nuclei of distinctly reticular structure. Such cases are termed undifferentiated cell leukaemia (stem-cell leukaemia, *myelosis indifferento-cellularis*).

The cytological classification, together with the nomenclature of various types of leukaemia, is based on morphological similarities between leukaemic and normal cells. It should, however, be emphasised that the classification of acute leukaemia as undifferentiated cell leukaemia, myeloblastic, paramyeloblastic etc. is of only relative value. The pathologic chan-

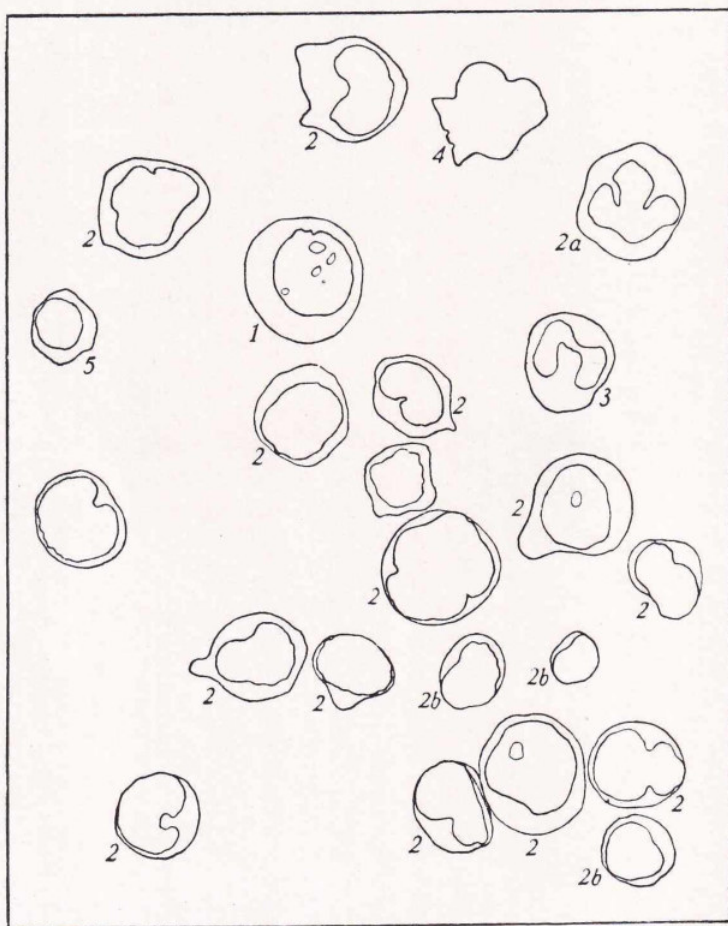
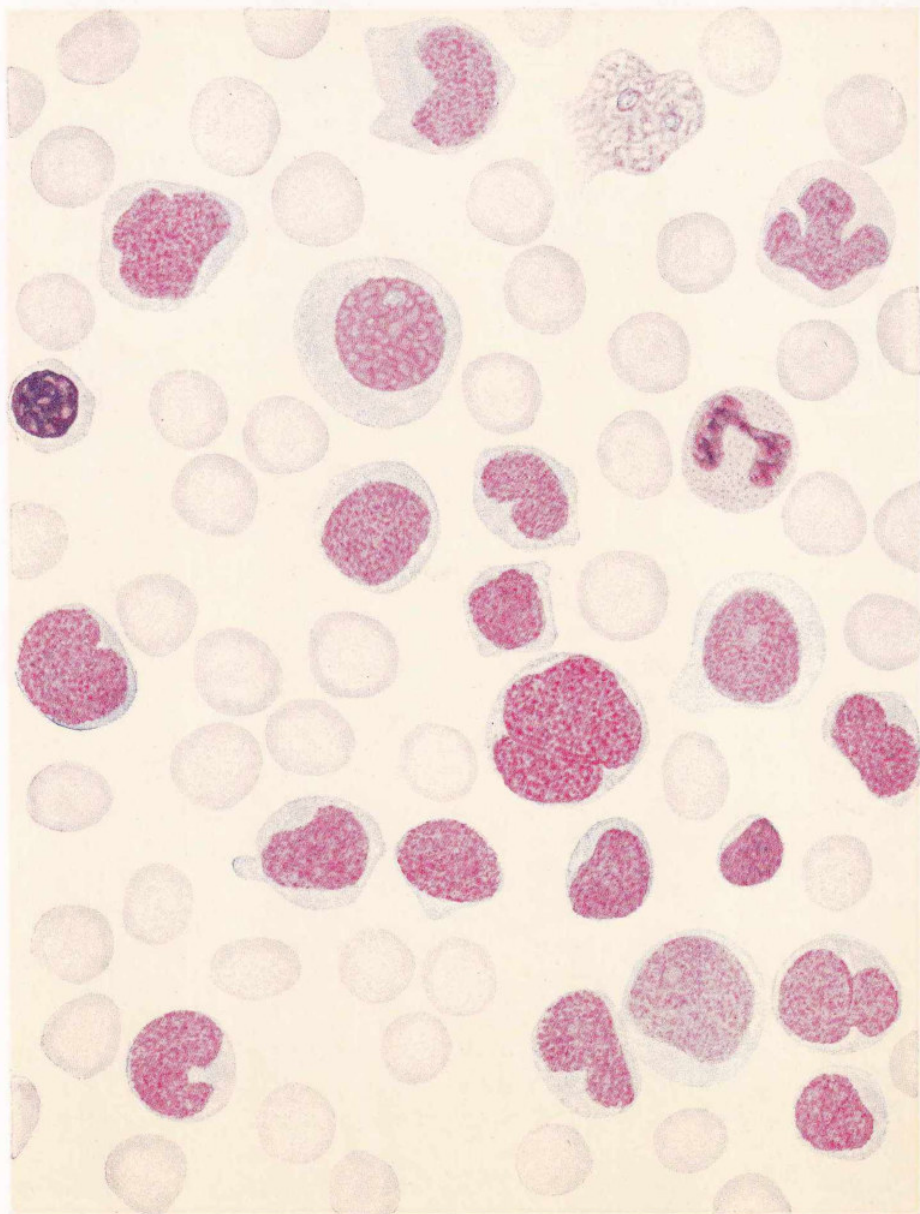


Plate 71. — Acute myeloid leukaemia (bone marrow): 1. Reticulum cell. 2. Atypical myeloblast. 2a. — myeloblast with lobulated nucleus. 2b. — micromyeloblast ("lymphocytoidal" myeloblast). 3. Staff cell. 4. Remnants of a myeloblastic nucleus. 5. Orthochromatic erythroblast.



*Plate 71. — Acute myeloid leukaemia (bone marrow).*



ges of the cell morphology may be very extensive and misleading (Krajevsky and Nemenova, Bykov). Leukaemia cells should not therefore be identified with myeloblasts or haemocytoblasts.

The Russian authors (Gubergric, Davydovsky, Vlados, Krajevsky and others) have with the object of evading any unprecise (and as pointed out above misleading) concepts, introduced the general term "haemocytoblastic leukaemia" for all leukaemias accompanied by an immature and atypical cytological picture. The term "haemocytoblastic leukaemia" suggests an early period of haematopoietic activity and all inferences as to the nature of cells proliferation are unnecessary.

The stem-cells of granulocytes do not contain peroxidases or oxidases, which become demonstrable at later developmental stages. More differentiated myeloblasts and paramyeloblasts are peroxidase-positive. Peroxidase reaction should be carried out in cases of acute myeloses, since the results indicate the degree of maturity of the cell<sup>1</sup>. If the results are distinctly positive, promyelocytes or parapromyelocytes are the prevalent cell type. In smears stained by the May-Grünwald-Giemsa method, the cells may lack the azurophilic granulation characteristic of promyelocytes. The results of peroxidase reactions should, however, be evaluated critically. The presence of peroxidase in an undifferentiated cell may indicate other properties, such as, for example, phagocytic activity.

The myelogram in acute myelosis usually demonstrates proliferation of stem-cells (myeloblasts), while other bone-marrow cells become suppressed. Although among myeloblasts normal cells may be encountered, the majority of them consist of atypical cells of variegated appearance (haemocytoblasts). It is therefore extremely difficult to describe a general cytological picture of acute myelosis. Rohr is of the opinion that every case of acute myelosis is characterised by a cell type specific for that case only. Leitner says that "atypical myeloblasts are a typical symptom of acute myelosis".

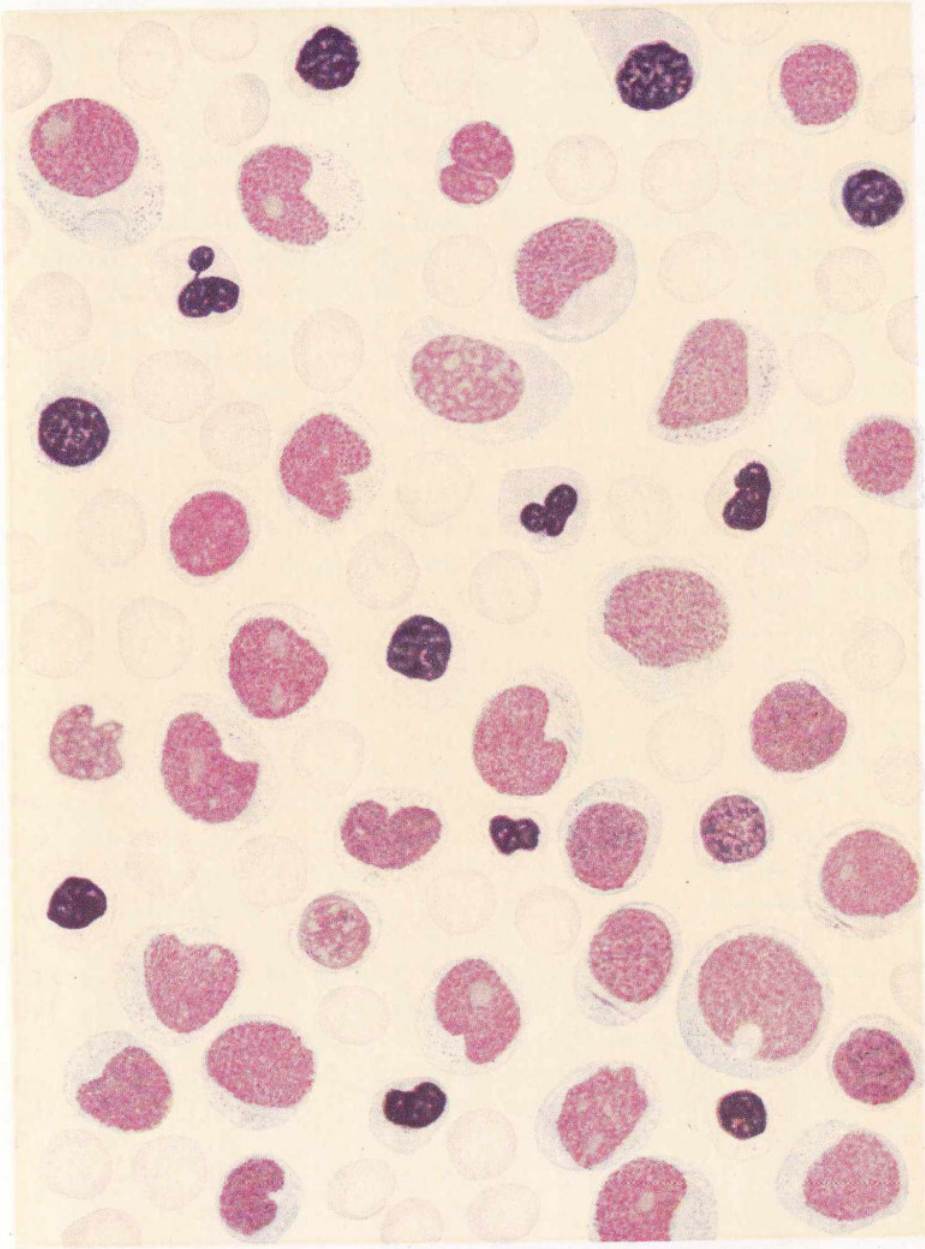
In bone-marrow smears, myeloblasts, micromyeloblasts and paramyeloblasts (including many monocytoidal in type) may be encountered.

Plate 71 illustrates the bone marrow in acute myeloid leukaemia. The myeloblasts are characterised by anisocytosis and variations in the shape of the nuclei — indented or lobulated. Micromyeloblasts are less numerous, and a single reticulum cell is present. The cytoplasm of several myeloblasts is vacuolated. The number of erythroblasts is markedly diminished.

Haematological literature stresses anaplastic and dysplastic disturbances in the bone marrow in the course of acute myelosis. Anaemia is among

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<sup>1</sup> Similar results are obtained with Sudan black B; this method is, however more sensitive. Myeloblasts by contrast with lymphoblasts are usually Sudan-positive.



*Plate 72. — Acute myeloid leukaemia (bone marrow).*

Numerous myeloblasts are present, several among them contain Auer's bodies in the cytoplasm. Larger cell with an azurophilic granulation suggest promyelocytes.

*Note:* a marked erythropoietic response, the presence of numerous erythroblasts, several of these cells contain atypical nuclei.



the regular symptoms of this disease. This has often been explained by the displacement of erythropoietic tissue by leukoblastic cells — a concept which seems to be oversimplified. Closer analysis demonstrates interesting changes and disturbances in the red-cell series.

In several cases followed by the present authors, megaloblastic features of erythrocyte stem-cells were noted. Cases have been reported in which Addison-Biermer's disease preceded the development of acute leukaemia. The question arises, are there created such conditions in the course of leukaemia, as lead to a megaloid (megaloblastic?) transformation of erythropoiesis.

The present authors also observed marked erythroblastic (normoblastic) responses in the course of acute leukaemia. The number of erythroblasts amounted to as much as 48 % of all nucleated bone-marrow cells. In certain cases, the erythroblasts demonstrated profound alterations in nuclear chromatin, as well as in the cytoplasm (paraerythroblasts). The cytoplasm was of irregular outlines and demonstrated abnormal haemoglobin content and pathological granulation. These observations indicate that disturbances in the course of acute leukaemia are complicated and concern the entire haematopoietic tissue.

Plate 72 illustrates an erythropoietic response in the course of acute leukaemia. Certain erythroblasts are abnormal in shape. The myeloblasts are atypical (paramyeloblasts). Note the nucleoli.

The myeloblastic proliferation may, particularly in early periods of the disease, be limited to several foci only. Therefore in cases of suspected myeloproliferative disease, negative findings obtained by examination of sternal bone marrow should be confirmed by puncture of other bones, such as, for example, the iliac crest or spinous processes of lumbar vertebrae.

Bone-marrow examination is helpful in diagnosis, and in cases of aleukaemic leukaemia it may be the only determining factor. It should be emphasised, however that repeated biopsies may be necessary before definitive diagnosis can be made. Reversible myeloblastic responses are known to occur. The possibility of aplastic anaemia (pancytopenia) accompanied by a hyperactive bone-marrow picture should be also borne in mind. In these cases, it is often extremely difficult to decide whether the bone marrow shows compensatory activity or preleukaemic proliferation.

Myeloblastic proliferation may be encountered in agranulocytosis. In all doubtful cases, conclusions may be drawn only on the basis of a prolonged period of clinical observation and repeated biopsies. No sharp borderline can be drawn between myeloblastic reactions, aplastic and dysplastic disorders and the true leukaemic bone-marrow picture. No criteria exist for the recognition of the preleukaemic states.

In diagnosis of acute myelosis, the following points may be helpful:

1. Haematologic findings; it is emphasised that the leukocyte count is not a deciding factor; the most significant findings are the abnormality and immaturity of the cells; nearly always progressive anaemia and thrombocytopenia; haemorrhagic diathesis; prolonged coagulation time; by contrast with aplastic anaemia — anisocytosis; poikilocytosis, polychromasia in erythrocytes, reticulocytosis, erythroblastosis in peripheral blood; in myeloid reactions in the course of an infection, it is only rarely that anaemia is severe;

2. Enlargement of the spleen (usually discrete), of the liver and in some instances of lymph nodes;

3. Febrile reactions; septicaemia;

4. Necrosis of the buccal mucosa;

5. Symptoms of bone and joint involvement;

6. Neurologic symptoms;

7. Increase in basal metabolic rate;

8. Examination of the eyegrounds (haemorrhages).

## CHLOROMA

### *Myeloblastoma — Cancer Vert*

The term chloroma refers to a form of leukaemia characterised by the occurrence of localised tumours of proliferating myeloblasts in the skeletal system (typically periosteal tumours are present in addition to changes in the bone medulla), lymph nodes and internal organs. This mode of growth resembles that of neoplastic tumours in the strict sense.

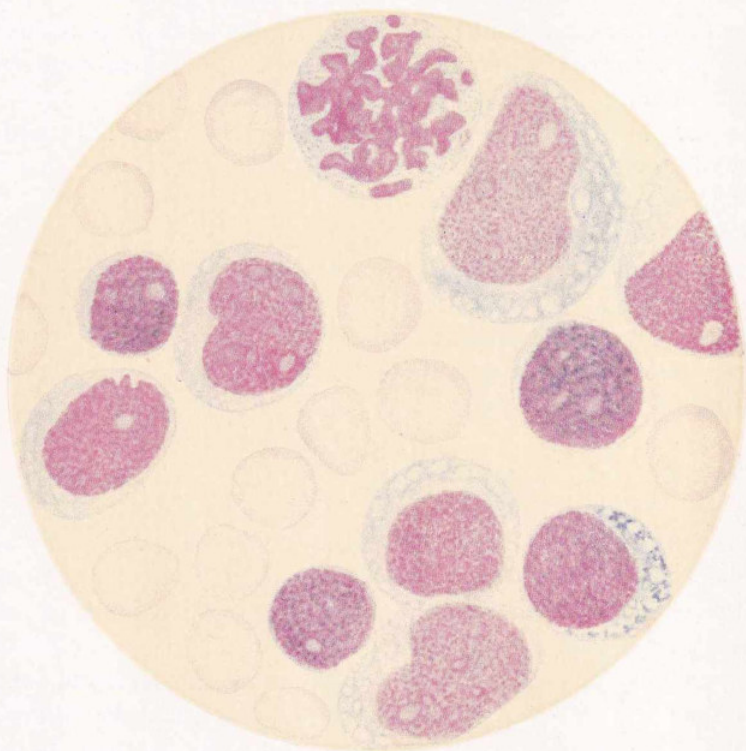
The clinical features of chloroma do not differ from the symptoms described above of manifest or latent leukaemia. The haematological picture is also similar. Periosteal tumours of the skull constitute the main differential feature.

The present authors followed two cases of chloroma in children. The diagnosis was based on bioptic examinations.

CASE I: a 4<sup>7</sup>/<sub>12</sub>-year old female child: leukocytosis from 11,400 to 11,800 per cu.mm.; no myeloblasts or atypical cells in peripheral blood smears. Progressive anaemia was present, the initial erythrocyte count 3,390,000 per cu.mm. (haemoglobin 53%) dropped to 2,670,000 per cu.mm. (haemoglobin 48%). Death ensued after a four-week period of clinical observation. In addition to sternal biopsy, a puncture of a periosteal tumour localised over the left orbit, was performed.

Bone-marrow smears were characterised by the exclusive presence of myeloblasts including many which were atypical, demonstrating marked anisocytosis and variegated appearance. The most striking changes were observed in giant myeloblasts some of which were multinucleated or showed lobulated nuclei. The cytoplasm of these cells was vacuolated. Cells in mitosis were frequent.

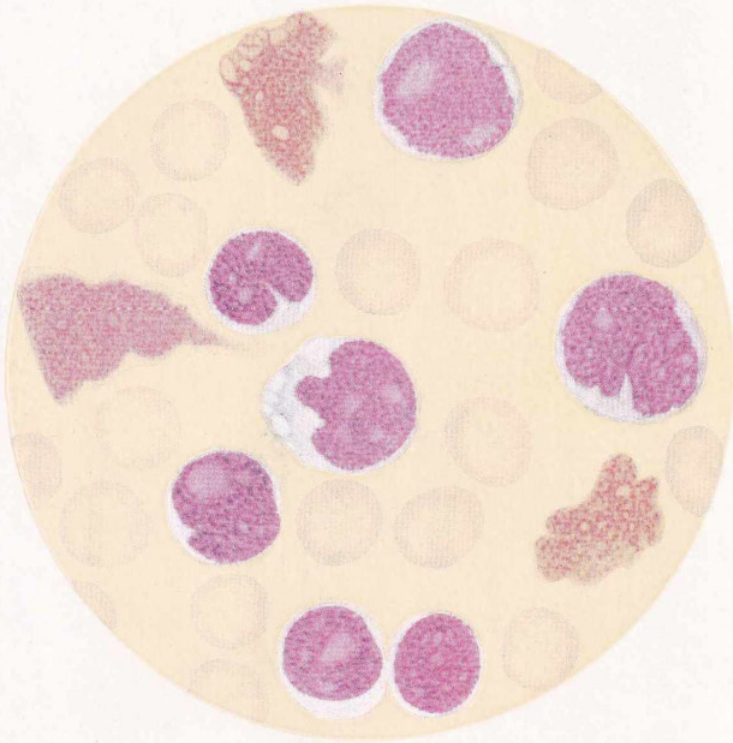




*Plate 73. — Chloroma (bone marrow). Case I.*

Atypical myeloblasts; one of them in mitosis.

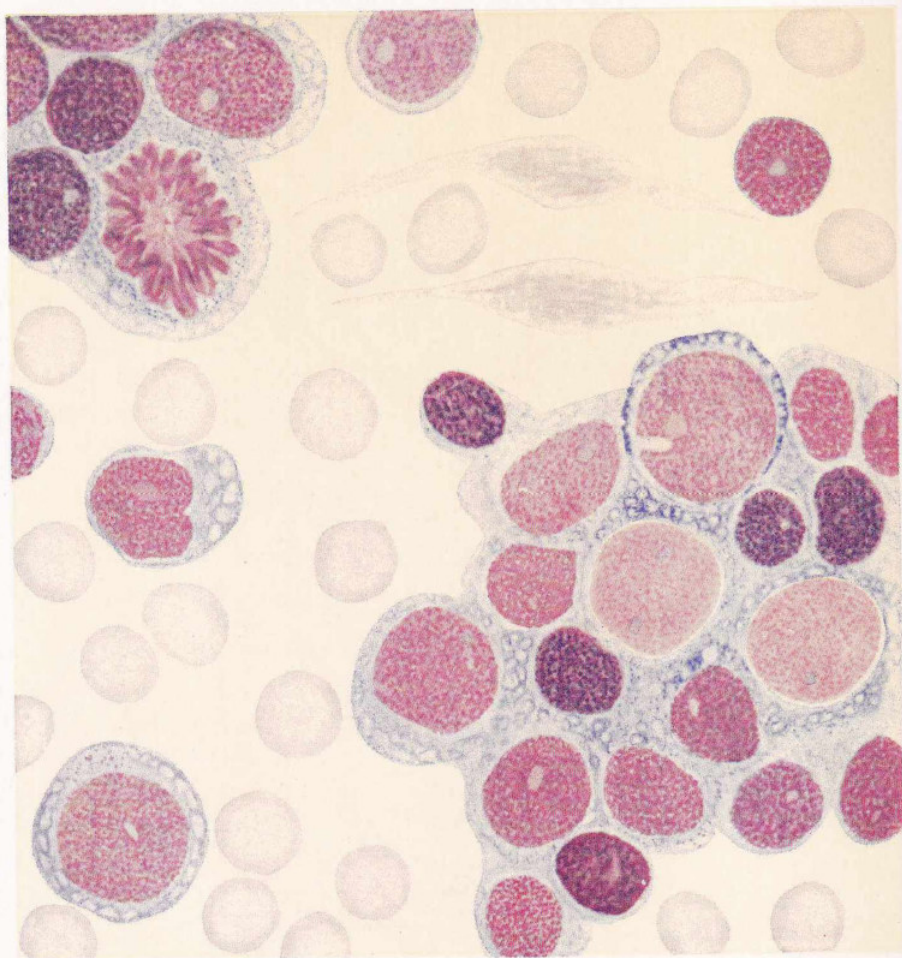
*Note:* characteristic vacuolation of the cytoplasm.



*Plate 74.* — Chloroma (bone marrow). Case II.

*Note:* numerous and distinct nucleoli; atypical nuclear shape; vacuolation of the cytoplasm in two myeloblasts and the presence of the basket-cells (remnants of myeloblastic nuclei).





*Plate 75.* — Chloroma (lymph node aspiration smear).  
Only myeloblasts, demonstrating typical abnormalities (of text), are present. One of these cells undergoes mitosis.

Plate 73 illustrates the bone-marrow picture in this case. Smears obtained by biopsy of the tumour were identical with those of the bone marrow.

CASE II: a 4-year old male child was characterised by severe anaemia, haemorrhagic diathesis and persistent leukopenia, which developed long before the remaining symptoms (leukocyte count from 2,400 to 3,400 per cu.mm.). The highest leukocyte count observed was 8,000. Micromyeloblasts amounted to from 50% up to 72% of leukocytes.

The bone marrow, consisting exclusively of myeloblasts, was less cellular than in the preceding case. On Plate 74, several of these cells are reproduced. The myeloblasts are more uniform in appearance than in case I, but are also atypical. The nuclei are large, of "immature" structure and contain nucleoli; the cytoplasm is deeply basophilic and scanty.

One of the posterior cervical lymph nodes was punctured in this case. Atypical myeloblasts, such as illustrated on Plate 75 dominated the lymph-adenogram. They are characterised by marked anisocytosis; certain cells may be considered giant forms. The nuclei are round or oval, rarely indented, leptochromatic and contain nucleoli, including several which are usually large. The agranular cytoplasm is basophilic, often vacuolated, particularly in the perinuclear region. In certain instances, the entire cytoplasm is filled with vacuoles. A few cells contain coarse azurophilic granules (promyelocytes).

Such a picture indicates myeloblastic transformation of lymphoid tissue. Special note should be taken of the abnormal appearance of myeloblasts and the marked vacuolation of the cytoplasm, such as is frequently encountered in neoplastic cells.

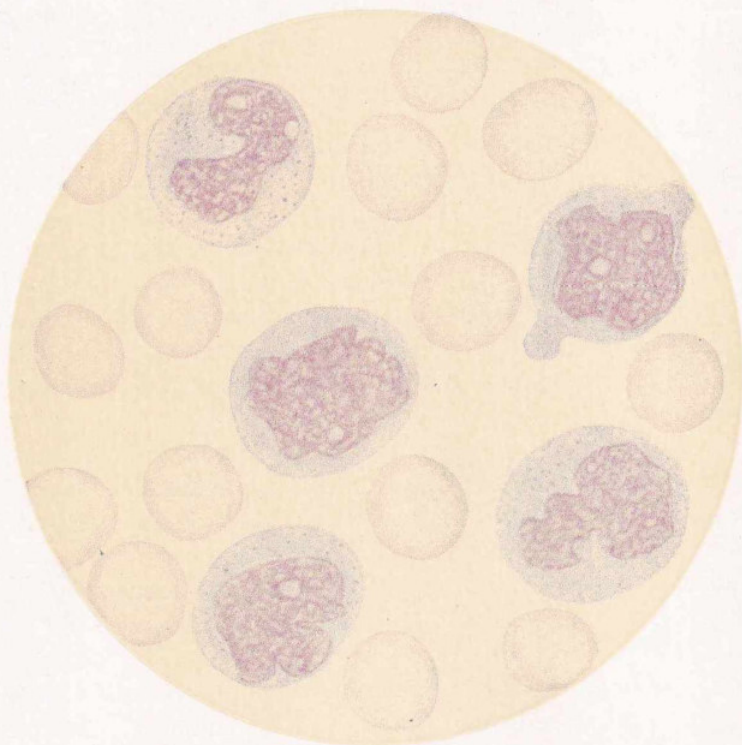
Among four cases of chloroma subsequently followed by the present authors, three were characterised by similar vacuolation of the cytoplasm of myeloblasts.

Among the outstanding clinical features of both cases described above were the specific localised tumours, and enlargement of the spleen and liver.

## MONOCYTIC LEUKAEMIA

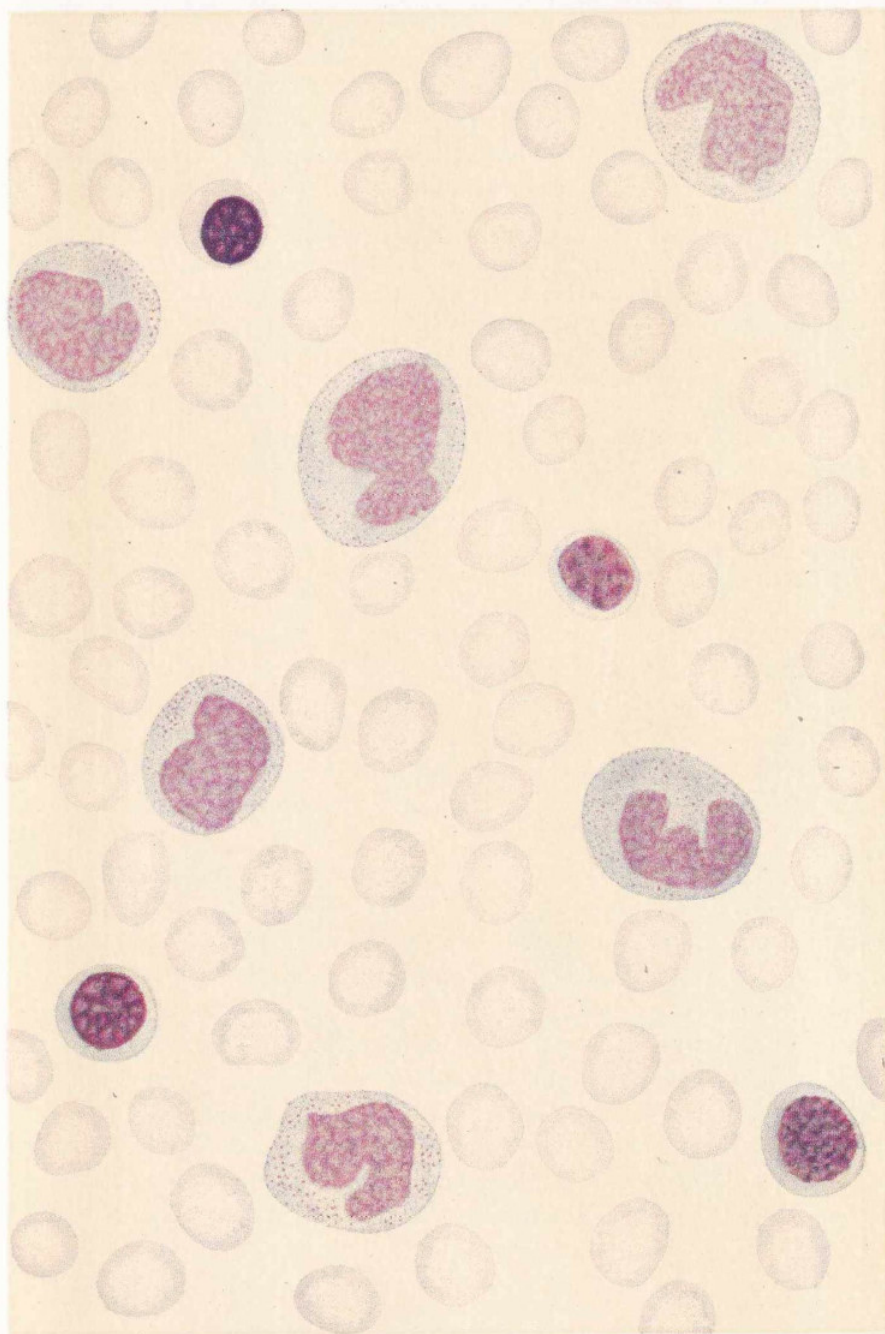
This entity is not universally recognised, because a great majority of suspected cases of monocytic leukaemia have proved, when examined by bioptic procedures, to be of the paramyeloblastic variety. The myelogram indicated a myeloblastic proliferation accompanied by profound changes in the morphology of these cells. Intermediate stages linking the typical myeloblasts with monocytoid paramyeloblasts (looked upon as monocytes) were usually demonstrated. Nevertheless, a careful study of the extensive literature on this subject indicates that all cellular elements of the haematopoietic system may be subject to leukaemic proliferation. All cases demonstrating distinct bone-marrow monocytosis (with-





*Plate 76.* — Monocytic leukaemia (peripheral blood). Wright's stain.

*Note:* the size of the cells and the presence of distinct nucleoli.



*Plate 77. — Monocytic leukaemia (bone marrow).*

Monocytoidal cells, monocytes, 3 polychromatic and 1 orthochromatic erythroblast are represented on the drawing.



out a concomitant myeloblastic proliferation) and peripheral monocytosis are, however, evaluated carefully and critically by most haematologists. The opinion prevails that peripheral monocytosis should be rather accompanied by a reticulum-cell proliferation in the bone marrow, since monocytes originate from these cells. This contention finds support in a case observed by one of the present authors.

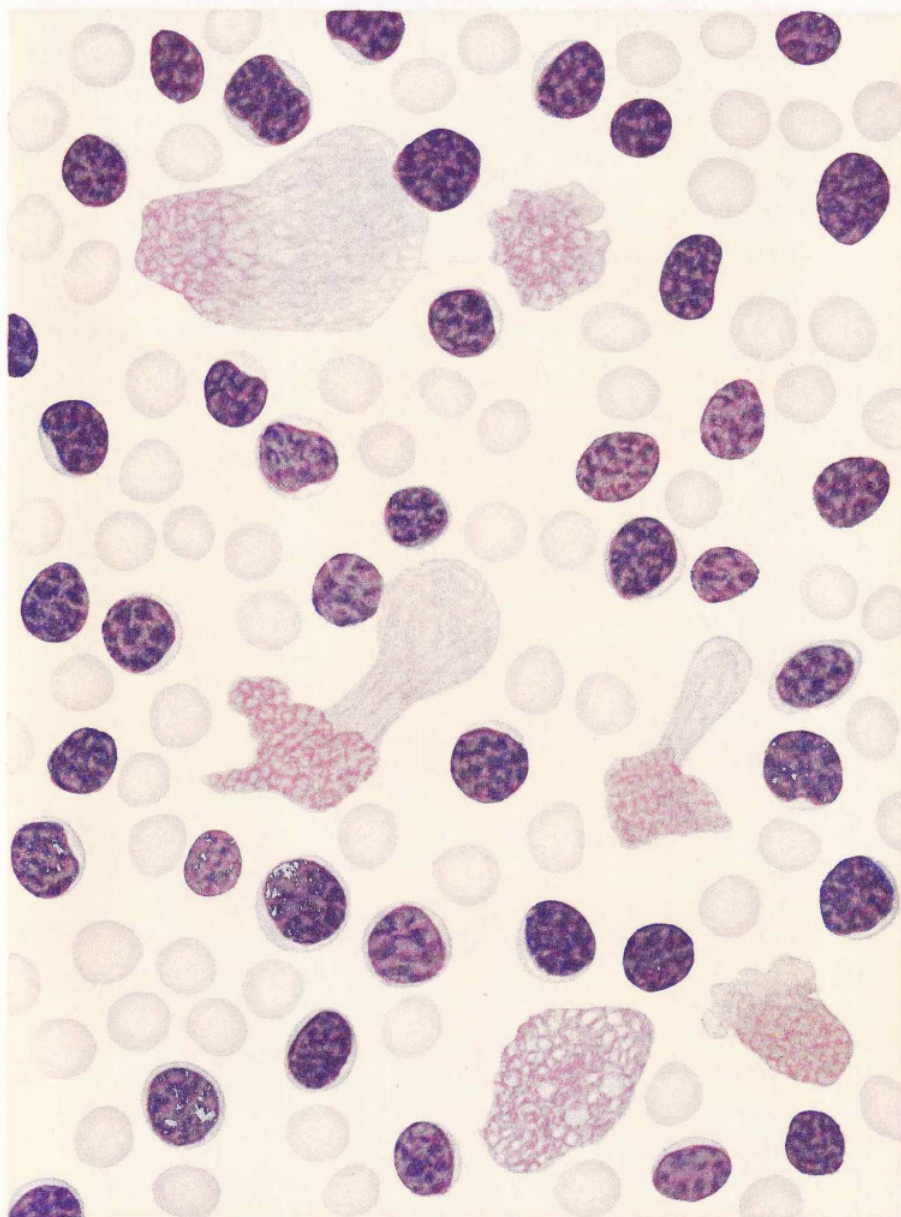
A 38-year old female showed mild anaemia, splenomegaly and generalised lymph-node enlargement. These symptoms persisted for about three years. During the mild course of the disease, subfebrile reactions and general weakness appeared periodically. In peripheral blood, the leukocyte count ranged from about 20,000 to 40,000 per cu.mm. A marked preponderance of atypical monocytes, certain of them faintly peroxidase-positive, was prominent. Sternal puncture, twice repeated, yielded negative results ("dry puncture"). A small greyish tissue fragment was, however, found in the lumen of the puncture needle. In smears prepared from this scanty material, numerous lymphoid reticulum cells of uniform morphological features were demonstrated. On the other hand, no myeloblasts or monocytoid cells were encountered. A similar picture was obtained on examination of the bone marrow aspirated from the iliac crest. Unfortunately, the patient was not cooperative, and refused hospitalisation. A similar case was examined by one of the present authors in his capacity as haematological consultant.

Cases characterised by both peripheral and bone-marrow monocytosis present a different picture. In these instances, the diagnosis of monocytic leukaemia may be challenged.

Large cells, corresponding morphologically to monocytes are reproduced on Plate 76. In spite of the apparent maturity of the cytoplasm (the presence of minute azurophilic granules), several of the nuclei contain nucleoli.

Plate 77 illustrates the myelogram in this case. The bone marrow was hypocellular; no foci of myeloblastic proliferation could be demonstrated. Numerous monocytoid cells and small stem-cells with an agranular basophilic cytoplasm and leptochromatic nuclei were present. No intermediate forms between these two cell types were encountered.

The origin of such cells cannot be elucidated by morphological methods only. It may be that questions arising out of similar observations will be answered by means of cytochemical studies and serological investigations. In the case, in which the cells reproduced on Plates 76 and 77 were encountered, a diagnosis of paramyeloblastic leukaemia was made improbable by the clinical symptoms and chronic course of the disease.



*Plate 78. — Chronic lymphatic leukaemia (peripheral blood).*

Numerous small lymphocytes with agranular cytoplasm and "naked lymphocytic nuclei" are represented on the drawing. The blood picture is characteristic by its monotony.

*Note:* the presence of several Gumprecht's cells.



## CHRONIC LYMPHATIC LEUKAEMIA

*Chronic Lymphocytic Leukaemia — Chronic Lymphadenosis*  
*Lymphadenosis Leukaemica Chronica — Leukaemia Lymphatica Chronica*

In typical cases of fully developed lymphatic leukaemia, examination of peripheral blood smears is sufficient for correct diagnosis.

As the disease becomes more advanced, lymphocytosis increases progressively. Anaemia, in some instances present at an early stage, is among

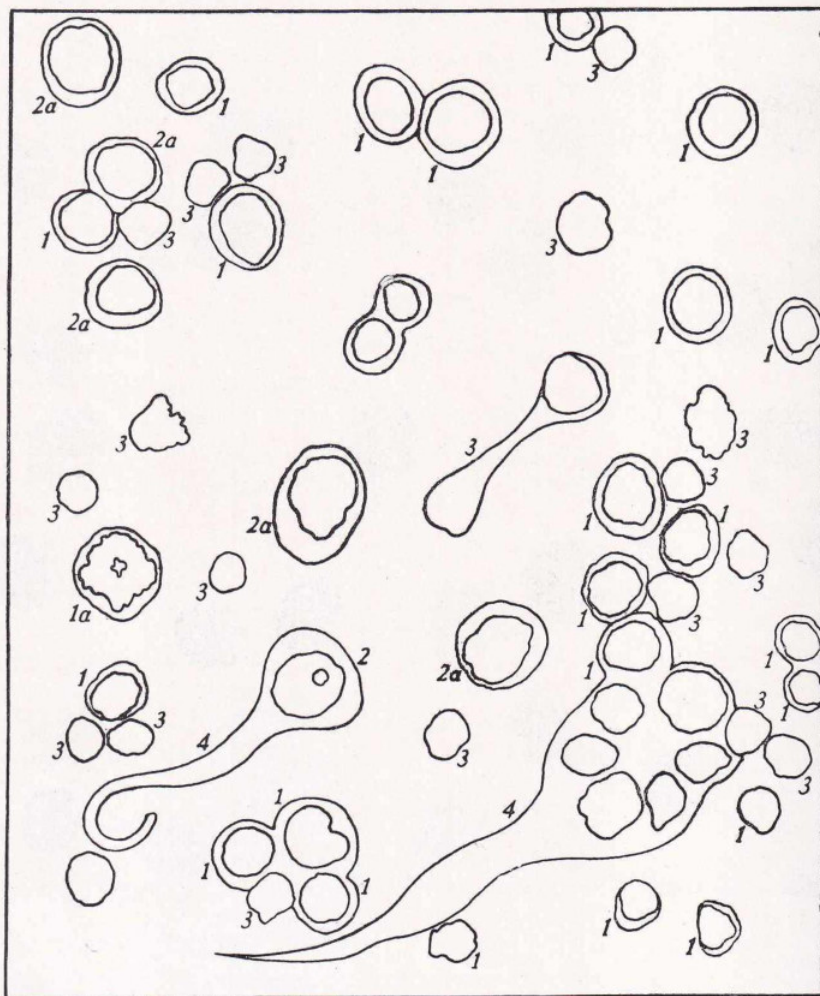
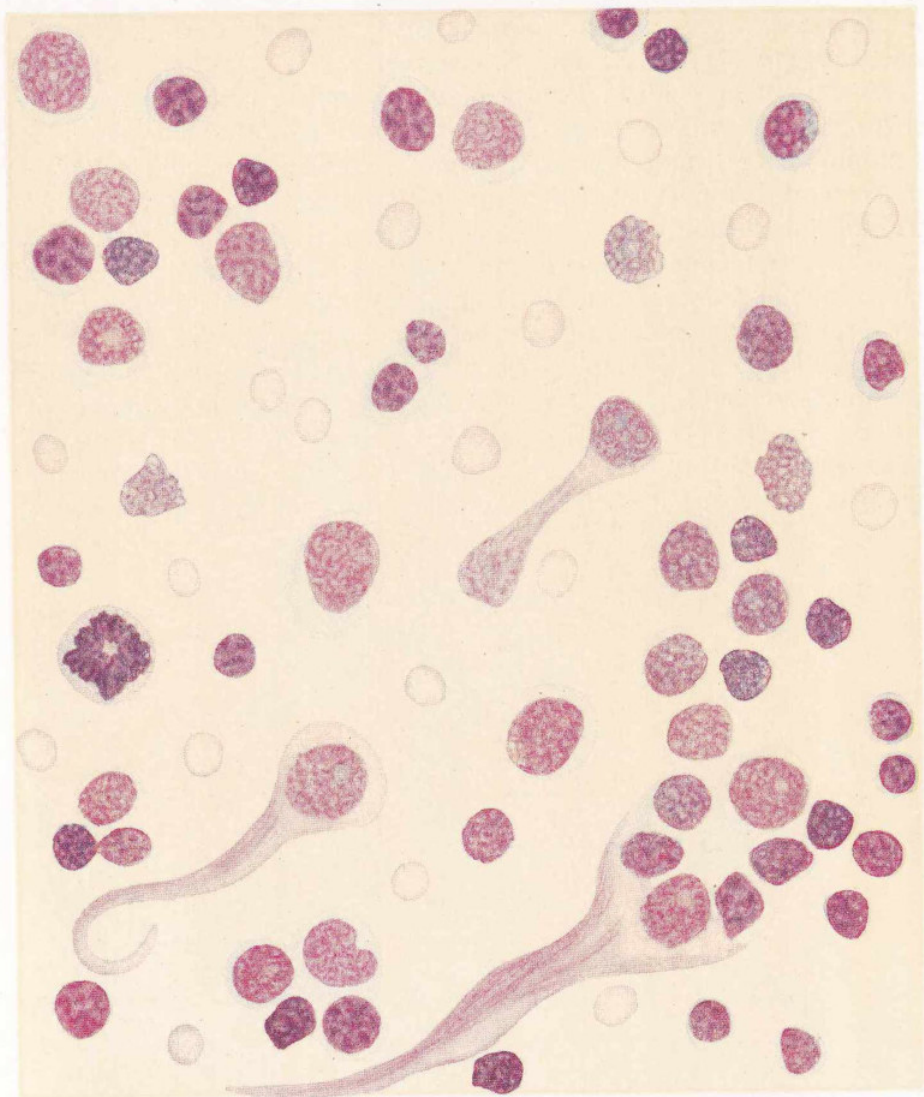


Plate 79. — Chronic lymphatic leukaemia (lymph node aspiration smear): 1. Lymphocyte. 1a. — lymphocyte in mitosis. 2. Lymphoblast. 2a. — prolymphocyte. 3. Naked lymphocytic nuclei and basket-cells (Gumprecht's shabows). 4. Connective tissue fibers.



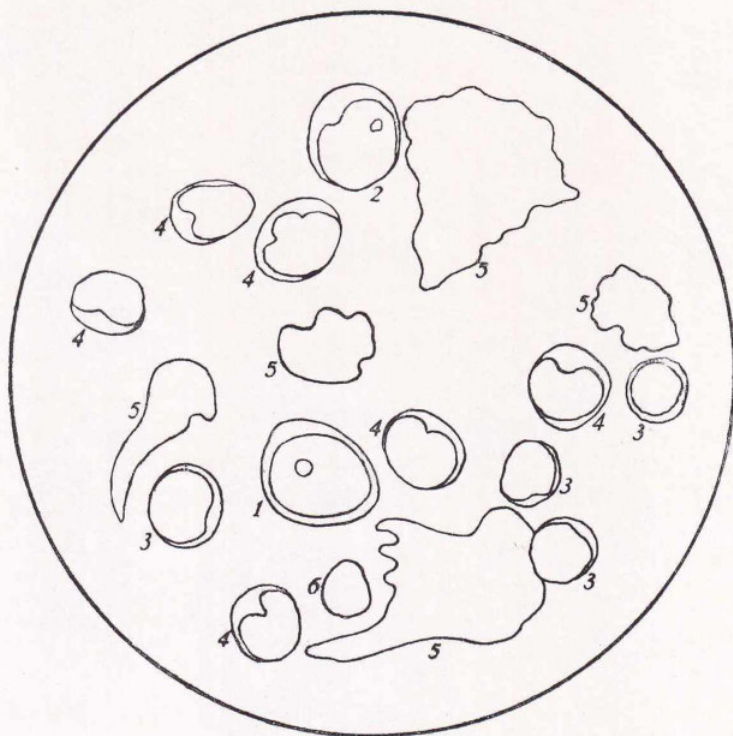
*Plate 79.* — Chronic lymphatic leukaemia (lymph node aspiration smear).



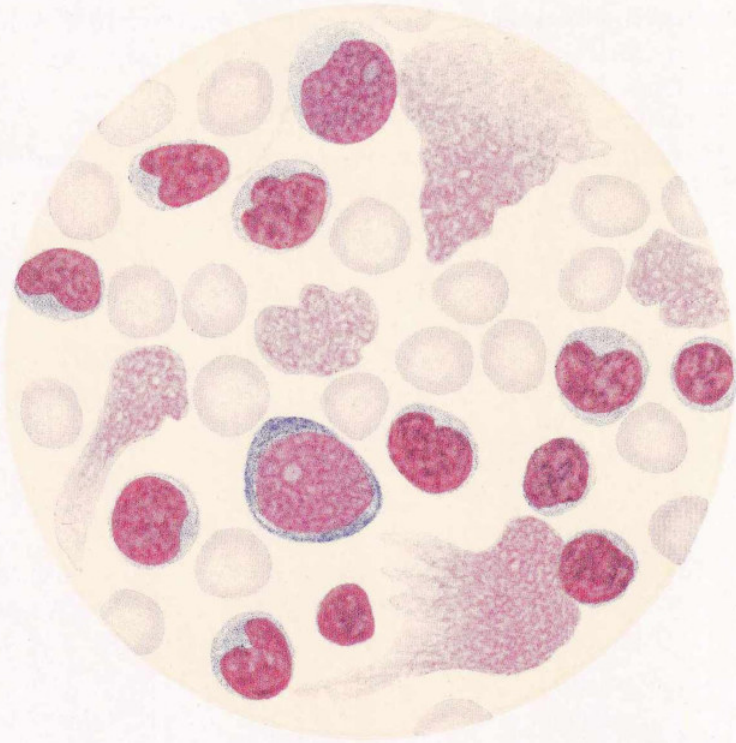
the fairly common symptoms. Thrombocytopenia, leading to haemorrhagic diathesis, may be occasionally observed.

The most characteristic feature of blood smears is the preponderance of lymphocytes, which may amount to over 90% of white blood cells. In chronic cases, small lymphocytes with a narrow agranular cytoplasmic rim and a round, pycnotic nucleus are present almost exclusively. "Naked lymphocytic nuclei" are also numerous by contrast with myeloid leukaemia, the blood picture is monotonous. Only rarely are a few lymphocytes with indented nuclei or immature cells (prolymphocytes, lymphoblasts) encountered. The cytoplasm of lymphocytes is nearly always agranular. Certain haematologists suggest that the presence of granular lymphocytes should be considered a favourable prognostic sign as to the duration of life.

The presence of Gumprecht's cells ("smudge cells"), Gumprecht's shadows, basket-cells confirms the diagnosis of lymphatic leukaemia.



*Plate 80.* — Chronic lymphatic leukaemia (bone marrow): 1. Lymphoblast. 2. Prolymphocyte. 3. Small lymphocytes. 4. Rieder's cells (lymphocytes with atypical nuclear shape). 5. Gumprecht's cells (degenerated lymphocytic nuclei). 6. "Naked" lymphocytic nucleus.



*Plate 80.* — Chronic lymphatic leukaemia (bone marrow).



Gumprecht's cells<sup>1</sup> (smear-cells) probably originate from damaged nuclei of lymphocytes or their stem-cells, and it is claimed that they may originate from damaged reticulum cells (Plate 78).

The lymphadenogram indicates a uniform proliferation of all elements of the lymphatic system (lymphocytes). Small, agranular lymphocytes, however, preponderate. Earlier developmental stages are relatively sparsely represented and mitotic forms may be encountered. Gumprecht's cells are less numerous than in peripheral blood (Plate 79).

In examination of a lymphadenogram from a case of chronic lymphatic leukaemia particular attention should be directed to:

1. Morphology of individual cells — their shape, size and degree of differentiation (maturity);
2. Which cell types are most abundantly represented;
3. Anisocytosis of white cells;
4. Occurrence and number of immature cells;
5. Appearance and number of nucleoli;
6. Mitotic forms; in chronic lymphatic leukaemia, these occur infrequently; the finding of numerous karyokineses may be suggestive of lymphosarcoma, although increase in cell divisions is not pathognomonic to this disease.

Reliable histological criteria for diagnosis of lymphatic leukaemia do not exist — the sections of lymph nodes present only the picture of lymphocytic proliferation, which obscures the normal histological structure. The diagnosis of chronic lymphatic leukaemia should therefore be based on the clinical picture and on findings of joint biopsies.

The lymphadenogram findings acquire particular value in cases, in which lymph-node enlargement is not accompanied by lymphocytosis in peripheral blood. In such cases, the picture of lymphocytic proliferation indicates the possibility of latent lymphatic leukaemia.

The bone-marrow findings depend on the advancement of the disease and lymphocytic proliferation. At initial stages of the disease the number of lymphocytes may be normal, in fully developed lymphadenosis they are of marked preponderance. Bone-marrow lymphocytosis occurs only rarely in adults and patients with this symptom should be suspected of lymphatic leukaemia.

Plate 80 illustrates lymphocytic proliferation in bone marrow. Note the preponderance of small lymphocytes and the presence of only two earlier developmental stages. Lymphoblasts and prolymphocytes are more

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<sup>1</sup> Since the term Gumprecht's cell (or in literal translation Gumprecht's shadow) is in common use on the Continent, it is used consistently in this book, although it may be less familiar to the English reader (translator's note).

frequently found in bone marrow than in peripheral blood. In chronic leukaemia, however, mature forms dominate the picture.

Since lymphocytic transformation may be present at an early stage, bone-marrow examination is helpful in diagnosis of latent lymphadenosis.

The diagnosis of chronic lymphatic leukaemia should be based on following symptoms: 1) lymph-node enlargement, often generalised; usually no tenderness of lymph nodes is demonstrable; 2) enlargement of the spleen; 3) haematological findings.

### ACUTE LYMPHATIC LEUKAEMIA

*Acute Lymphadenosis — Lymphadenosis Leukaemia Acuta*  
*Lymphadenosis Lymphoblastica*

The differentiation of this disease from acute myeloblastic myelosis is often impossible and of academic value only. Numerous haematologists therefore regard such attempts as superfluous. Aleksandrowicz states:

... "if, in initial phases of the disease, numerous questionable mononuclear cells are encountered, myeloblastosis may be assumed and the bone-marrow tissue looked upon as the matrix of leukaemic proliferation. In cases of peripheral lymph-nodes involvement and an unchanged bone marrow, lymphadenosis may be suspected. Of course, such criteria are in many instances fallacious"...

Acute lymphadenosis develops mainly in infants although its occurrence has been reported also in adults.

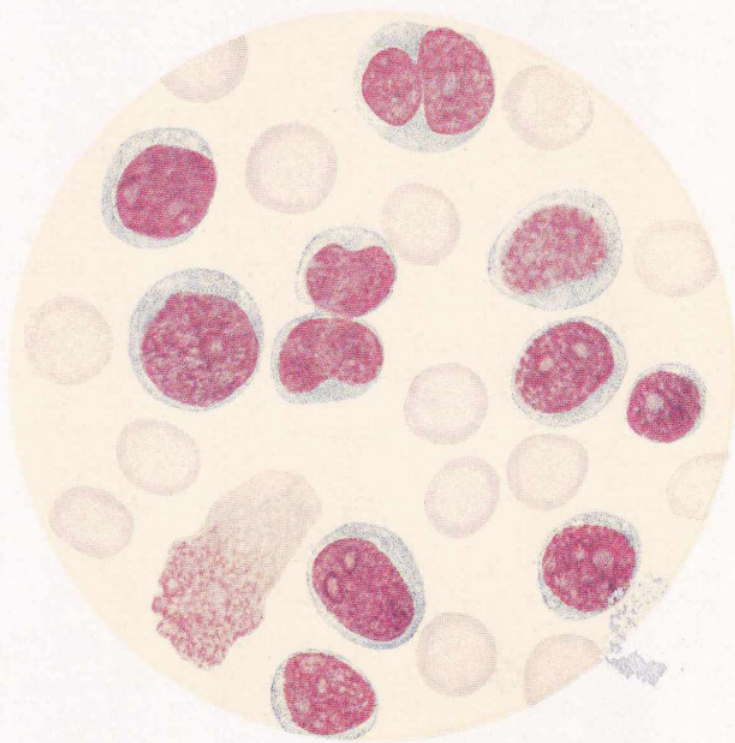
The present authors followed a case of acute lymphatic leukaemia in a 31-year old female with generalised lymph-node enlargement. By contrast with the chronic form, the lymphocyte types encountered in peripheral blood were more varied.

On Plate 81 the anisocytosis of mononuclear cells, the round lightly stained nuclei containing nucleoli should be noted. One of the cells is binucleated. The cytoplasm is narrow, agranular and faintly basophilic. A few Gumprecht's cells were encountered.

The blood picture indicates acute leukaemia (immaturity of the cells). The cells are peroxidase-negative. The diagnosis cannot, however, be based only on peripheral blood examination, because these cells may correspond to atypical myeloblasts. The presence of two nucleoli only in the nuclei of the cells may indicate lymphoid origin, but such differential criteria are not to be relied upon.

In the bone-marrow smears, numerous mononuclear cells are present. They show marked anisocytosis, the nuclei are round of immature structure. The cytoplasm is scanty, almost invisible. Among the most striking features is the presence of atypical mitoses with blurred outlines of chromosomes, clumped together. Such mitotic forms resemble those of lymphoid cells (Plate 82).

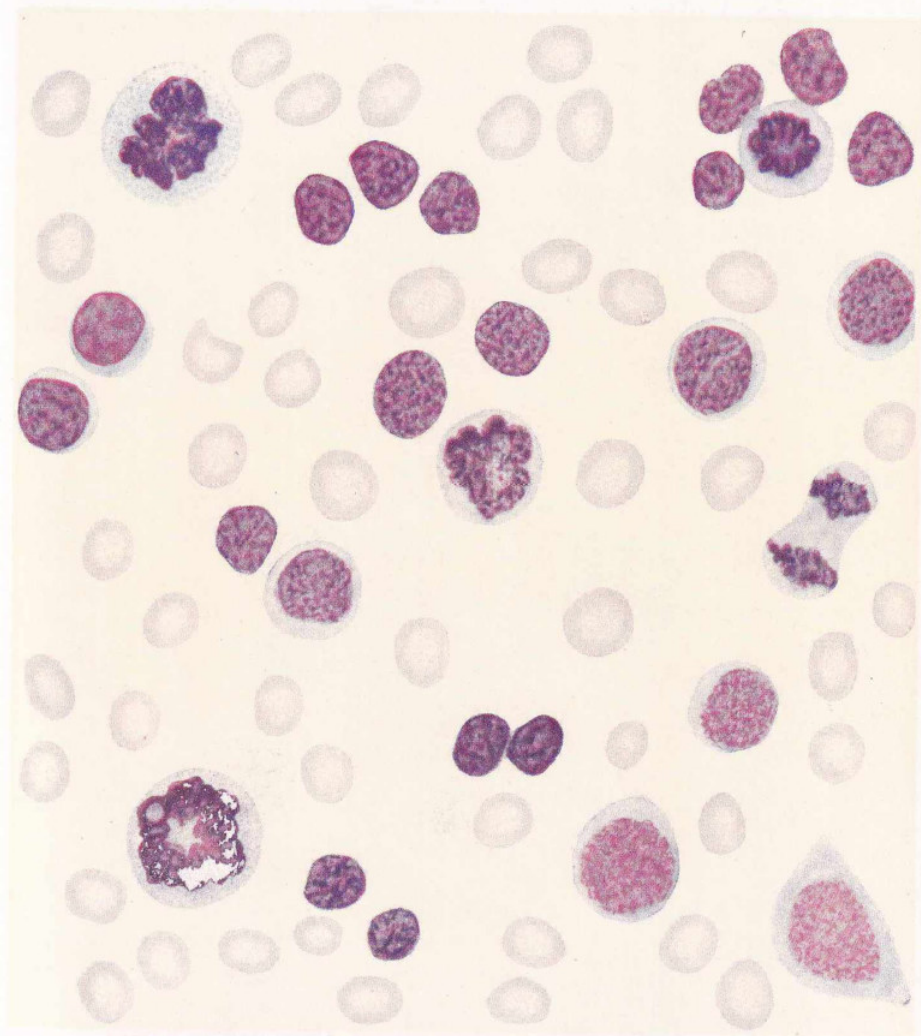




*Plate 81.* — Acute lymphatic leukaemia (peripheral blood).

Lymphoblasts and prolymphocytes preponderate.

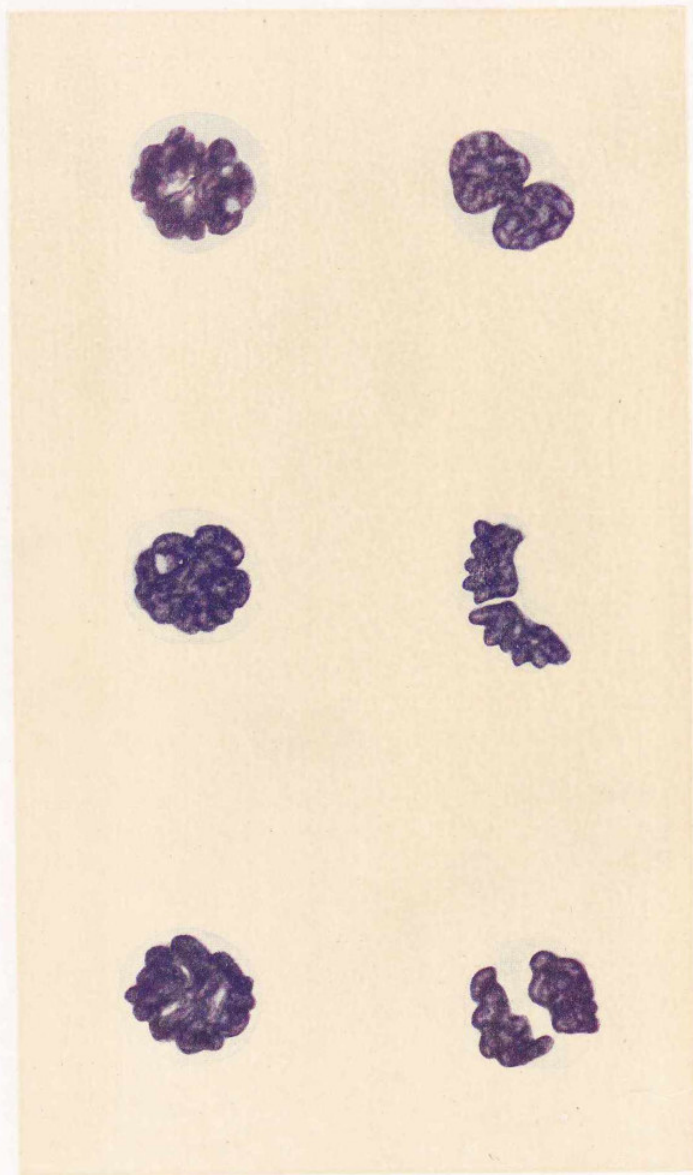
*Note:* one binucleated cell and one Gumprecht's cell.



*Plate 82.* — Acute lymphatic leukaemia (bone marrow).

*Note:* the exclusive presence of the cells of the lymphatic series and numerous atypical mitoses.





*Plate 83.* — Acute lymphatic leukaemia. Lymphoblastic mitoses.  
*Note:* the blurred outlines and clumping together of chromosomes, distributed in an un-  
orderly manner.

In normal conditions, mitoses of lymphatic cells are extremely rarely encountered. The present authors observed numerous mitoses in the case of acute lymphadenosis described above and in lymphosarcoma. The chromosomes are short and thick, clumped together — which causes the blurring (Plate 83).

Among criteria applied to the cytological diagnosis of the case described above, the abnormality of the mitotic forms was taken into account. This feature, usually overlooked in haematological considerations, seems to deserve greater attention. It should be emphasised that purely morphological criteria are unsatisfactory for differentiation between stem-cells of the myeloid and lymphoid cell series. It may be that cytochemistry will find way to meet the need for such criteria. Cytoenzymatic studies indicates that the cells of the myeloid and lymphoid series react differently when treated by ribonuclease. Fluorescence microscopy constitutes another advance in this field. In smears stained by acridine orange, lymphoid cells show orange fluorescence, whilst cells of the myeloid series fluorise green.

It is to be expected, moreover, that cytoimmunological methods will be helpful. Investigations in this field have demonstrated differences in the antigenic properties of myeloblasts and lymphoblasts.

### LYMPHOSARCOMA

Proliferative processes in the lymphatic system may concern all cellular elements, or may be limited to individual cell types only. These pathological phenomena lead, according to the dynamics of their progress, to various changes in the structure of the lymph node, and to its enlargement.

There is considerable confusion in the nomenclature of primary diseases of the lymphatic system. The terms "lymphoma" or "lymphoblastoma" are often misused for the description of a wide variety of pathological conditions. These terms embrace Hodgkin's granuloma, Hodgkin's sarcoma, lymphatic leukaemia, Brill-Symmers disease together with different cytological varieties of sarcoma (retiotheliosarcoma, lymphosarcoma etc.). Strict differentiation between proliferation of the lymphocytic cell series and proliferation of stroma elements seems to be indicated. The term lymphosarcoma should, not, therefore, be used for all primary neoplastic diseases of lymph nodes. Logically, this term should be applied only to the neoplastic proliferation of lymphocytes. Its use is, however, not restricted to description of such conditions. Gall and Mallory are of opinion that lymphosarcoma describes an intermediate stage of lymphatic leukaemia, when proliferation is limited to the lymph node only and not accompanied by generalised symptoms. It seems, however, that lymphosarcoma



cells cannot be identified with leukaemic cells. Although the morphology of these two cell types is similar, they differ in their susceptibility to X-rays.

It was demonstrated that the susceptibility to X-rays of sarcoma cells is about ten times and of leukaemic cells about three times that of normal cells.

The occurrence of acute lymphadenoses is disputed. Wiseman identifies them with leukosarcoma.

The meaning of the term lymphosarcoma should be strictly defined. We therefore use this term only for the description of a neoplastic transformation of lymphocytes. Initially, the disease is limited to the primary site; in later stages, the proliferating cells spread beyond the lymph node, infiltrating the capsule and neighbouring tissues or disseminating through the lymphatics. Atypical lymphocytes may be absent from peripheral blood. At advanced stages normochromic anaemia develops in certain instances accompanied by lymphocytosis in peripheral blood. Cases in which atypical lymphocytes and lymphoblasts are present in peripheral blood may also be encountered.

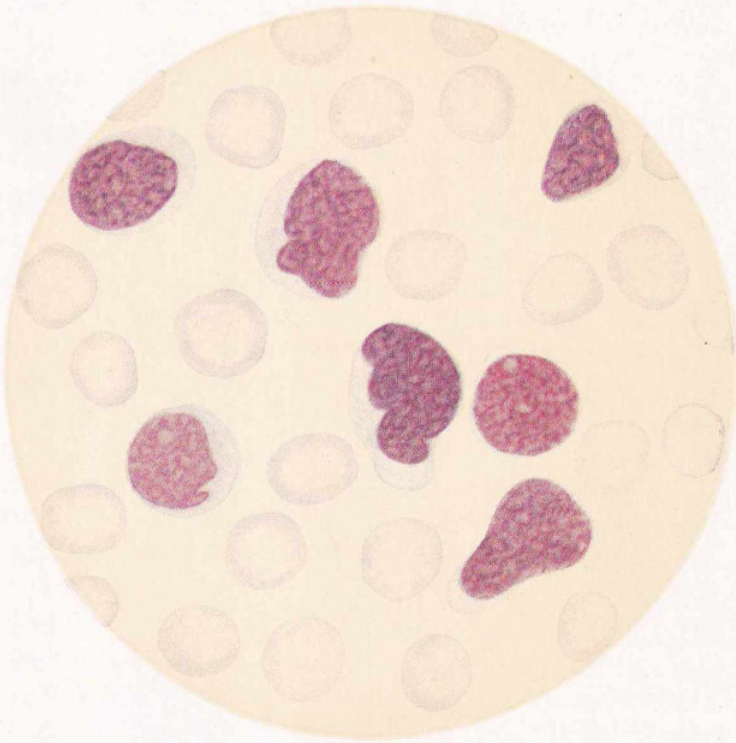
The present authors followed a case of lymphosarcoma in a 52-year old male. Cervical, submaxillar and axillar lymph nodes were enlarged. No enlargement of the spleen could be demonstrated. Peripheral blood findings were as follows: haemoglobin 95%, erythrocytes 6,520,000 per cu.mm., leukocytes 5,800 per cu.mm.; erythrocyte-sedimentation rate 2 mm. in 1 hour, 5 mm. in 2 hours. Peripheral blood smear: lymphocytes 30.24% (prolymphocytes 0.12%, granular lymphocytes 11.63%, agranular lymphocytes 18.49%); peripheral erythroblastosis was present. The simultaneous occurrence of a high erythrocyte count and peripheral erythroblastosis constituted a striking feature of this case. Peripheral erythroblastosis is looked upon as a rather frequent symptom of tumour metastases to the bone marrow. Accordingly, on the basis of this symptom occurring in a patient with lymph-node enlargement, proliferation of foreign tissue in the bone marrow was suspected.

Bone-marrow examination revealed lymphocytic proliferation, the lymphocytes amounting to 80.2% of all cellular elements.

Plate 84 illustrates the bone-marrow picture in this case. Lymphocytes, with nuclei atypical in shape, of immature nuclear structure and with basophilic, agranular cytoplasm are present. The bone-marrow findings demonstrate lymphocytic proliferation. Such findings may be suggestive of lymphatic leukaemia, but lymphosarcoma cannot be ruled out.

Examination of lymph node aspiration smears is essential to diagnosis.

Plate 85 illustrates the lymph-node biopsy findings. Numerous cells of the lymphatic series are present. They are characterised by marked anisocytosis. The nuclei are round in almost all cells. Naked nuclei are frequent. A feature uncommon in chronic lymphatic leukaemia is that immature lymphoid cells, corresponding to prolymphocytes and lymphoblasts,



*Plate 84. — Lymphosarcoma (bone marrow).*  
The nuclear structure of the cells suggests lymphoidal origin.  
*Note:* the diversity of cell-forms.



preponderate. The cytoplasm is basophilic and agranular. Numerous nuclei contain nucleoli of normal size.

Cells, undergoing mitosis, are abundant. Single fields of vision occasionally contain several karyokinetic forms (Plate 85).

Such a picture constitutes ample evidence of lymphocytic proliferation. The presence of numerous immature cells and the unusual frequency of mitoses suggest a neoplastic type of proliferation of lymphocytes — lymphosarcoma.

The satisfactory general condition of the patient, absence of leukaemic blood picture, absence of spleen enlargement, normal temperature, and normal erythrocyte-sedimentation rate do not favour the diagnosis of acute lymphatic leukaemia.

The above case history demonstrates the diagnostic value of lymph-node biopsy. In this instance lymph-node biopsy findings compared with clinical symptoms were the deciding factor in diagnosis.

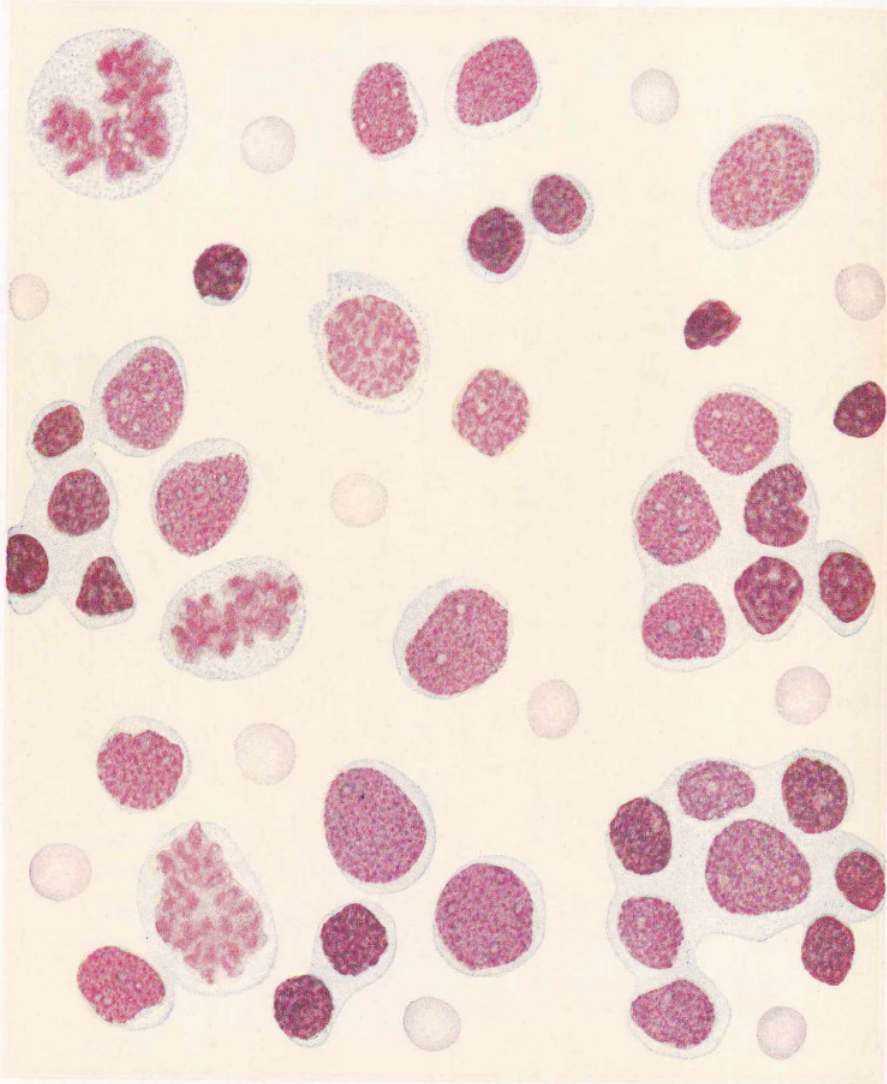
It should be emphasised that an increase in mitotic forms does not constitute a constant symptom of lymphosarcoma.

Neoplastic cells of lymphosarcoma may be characterised by a marked polymorphism and profound degenerative changes. In consequence, it may be difficult to determine the type of the proliferating cell. Another case of lymphosarcoma may serve as an example.

Atypical, giant, vacuolated cells (paralymphoblasts) were demonstrated by lymph-node biopsy. The nuclei were abnormal in shape, the nuclear structure was blurred (Plate 86).

Sternal biopsy performed in this case demonstrated a marked lymphocytic proliferation in the bone marrow, prolymphocytes and lymphocytes of normal appearance preponderating in the myelogram.

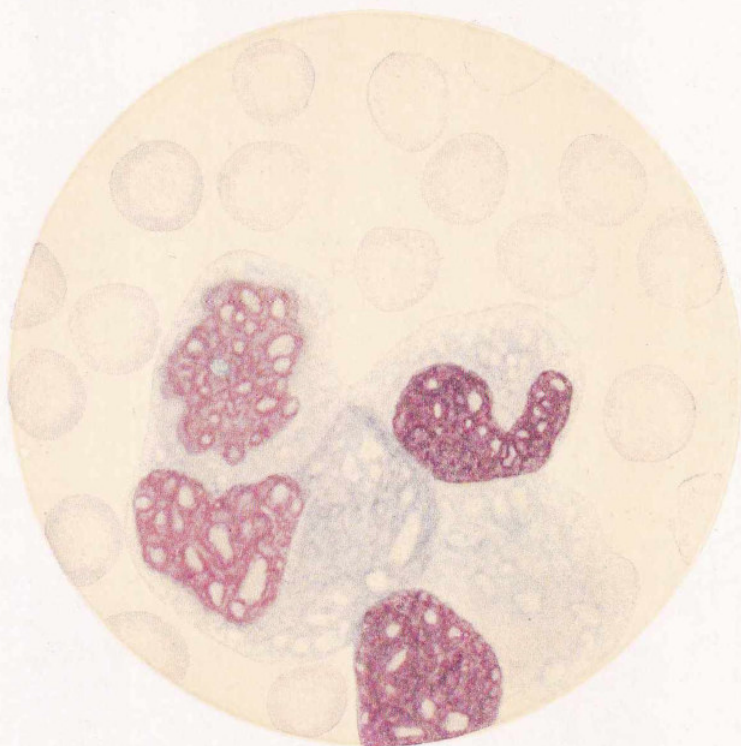
The diagnosis of lymphosarcoma should be based on the entire clinical picture supplemented by joint bioptic examinations. In addition, a diagnostic excision of a lymph node, and histological examination, is recommended. Changes in structure of the lymph node and infiltration of the capsule cannot be demonstrated by puncture. Aspiration biopsy may also yield negative results in cases where neoplastic proliferation is limited to a restricted area of the lymph node.



*Plate 85. — Lymphosarcoma (lymph node aspiration smear).*

*Note:* the diversity of cell types; numerous cells contain several, small nucleoli; 3 atypical mitotic forms are to be seen.





*Plate 86.* — Lymphosarcoma (lymph node aspiration smear).

*Note:* the marked abnormality of the cells — giant dimensions, vacuolation of the cytoplasm and of the nucleus.

## PLASMOCYTOMA

*Multiple Myeloma — Myeloma Multiplex*  
*Rusticky-Kahler's Disease*

Plasmocytoma refers to a malignant tumour originating from bone-marrow plasmocytes. Extramedullar solitary plasmocytic tumours occurring in the region of hilar, oesophageal or tracheal lymph nodes have also been reported. These tumours develop slowly, remain localised for a long time, and do not impair the general condition of the patient to any marked degree. They may, however, metastasise to the bone marrow, resulting in the development of a clinical picture similar to that of multiple myeloma.

In extremely rare cases multiple myeloma may be accompanied by a leukaemic blood picture, the majority of cells present in the peripheral blood corresponding to plasmocytes. Such a condition may be termed plasma cell leukaemia. In the cases described in literature plasma cell infiltration of internal organs, such as spleen liver, was found at autopsy. Such cases may be considered as a link between localised tumour-like proliferation and systemic proliferative diseases. The existence of stepwise gradual transition between localised and systemic proliferative processes is even more clearly demonstrated in the case of myeloblastic leukaemia and chloroma, or intermediate forms between lymphosarcoma and lymphatic leukaemia.

Haematologists — clinicians, biochemists, radiologists and pathologists alike — have devoted a great deal of attention to plasmocytoma because of its typical and manifold symptoms. The clinical and haematological picture of this disease is of great interest. Perhaps the most striking changes are observed in blood proteins. The demonstration of disturbances in protein metabolism accompanying plasmocytoma sometimes requires special methods. Though the total plasma-protein content may be normal, there are pathological variations in protein fractions. Investigations of Wuhrmann and Wunderly demonstrated that cases of plasmocytoma with a normal electrophoretic serum protein pattern are extremely rare. Individual globulin fractions are increased and the albumin-globulin ratio becomes reversed. This phenomenon is reflected by an increased erythrocyte-sedimentation rate, caused by, among other factors, the tendency to rouleaux formation. Such pseudo-agglutination creates difficulties in performing erythrocyte counts and impedes the execution of smears.

Peripheral blood examination reveals anaemia usually of the hypochromic type. It varies in degree according to the stage of advancement of the disease. In certain instances, anaemia may be refractory to treatment and in such cases is severe. Rouleaux formation demonstrable in smears constitutes a significant finding.



The leukocyte count may be normal, but often varies within wide limits; few immature granulocytes and atypical plasmocytes may be present in blood smears. Their occurrence is not among constant symptoms. In certain instances, in particular during the terminal phase of the disease, neoplastic cells may be released into the blood, leading to the development of a plasma cell leukaemia picture.

Reliable diagnostic data may be obtained by bone-marrow examination, frequently the sole means for establishing the correct diagnosis. It is no exaggeration to say that plasmocytoma is among those rare diseases accompanied by a bone-marrow picture so specific that the diagnosis can be in many cases established almost at first glance.

Following the demonstration of neoplastic proliferation in the bone marrow, attention should be directed to — 1) establishing the type of cells involved, and 2) estimation of malignancy on the basis of cytological criteria. It is generally known that plasma cells are the matrix from which all forms of myeloma originate. Reticulum elements are stem-cells of the plasmocytic series. Therefore plasma cells may according to the advancement of differentiation, vary greatly.

Morphologically, they range from forms similar to reticulum cells to that of mature plasmocyte, including numerous intermediate forms. The course of pathologic differentiation, or rather of neoplastic transformation results in an even greater variety of cells, atypical forms being present. Cases are encountered of plasmocytoma characterised by the preponderance of large, immature cells or, in other instances, of small, round plasmocytes. These cytological differences are reflected by the clinical course, the malignancy of the disease and plasma protein abnormalities<sup>1</sup>.

Neoplastic plasmocytes demonstrate, as stated above, a great variety of types. Large cells with round excentric nuclei are the most frequent. The nuclear chromatin does not usually show the "wheel-spoke" arrangement characteristic of mature plasmocytes; its distribution resembles rather that of early developmental stages. The nuclei may contain one or two large nucleoli. The cytoplasm is blue, occasionally dark blue, not infrequently vacuolated. The clear perinuclear zone is absent.

In some cases the plasmocytes may contain inclusion-bodies in the cytoplasm. These may be present in form of rods (similar to Auer's rods), drops or irregular masses. Their appearance is usually hyaline and they are azurophilic, in some instances eosinophilic. Probably these inclusions correspond

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<sup>1</sup> The comparison of the cytological picture with the electrophoretic pattern of plasma proteins has led to the following classification of plasmocytoma types: *type gamma* relatively benign, characterised by an advanced differentiation of plasma cells; and *types alpha* and *beta* — malignant, undifferentiated plasma cells. This classification is based on the electrophoretic mobility of the predominating plasma-protein fraction.

to pathologic proteins secreted by cells, which underwent neoplastic transformation. Such inclusions may be also met with in normal conditions, in form of the generally known Russel-bodies. It is also of interest to note that there appear specific inclusions in the cytoplasm of plasmocytoma — cells during the therapy by Stilbamidine, Pentamidine and Antimony. It is supposed that the inclusions appearing in the course of therapy with these compounds represent the results of a chemical reaction between the drug and the ribonucleic acids of the cytoplasm. The precise mechanism of these phenomena is not known. More cytochemical and cytophysiological investigations are needed. It seems that these problems deserve more attention and will amply repay the efforts of a detailed study.

Plates 87 to 90 illustrate the bone-marrow pictures from three cases of plasmocytoma.

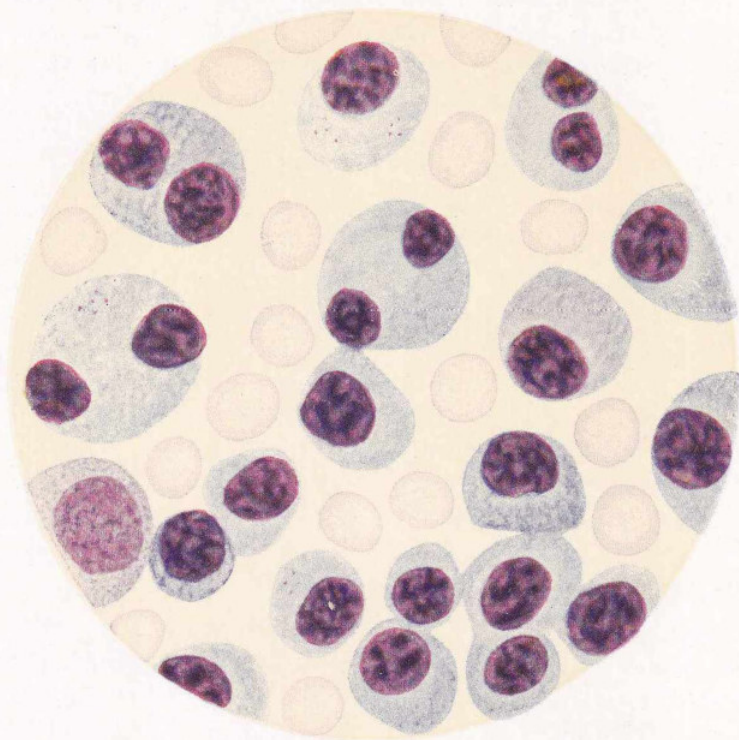
The entire field of vision contains plasmocytes, demonstrating anisocytosis. In addition to small plasma cells, large binucleated plasmocytes are present. Cells possessing three nuclei were also encountered in this preparation. The recognition of these cells presents no difficulties. The nuclei are pycnotic and excentric; nucleoli are invisible. The blue cytoplasm differs from that of mature plasmocytes, being "smooth" in appearance and lacking the whitish perinuclear zone (Plate 87).

The myelogram demonstrates plasmocytic proliferation in the bone marrow. On casual examination, the plasma cells resemble fully differentiated forms, only slightly abnormal.

The plasmocytes reproduced on Plate 88 are more variegated. Intermediate forms between undifferentiated elements and recognisable plasmocytes are present. The greyish-blue cytoplasm and distinct nucleoli indicate the immaturity of the cells. In early plasmocytes, the nuclei are located centrally. The outlines of certain of these cells are indistinct, resembling reticulum cells. Diagnosis in this case may be more difficult, because of abnormality and immaturity of the cells encountered. The presence of intermediate forms is helpful in recognition of all types. According to cytological features, this case may be termed plasmoblastoma or paraplasmocytoma.

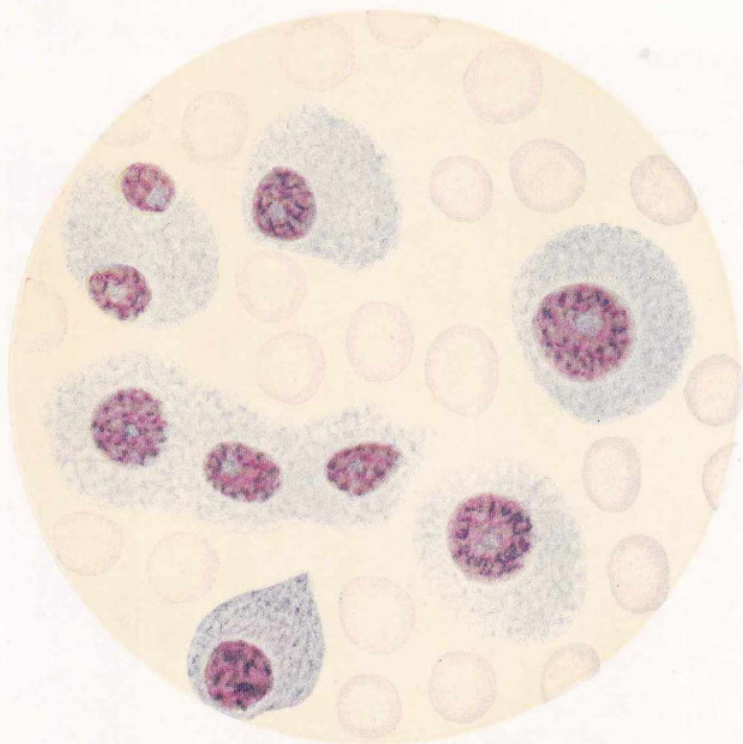
The bone-marrow picture in the third case is characterised by an abundance of various cell types. Immature cells profoundly changed preponderate. Normal plasmocytes are rarely encountered, the majority of the cells resembling undifferentiated reticulum cells, atypical plasmoblasts, or pathologically transformed plasmocytes; only the presence of intermediate forms makes recognition possible. The cells occur in clusters, some of them forming syncytial "flakes". Giant cells with large reticular nuclei and distinct nucleoli should be noted (Plate 89).





*Plate 87. — Plasmocytoma (bone marrow).*

The plasmocytes show no differences from normal cells of this series. Several contain a few azurophilic granules in the cytoplasm. One neutrophilic myelocyte is represented on the drawing.



*Plate 88.* — Plasmocytoma (bone marrow).

*Note:* the distinct blue nucleoli.

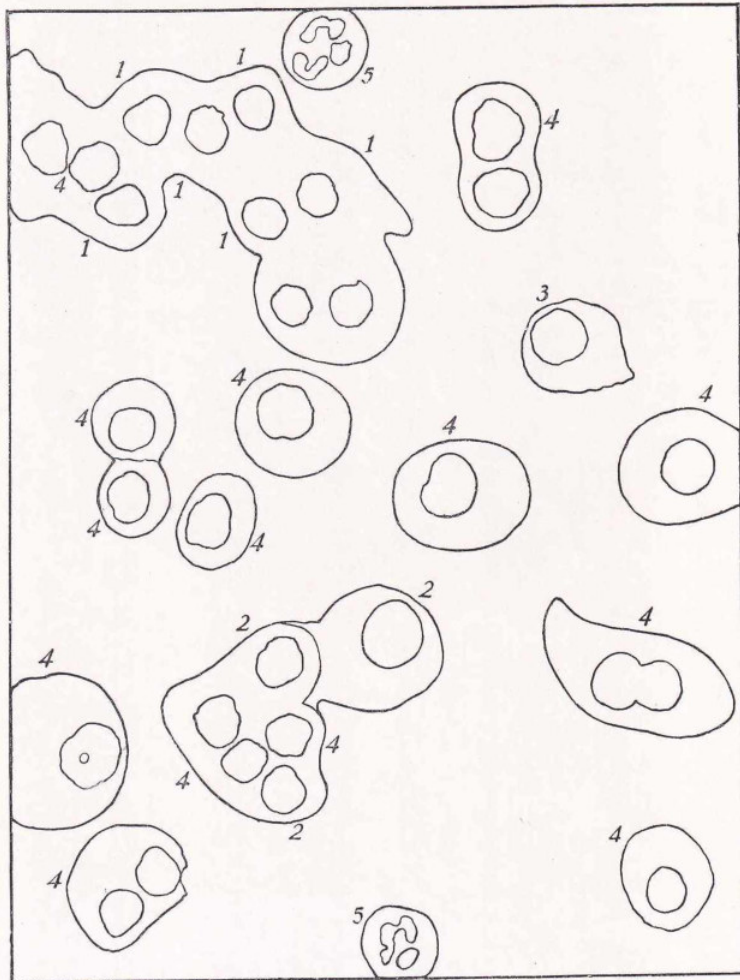


The proliferation of reticulum cells, which show a tendency to transformation into atypical, neoplastic plasmocytes, indicates the particular malignancy of the disease (plasmocytic reticulosarcoma).

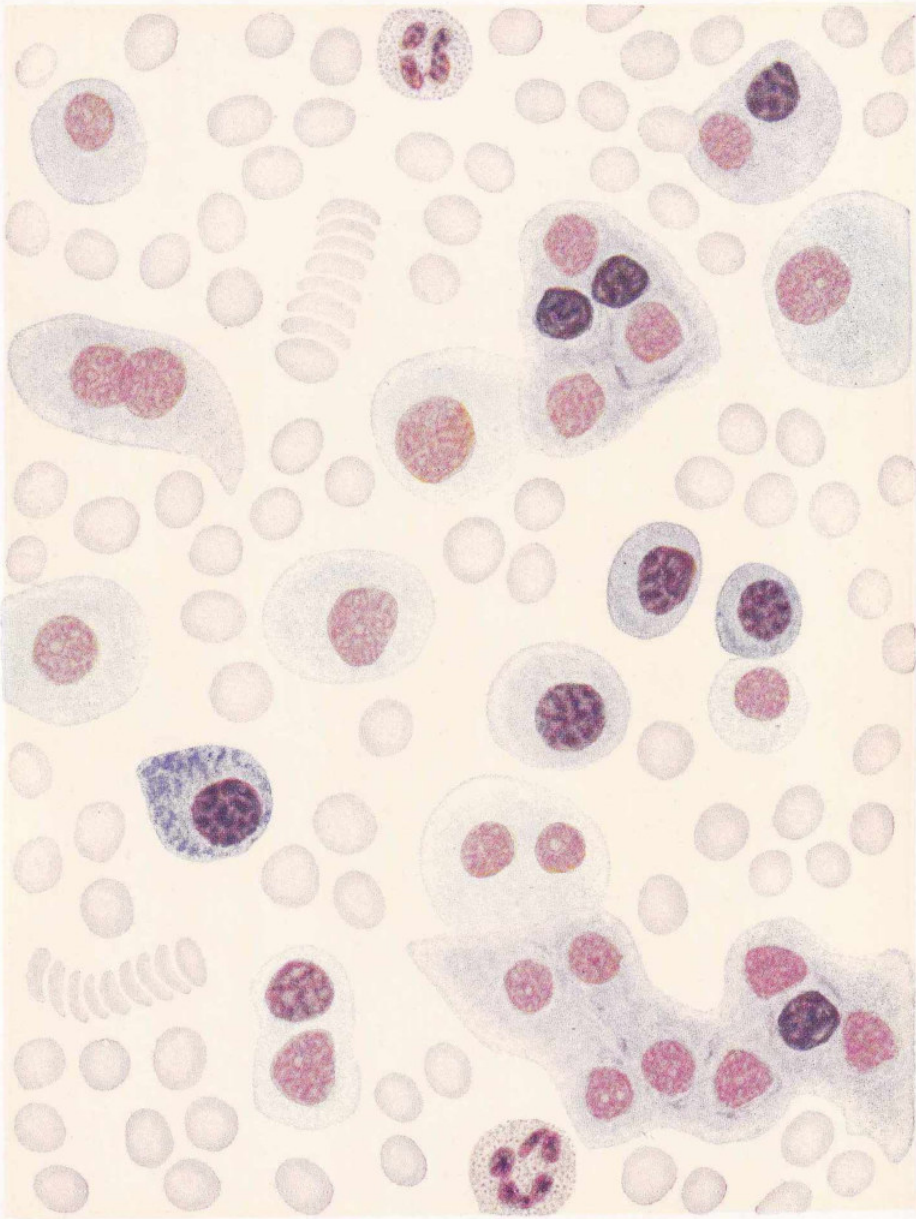
Plate 90 illustrates a fragment of the myelogram characterised above.

Bone-marrow biopsy is essential to the diagnosis of plasmocytoma. Significant clinical symptoms include:

1. Intractable bone pain, usually in the sacral region, spine and ribs; bone tenderness under pressure;



*Plate 89.* — Plasmocytoma (bone marrow): 1. Undifferentiated reticulum cells. 2. Reticulum cells, which show some differentiation — intermediate forms between reticulum cells and plasmocytes. 3. Typical plasmocyte. 4. Abnormal plasmocytes; intermediate forms between these and typical plasmocytes, as well as reticulum cells are to be encountered. 5. Neutrophils.



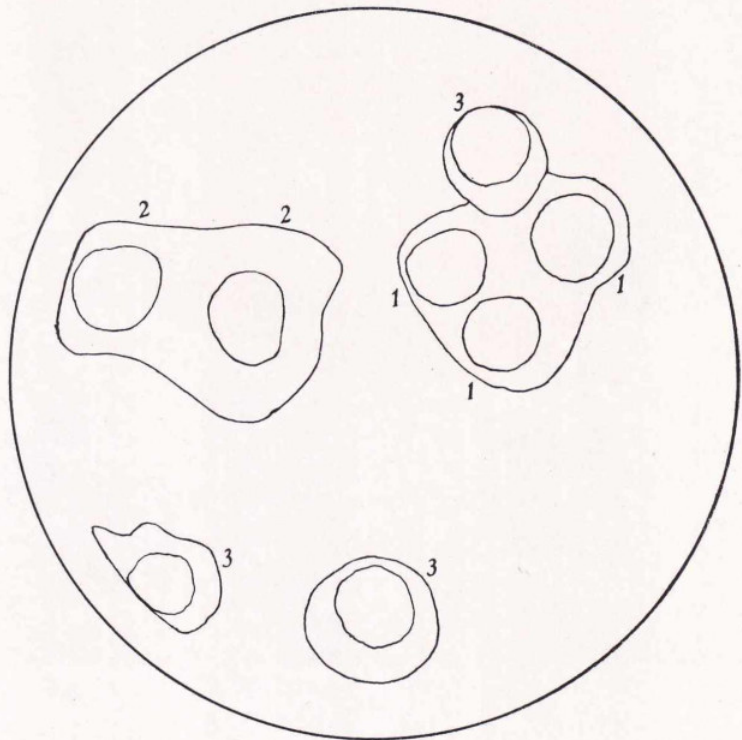
*Plate 89.* — Plasmocytoma (bone marrow).



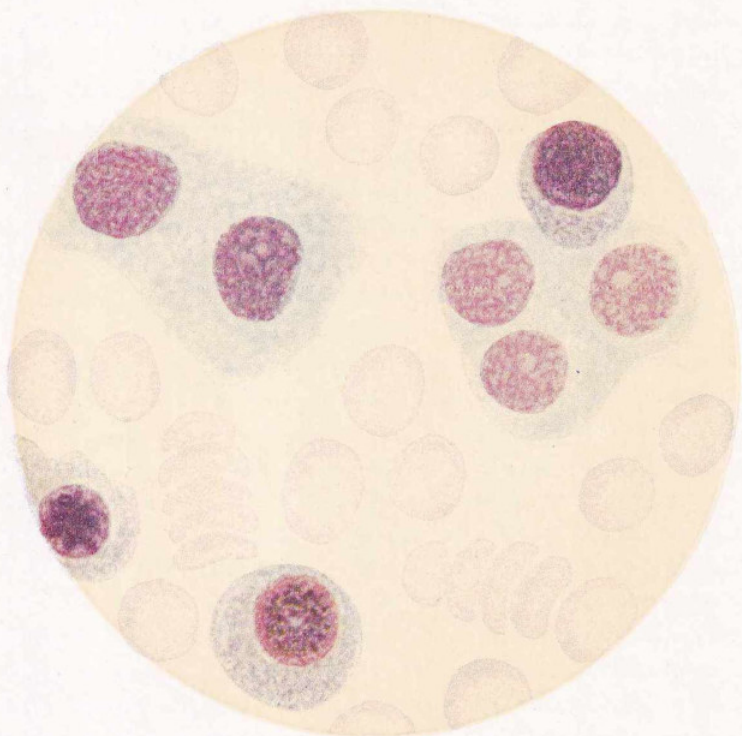
2. Spontaneous bone fractures;
3. X-ray findings (disseminated foci of bone rarefaction);
4. Progressive refractory anaemia;
5. Hyper- or paraproteinaemia;
6. Rapid erythrocyte-sedimentation rate;
7. Findings of urine analysis: the presence of albumin and Bence-Jones protein (inconstant), renal casts and red cells.

The onset of the pain is often attributed by the patient to motion, trauma or even pressure. At the onset of the disease the pain is wandering and intermittent, as the pathologic changes progress it becomes almost intolerable and lasts longer — hours or days, and gradually may become constant. In some instances the initial symptoms may be mistaken for signs of rheumatic disease.

Spontaneous bone fractures concern usually the bones of the trunk and patient may be unaware of their existence. They may even pass unrec-



*Plate 90.* — Plasmacytoma (bone marrow): 1. Undifferentiated reticulum cells. 2. More "mature" reticulum cells; the nuclear structure is no longer reticular and nucleoli are not demonstrable. 3. Paraplasmocytes — the nuclear structure is abnormal, homogenised and blurred; the cytoplasm is atypical.



*Plate 90.* — Plasmocytoma (bone marrow).



ognised by the physician, and are diagnosed on X-ray examination. The lesions seen on roentgenograms are usually in form of round, sharply delineated areas of bone rarefaction, only in rare cases diffuse osteoporosis may be met with. The most frequent sites of osseous lesions are the skull, the sternum, the vertebral column and the bones of the pelvis.

The anaemia presents no specific features.

The presence of hyper- or paraproteinaemia is demonstrated, among others, by the increased sedimentation rate and the findings of urine lysis. The most reliable means, however, of diagnosing changes in serum proteins institutes electrophoretic examination. Among other methods, the nephelogram after Weltmann, the solubility curve of Wuhrmann and Leuthardt, and the cadmium-reaction of Wuhrmann and Wunderly should be mentioned. These methods do not permit any quantitative determination of particular protein fractions; indicate, however, the presence of pathologic deviations. Among pathologic protein cryoglobulins, amyloid and paramyloid, as well as the mentioned above Bence-Jones protein may be found. In connection with disturbances in the protein metabolism the frequent occurrence of falsely positive Wassermann — and other serological tests should be mentioned.

Symptoms of chronic nephritis with nitrogen retention, low blood pressure and usually without oedema or retinitis are often encountered. At autopsy characteristic changes of the kidney tissue are an almost constant finding, which led even to the term "myelomatous kidney" for the description of this condition.

The occurrence of amyloidosis is, in the course of plasmocytoma, a not infrequent phenomenon.

Differential difficulties may be encountered in plasmocytic responses accompanying cancer metastases to the bone marrow. The plasmocytes may be atypical, but in such cases they rarely exceed six percent in number. In plasmocytoma, plasma-cell proliferation is strikingly conspicuous. An increase in bone-marrow plasmocytes occurs also in rheumatoid diseases, Hodgkin's disease, cirrhosis of the liver, and in the hitherto ill-defined group of disorders, referred to as collagenoses.

From the clinical point of view, the varying clinical picture of the disease offers additional difficulties. In a number of cases the initial diagnosis may be erroneous. In some instances rheumatic arthritis, Paget's disease and "refractory anaemia" are diagnosed. A closer scrutiny, however, and the finding of the above described symptoms usually clarifies the case. As said already, the most significant data are furnished by bone-marrow puncture. In some cases, repeated bone-marrow examination may be needed or even biopsy of a tumour (site of the plasmocyte proliferation). Such cases are, however, rare.

## CHRONIC LEUKAEMIC RETICULOSIS

*Reticulum-Cell Leukaemia*

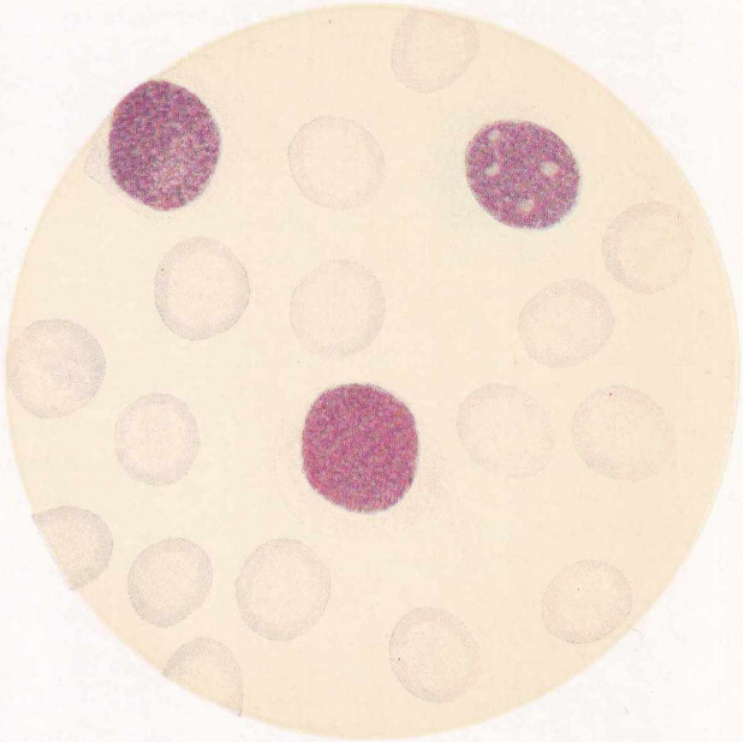
In the foregoing chapters, myeloid leukaemia, lymphatic leukaemia and monocytic leukaemia (a disputed entity) have been described. These are among the more frequently encountered and, in consequence, better understood of such diseases of the haematopoietic system, characterised by irreversible and uncontrolled proliferation of the individual cell series. For example, in the same way as the leukaemic proliferation in lymphadenoses finds its origin in lymphatic tissue, so the granulopoietic cells constitute the matrix from which myeloses develop. Since all cells of the haematopoietic system originate from multipotent reticulum cells it may be stated that all disorders of the blood and blood-forming system come within the pathology of the reticulo-endothelial system. However logically correct, such a statement cannot, for practical purposes, be accepted, in view of the wide and varied range of blood disorders. The pathology of cells arising from transformation of the RES elements should be separately considered apart. Therefore, we use the term pathology of the RES in a restricted sense, referring only to disorders concerning those cells, which form the RES as a histophysiological entity. Since RES cells occur in all tissues and organs, pathological lesions may become manifest in any of them in the course of RES diseases. The clinical picture therefore varies in the extreme according to the site of the morbid process. Moreover RES disorders may become generalised, a condition which complicates the clinical picture.

Localised lesions may become manifest as enlargement of an organ or as disturbances of its function. In other instances, the disease remains latent because of the inaccessibility of its site or because of compensatory mechanisms masking functional insufficiency.

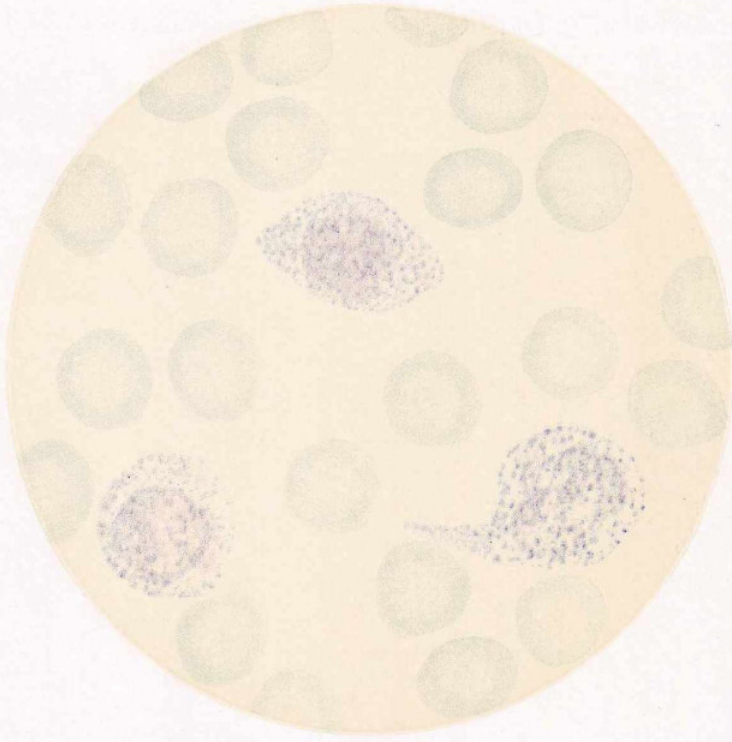
The liver, the spleen, the bone marrow and the lymph nodes contain numerous highly active RES cells. Involvement of these organs in the course of RES disorders gives rise to symptoms perceptible on clinical examination. The finding of an enlarged spleen, liver or lymph node should, among other possibilities, direct the clinician's consideration to diseases of the RES. Unfortunately, the knowledge concerning these is incomplete and most basic works on haematology devote too little attention to this problem.

Reticulum cells are among stroma elements, and their proliferation may be strictly localised to an organ (*e.g.* bone marrow or lymph nodes), no signs of the proliferation being present in the peripheral blood. Numerous instances of such conditions, have been reported and are usually referred to as various forms of reticulosarcoma (*e.g.* Ewing's sarcoma. "Retothelsarkom" — reticulum-cell sarcoma of Roulet) and so-called





*Plate 91.* — Chronic leukaemic reticulosis (peripheral blood).  
Large lymphoidal reticulum cells are present. The nuclear structure of these cells bears a slight resemblance to that of myeloblasts.



*Plate 92.* — Chronic leukaemic reticulosis (peripheral blood stained with toluidine blue).

This stain reveals the presence of numerous minute granules in the cytoplasm.



acute reticulosis of infancy (Abt-Letterer-Siwe disease). On the other hand, reticulum cells may appear in peripheral blood as a symptom accompanying such various blood disorders, as myeloid or lymphatic leukaemias.

The question arises as to whether primary reticulum-cell proliferation is always localised to organs ("latent" reticulosis), or whether there are leukaemic forms in which the reticulum cells become manifest in peripheral blood.

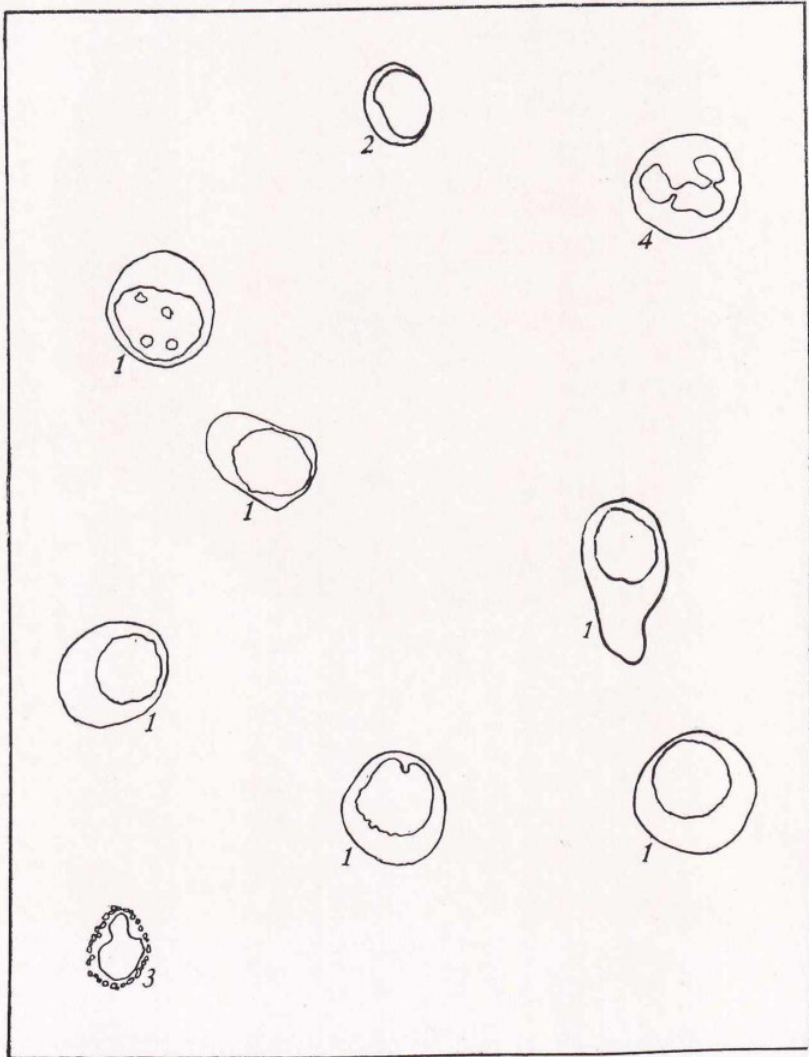
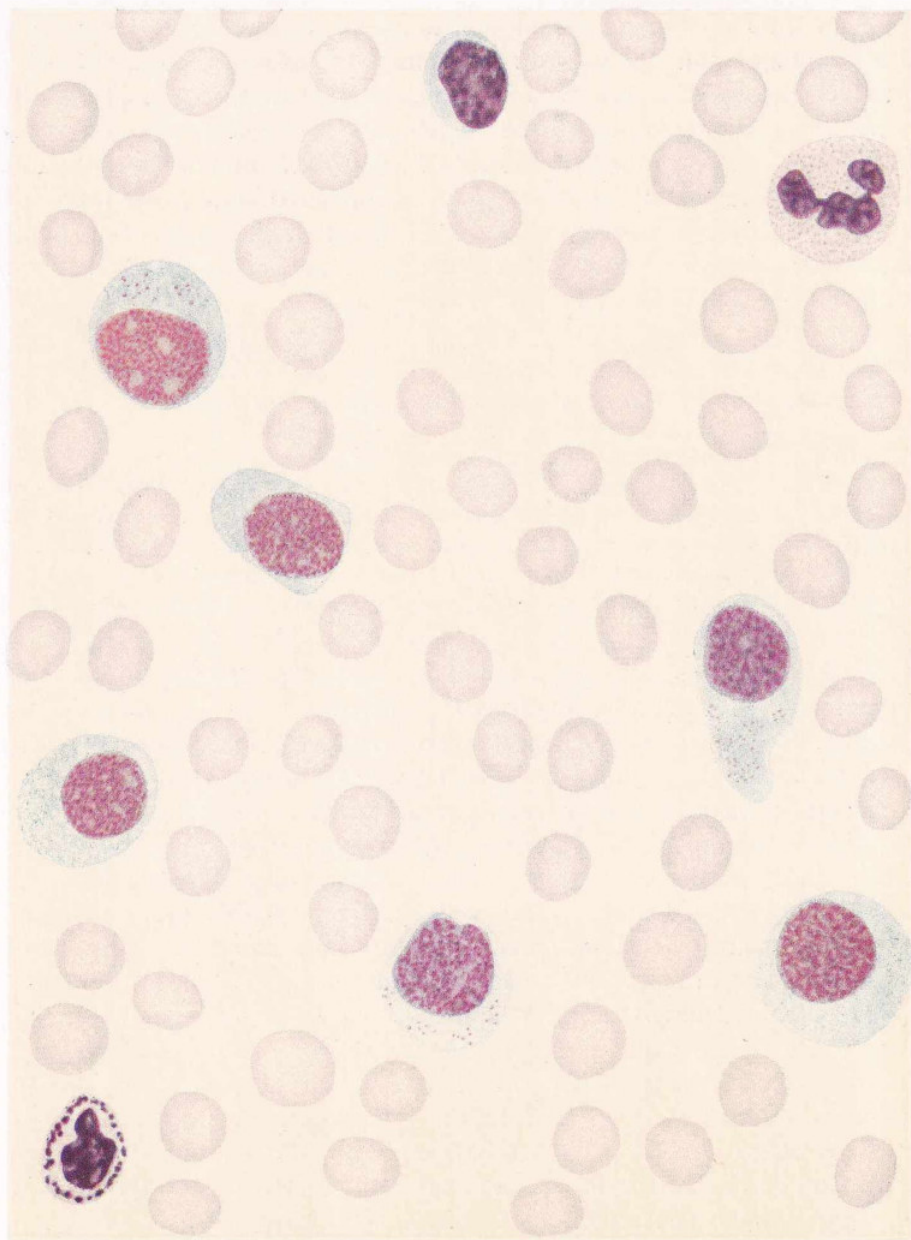


Plate 93.—Chronic leukaemic reticulosis (bone marrow): 1. Lymphoidal reticulum cell. 2. Small lymphoidal reticulum cell. 3. Paraerythroblast. 4. Segmented neutrophil.



*Plate 93.* — Chronic leukaemic reticulosis (bone marrow).



Not so long ago only "latent" or localised forms were known, apart from monocytic and plasmocytic leukaemias which are separate disorders. In recent years several instances of leukaemic myeloses corresponding to the di Guglielmo's histioleukaemia (A. Fieschi) have been reported. The present authors followed a case of leukaemic reticulosis (lymphoidal reticulum-cell leukaemia). On the basis of these observations reticuloses may be classified in a manner similar to that used for myeloses (latent or aleukaemic myelosis — manifest or leukaemic myelosis; latent or aleukaemic reticulosis — manifest or leukaemic reticulosis).

CASE. A 69-year old male was for ten years before admission treated for anaemia, subfebrile reactions and weakness. The erythrocyte count ranged from 2,500,000 to 3,550,000 per cu.mm.; haemoglobin — from 62 to 70%; leukocyte count from 10,000 to 20,000 per cu.mm. The lymph nodes were not enlarged. On the basis of persistent lymphocytosis (86 to 93%), atypical lymphatic leukaemia was diagnosed. On examination, a marked enlargement of the spleen was found. Peripheral lymph nodes were not enlarged. X-ray examination of the chest demonstrated the presence of enlargement and calcification of hilar lymph nodes. Bone lesions could not be demonstrated on thorough X-ray examination of the skeletal system. Erythrocyte-sedimentation rate was usually rapid, the general condition of the patient fairly satisfactory. Peripheral blood findings — mild hypochromic anaemia and the presence of numerous (72%) atypical cells (cf Plates 91 and 92) were present.

Plate 91 illustrates large cells with round or oval leptochromatic nuclei. In certain cells, nucleoli are present. Numerous cells — one of them is reproduced on the plate — were characterised by the excentric position of the nucleus. The cytoplasm is faintly basophilic (pale blue or greyish-blue), and, in the majority of cells, agranular. The outlines of many of the cells were irregular. Certain cells contained minute granula, which could be seen more distinctly in preparations stained with toluidine blue. Morphologically, these cells are similar to lymphoidal reticulum cells.

Plate 92 illustrates a blood smear stained with toluidine blue. Numerous minute granules are present in the cytoplasm.

Myelogram: hypocellularity, erythroblasts 9.7%, granulocytes 10.5%, the majority of the cells — 76.96% — are difficult to classify.

These cells are reproduced on Plate 93. They show anisocytosis and somewhat resemble large and small lymphoidal reticulum cells. The nuclei are round or oval and contain from 1 to 3 small nucleoli. The nuclei are usually excentric; in some instances they lay on the periphery of the cell, suggesting a resemblance to plasmocytes. The nuclear structure is leptochromatic, the cytoplasm greyish-blue, inhomogeneous, of spongy or reticular structure. The cytoplasm of a few cells contains several azurophilic granules, the clear perinuclear zone being absent. The outlines of the cells are irregular and indistinct. A few typical reticulum cells were also present. The cells described above were peroxidase-negative. The bone-marrow picture may be interpreted as a result of reticulum-cell

proliferation. On the basis of haematological findings and clinical features (an atypical proliferative disorder accompanied by anaemia and splenomegaly), chronic leukaemic reticulosis — reticulosis chronica manifesta — was diagnosed. It was demonstrated by cytoimmunological methods that the cells (lymphoidal reticulum cells) present in this case differed in their antigenic properties both from myeloblasts and lymphocytes (Ławkowicz, Lille-Szyszkowicz, and Zawadzki).

## GAUCHER'S DISEASE

### *Lipoidosis Cerebrosidica — Morbus Gaucher*

This rare disease is probably caused by an abnormal function of reticulum-cell enzymes leading to accumulation of lipoidal substances (kerasin) in the cytoplasm. These pathological cells proliferate, and spleen enlargement, anaemia, thrombocytopenia and granulocytopenia develop in consequence. Cell infiltrates may also invade the bone tissue, which becomes manifest as rarefaction areas.

The finding of pathological reticulum cells (Gaucher cells) in bone-marrow smears or in splenograms establishes the diagnosis.

Plate 94 illustrates Gaucher cells. These may be exceptionally large reaching even 80  $\mu$  in diameter. Gaucher cells often occur in clusters discerned under low magnification. The nuclei are small, excentric, of reticular structure and contain nucleoli. The chromatin strands are sometimes coarse. Certain cells may be multinucleated. The cytoplasm is abundant, pale blue, and contains numerous wavy fibrillae. The cells are round, oval or spindle-shaped.

When the following symptoms are present Gaucher's disease should be suspected:

1. Marked enlargement of the spleen, in certain instances of lymph nodes also; peripheral lymph nodes are, however, only rarely involved;
2. Enlargement of the liver;
3. Skeletal pains and bone rarefaction areas;
4. Spontaneous bone fractures;
5. Anaemia and thrombocytopenic purpura;
6. Brownish pigmentation of the skin; particularly of the face, neck, palms and legs from below the knee to the instep; in some instances, yellowish-brown tuberculi may be found on the conjunctiva (pingueculae).

Gaucher's disease belongs to a group of diseases characterised by pathological lipid metabolism and the presence of pathological reticulum cells. These cells accumulate cholesterol and its esters (Hand-Schüller-Christian disease), sphingomyelin (Niemann-Pick disease) or kersin (Gaucher's disease). These traits — pathological metabolism of lipoids



and intracellular accumulation — are common to the whole group of diseases, as specified above. Because of these traits this group of diseases is sometimes denominated as lipoidoses or thesaurysmoses. The pathogenesis of these disease remains obscure. Even more obscure is a somewhat similar disease — thesaurysmosis with accumulation of glycogen — the morbus von Gierke.

Because of the rareness of these diseases no adequate statistical data are available. It should be stressed that detailed biochemical investigations are needed in the first order. Even comparatively large series of cases were published (up to 69 cases), but no adequate biochemical data were included. Cytochemical investigations could also contribute to the understanding of the pathogenetic mechanisms of these diseases. The problems of the aetiology and pathogenesis of thesaurysmoses lie on the borderline of haematology, cytology and biochemistry. Among the most interesting questions pertaining to these diseases is problem of the relationship between the cytological and biochemical disturbances. One of the present authors followed in his capacity of a haematological consultant a case of generalised reticulosis accompanied by hyperlipaemia, in which no cytological signs of abnormal accumulation of lipids in the reticulum cells were present.

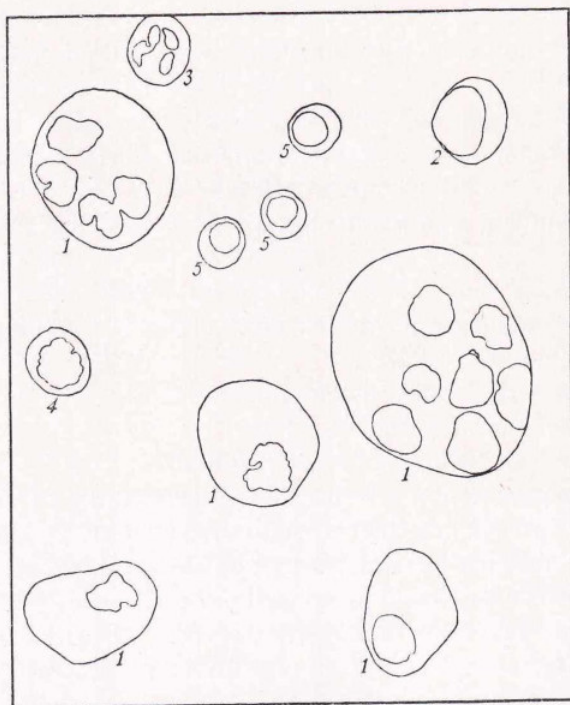
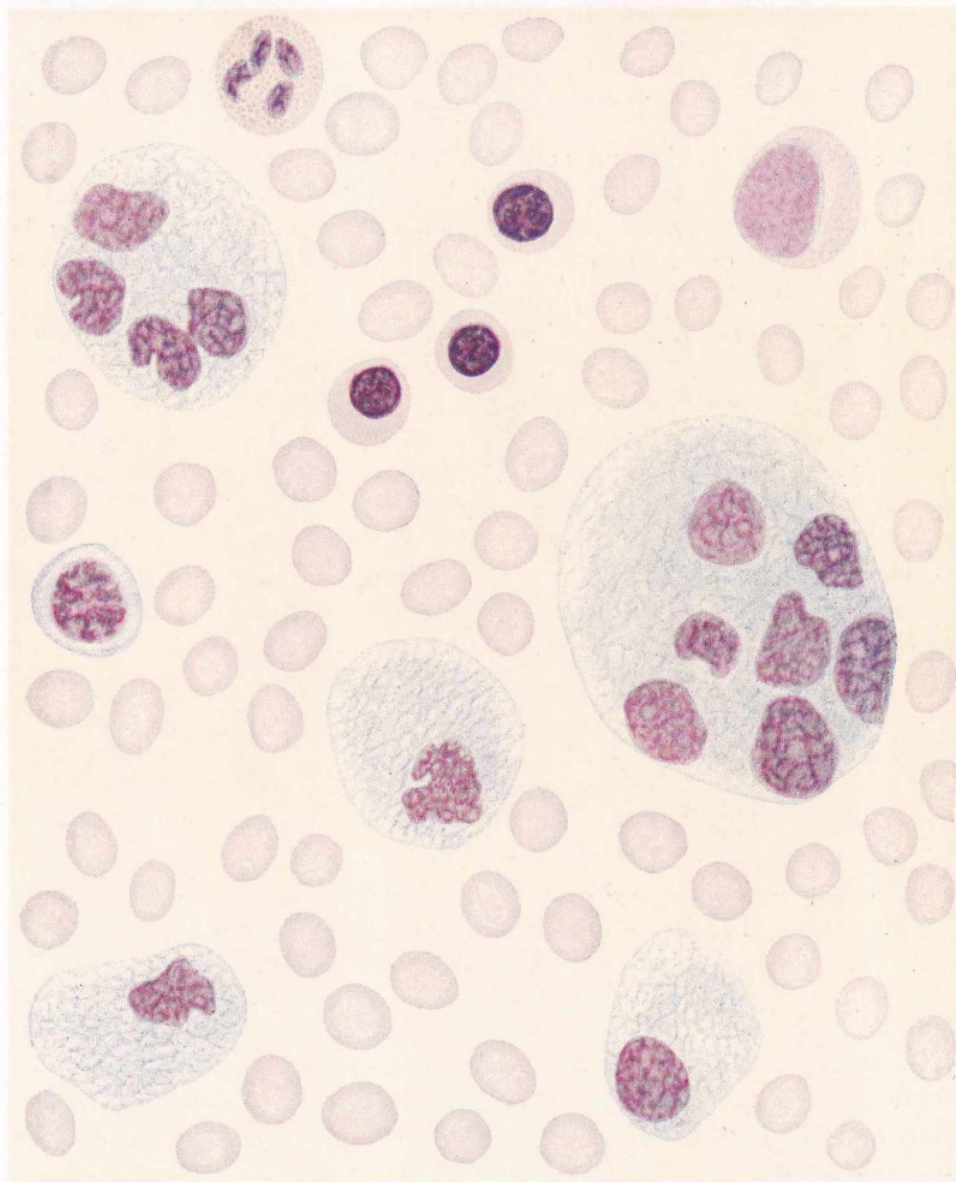


Plate 94. — Gaucher's disease (bone marrow): 1. Gaucher cells. 2. Myelocyte. 3. Segmented neutrophil. 4. Polychromatic erythroblast. 5. Orthochromatic erythroblast.



*Plate 94.* — Gaucher's disease (bone marrow).



## HODGKIN'S DISEASE

*Lymphogranulomatosis Maligna — Morbus Hodgkin — Morbus  
Paltauf-Sternberg — Granuloma Malignum — Hodgkin's Lymphoma*

This disease of unknown aetiology manifests itself by reticulum-cell proliferation leading to characteristic histological pictures. The ubiquity of the reticulo-endothelial system implies that almost all organs may be the primary site of this disorder. The lesions may remain localised for a long time, while in other instances the disease becomes generalised.

Hodgkin's disease chiefly affects the lymph nodes, among other organs the spleen, the liver, the respiratory tract, the digestive tract, the skeletal system, the skin and the nervous system are frequently involved.

There is a vast literature concerning blood findings in Hodgkin's disease. The symptoms vary, however, over a wide range and it is generally agreed that pathognomonic blood changes do not exist. At the utmost, it may be stated that certain findings are suggestive of Hodgkin's disease. Among these, however, inconstant, symptoms is moderate neutrophilic leucocytosis accompanied by lymphopenia and occasionally eosinophilia. The presence of "toxic" (persistent) granulation in neutrophils is also suggestive. In certain instances, monocytosis may be encountered. The blood picture varies in the course of the disease. Refractory anaemia is among frequent symptoms, particularly in advanced stages. It is usually hypochromic, while in other instances the colour index may remain about 1.0. In cases of spleen involvement, the occurrence of haemolytic anaemia accompanied by macrocytosis and sphaerocytosis has been reported. The thrombocyte count remains normal or may be diminished.

Focal granulomatous lesions of the bone marrow are not infrequent, although only rarely demonstrated by aspiration biopsy. Among numerous bone-marrow findings, reported by various authors, maturation disturbances and shift to the left in granulocytes, persistent granulation in neutrophils, increase in reticulum cells, megakaryocytes, plasmocytes and eosinophils call for mention. The presence of large, vacuolated cells ("Lymphogranulomzellen"—Klima) has also been noted, this symptom being, however, rather rare. At an advanced stage of the disorder bone-marrow eosinophilia and an increase in plasma cells is fairly frequent (cf Plate 95).

Bone-marrow examination in Hodgkin's disease rarely contributes positive diagnostic findings, though it may be useful in ruling out other syndromes, which are characterised by lymph-node enlargement.

Typical findings, which make it possible to establish the diagnosis, are obtained by lymph-node examination. Until recently, the view prevailed that reliable diagnostic data can be obtained in histological sections

only. Nowadays, aspiration biopsy has numerous supporters, who claim that this procedure yields findings sufficient for diagnostic purposes.<sup>1</sup>

The histological patterns of granulomatous lesions were classified by Jackson and Parker into three categories: 1) Hodgkin's paraganuloma, 2) Hodgkin's granuloma, 3) Hodgkin's sarcoma.

Type 1 (paraganuloma) is usually characterised by a mild course and involves mostly cervical and mediastinal lymph nodes. The life span in this form is long, cases of 10 years duration having been reported. The histological picture is characterised by proliferation of mature lymphocytes, the presence of the pathognomonic Sternberg cells, reticulum cells, plasmocytes and eosinophils. The number of eosinophils is, however, moderate. The structural pattern of the lymph node is preserved, necrotic foci are absent.

Type 2 (Hodgkin's granuloma) is the most frequently encountered. The clinical picture varies greatly; the life span is shorter, death ensuing after a few years' duration. The histological pattern is characterised by the diversity of cell types present. These include Sternberg cells, neutrophilic and eosinophilic granulocytes, plasmocytes, lymphocytes, reticulum cells and numerous mitotic forms. The lymph node structural pattern is obscured. Necrotic foci are usually demonstrable.

Type 3 (Hodgkin's sarcoma) refers to a malignant variety of short duration. The histological pattern is less varied, large, round undifferentiated cells preponderating. These are considered by certain authors as undifferentiated Sternberg cells. In addition, typical Sternberg cells and a few lymphocytes are present. Plasmocytes, neutrophils and eosinophils rarely only occur. The structural pattern of the lymph node is totally obscured. Necrotic foci are numerous. The pathological cell infiltrates the capsule and encroaches upon perinodal tissues.

The finding of Sternberg-Reed cells establishes the diagnosis of Hodgkin's disease. This feature unites all three types of this disorder; one type may, moreover, become transformed into another, benign forms taking a more malignant course. A few instances of coexistence of all Hodgkin's disease types in a patient have been reported.

Plate 96 illustrates the lymph node aspiration smear picture from a case of fully developed Hodgkin's disease (Hodgkin's granuloma).

By contrast with the monotonous picture presented by the normal lymphadenogram, there is a striking diversity of cellular elements. Among mature lymphocytes, neutrophils, eosinophils, plasmocytes and reticulum cells are to be seen.

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<sup>1</sup> The pioneer work of Tempka and Kubiczek on lymph node aspiration biopsy deserves special mention in discussion of this subject.



Large cells with a single, round or slightly indented nucleus are also present. The nuclear structure is fine. Nucleoli are occasionally encountered. The cytoplasm is abundant, agranular, pale blue or distinctly basophilic, often vacuolated. These cells correspond to early developmental stages of Sternberg-Reed cells and are usually referred to as "small giant cells". Such forms occur in initial stages of granulomatous proliferation.

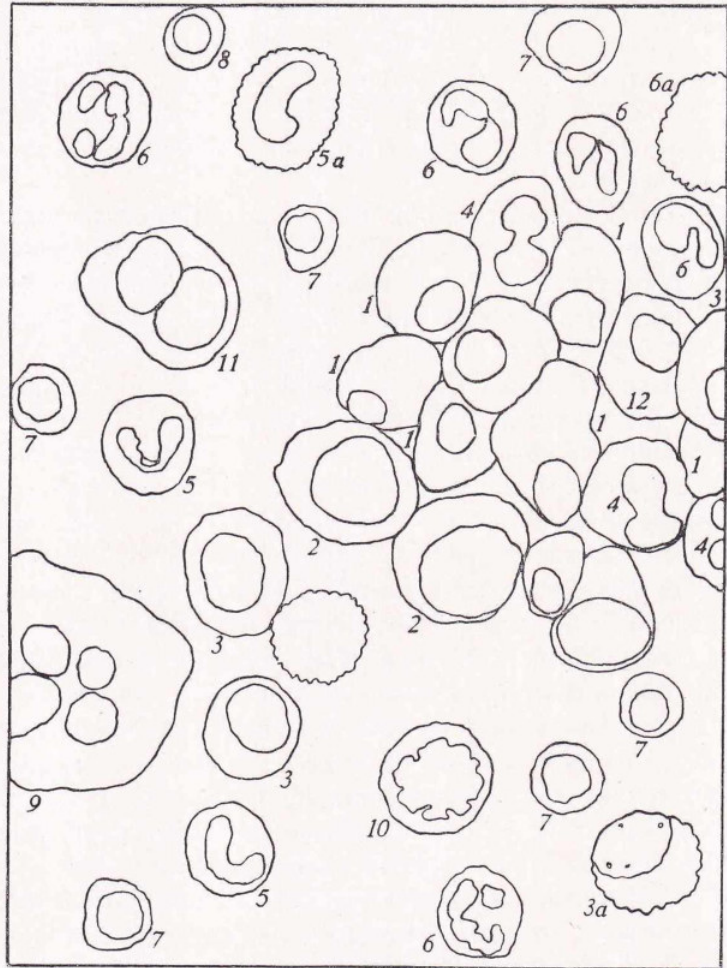
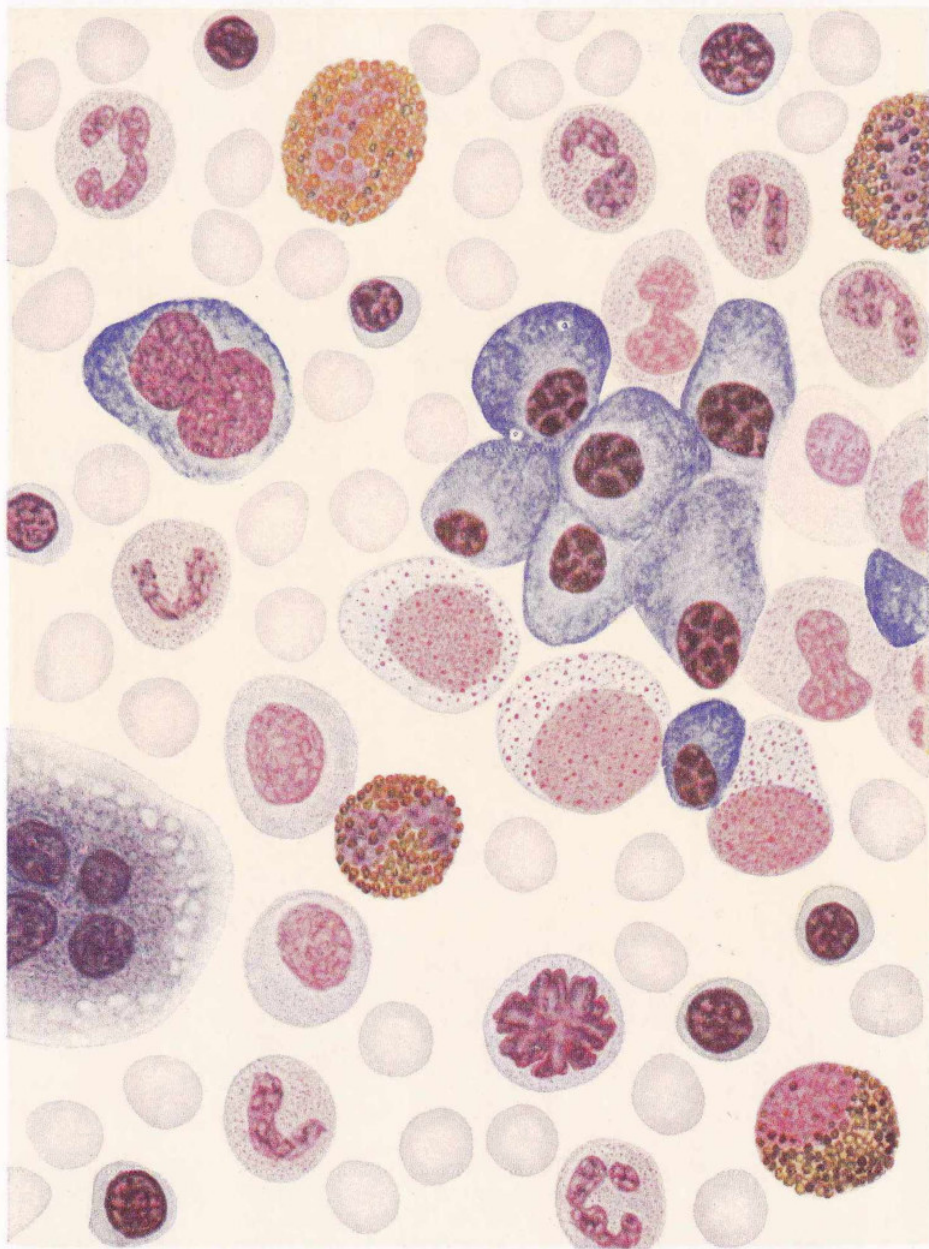


Plate 95. — Hodgkin's disease (bone marrow): 1. Plasmocyte. 2. Promyelocyte. 3. Neutrophilic myelocyte. 4. Neutrophilic metamyelocyte. 5. Neutrophilic staff cell. 5a. — eosinophilic staff cell. 6. Segmented neutrophil. 6a. — segmented eosinophil. 7. Polychromatic erythroblast. 8. Orthochromatic erythroblast. 9. Degenerative form of megakaryocyte — note the atypical nuclear structure, the blurring of the azurophilic granulation and the presence of vacuoles. 10. Granulocyte in mitosis. 11. Binucleated stem-cell — a precise recognition is difficult. 12. Lymphoid reticulum cell.

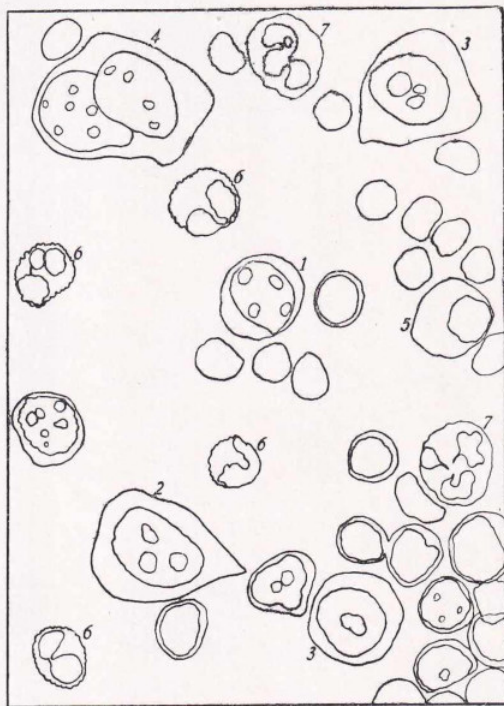


*Plate 95.* — Hodgkin's disease (bone marrow).

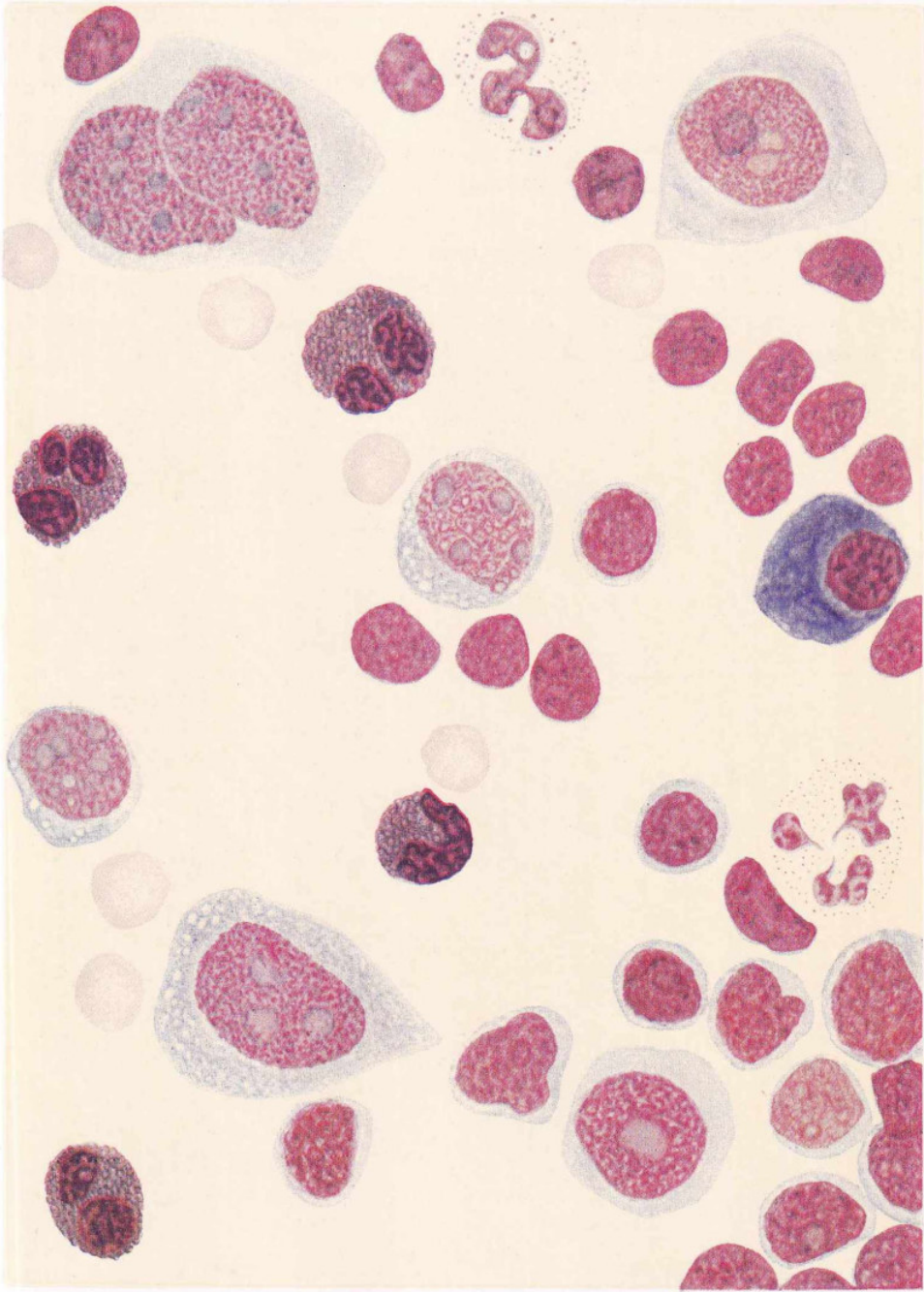


Typical giant Sternberg-Reed cells are also present (cf Plate 97). These are often multinucleated, the nuclei being usually even in number. The chromatin forms coarse strands, among which clear areas are discernible. Blue, large nucleoli are among characteristic features of these cells. The cytoplasm is pale blue, of indistinct outlines, often containing vacuoles. Intermediate forms between immature and differentiated Sternberg cells are frequently encountered.

Small Sternberg-Reed cells are by reason of their frequent occurrence in smears from aspiration-biopsy specimens of great diagnostic value. Typical Sternberg cells, become often destroyed during the execution of the smear, and are better demonstrable in paraffin sections.



*Plate 96.* — Hodgkin's disease (Hodgkin's granuloma) — lymph node aspiration smear: 1. Reticulum cell — the nuclear structure resembles that of a Sternberg cell; the chromatin strands demonstrate a reticular arrangement; such cells are not pathognomonic for Hodgkin's disease; Stahel terms these cells: "young round cells" (junge Rundzelle). 2. Intermediate cell between a young round reticulum cell and a Sternberg cell. 3. Cells suggesting small Sternberg cells. 4. Small binucleated Sternberg cell. 5. Plasmocyte. 6. Eosinophils. 7. Neutrophils. The remaining cells belong to the lymphocytic series. Lymphoblasts (loose nuclear structure, nucleoli), prolymphocytes and mature lymphocytes are present. Such a variegated, "motley" picture is characteristic, all cells specific for Hodgkin's disease being represented. Tempka terms such picture a "complete lymphadenogram".



*Plate 96.* — Hodgkin's disease (Hodgkin's granuloma).  
Lymph node aspiration smear.



At advanced stages of the disease or following X-ray therapy, the number of Sternberg cells and lymphocytes in smears decreases, these elements being replaced by numerous fibroblasts. This phenomenon is explained by progressive fibrosis of the lymph node.

The diagnosis of Hodgkin's disease should be established by lymph-node examination, characteristic cellular polymorphism and the demonstration of Sternberg-Reed cells being positive findings.

The cytological examination of lymphadenograms requires long experience and critical evaluation. The diagnosis should be made with prudence, taking the entire clinical picture into account. In addition to typical cases, there exist numerous instances fraught with serious differential difficulties (*e.g.* differentiation between an initial period of Hodgkin's disease and an unspecific inflammatory reaction or Hodgkin's and other types of sarcoma).

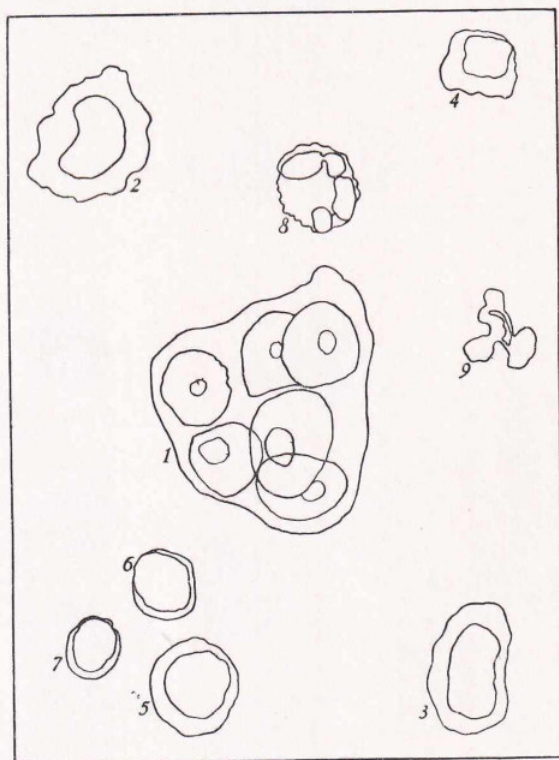
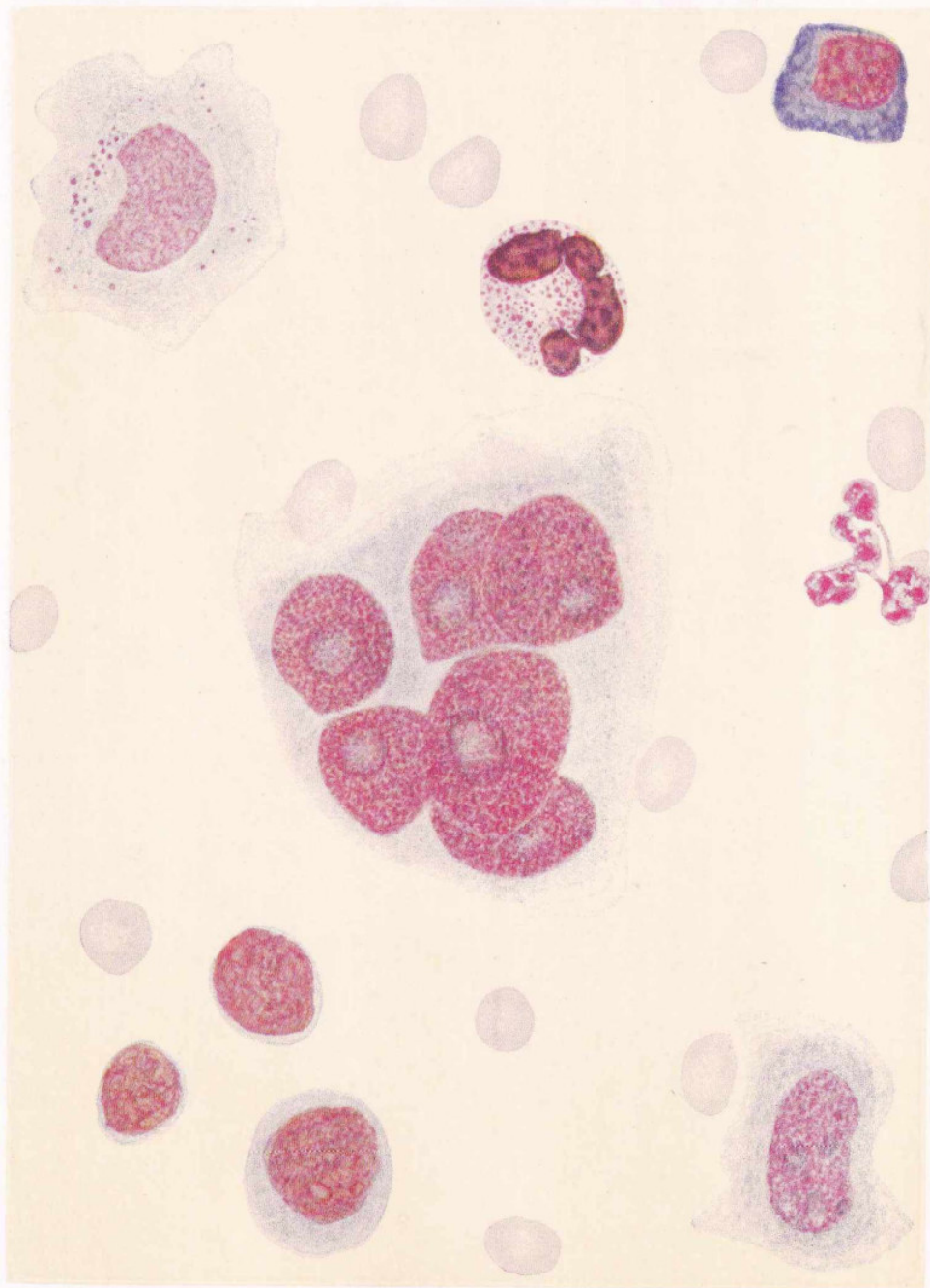


Plate 97. — Hodgkin's disease (lymph node aspiration smear): 1. Sternberg-Reed cell — note numerous nuclei typically fine in structure containing large nucleoli. 2. Reticulum cell. 3. Reticulum cell — the nuclear structure suggests a "young" Sternberg cell. 4. Plasma cell. 5. Lymphoblast. 6. Prolymphocyte. 7. Lymphocyte. 8. Neutrophil. 9. Nucleus of a granulocyte undergoing destruction.



*Plate 97.* Hodgkin's disease (lymph node aspiration smear).





## CHAPTER IX

# PARASITIC DISEASES

## MALARIA

*Plasmodium malariae* belongs to the family of *Sporozoa*, intracellular parasites of various animal species. In man, four varieties of plasmodium are encountered: *Plasmodium vivax* (causing tertian malaria), *Plasmodium malariae* (causing quartan malaria), *Plasmodium falciparum vel immaculatum* (causing tropical malaria, often termed aestivo-autumnal), and *Plasmodium ovale* (causing mild forms of tropical malaria). The latter variety is from the clinical point of view less important.

The diagnosis is established by peripheral blood examination, the *Plasmodia* being, in man, intracellular erythrocyte parasites.

The development of *Plasmodium* consist of two cycles. The first — sexual, takes places in the organism of mosquitoes; the second — asexual (termed the schizogonial stage), in human circulatory system. In human blood, sexually differentiated forms of the parasite may be encountered (macrogametocytes and microgametocytes); these are, however, immature and become functionally active only in a specific environment (organism of the mosquito).

Asexual forms are termed schizonts and the process of their division — schizogonia. Each species of *Plasmodium* is characterised by specific morphological and developmental features.

Immature parasites are blue, ring-shaped and possess a distinctly outlined red chromatin-aggregate (May-Grünwald-Giemsa stain). These forms are on ground of resemblance termed signet-ring forms.

The maturation cycle of schizonts is accompanied by morphologic transformations, specific for each species. The mature forms undergo division, causing the destruction of the red cell in which they developed. Merozoites are liberated, giving rise to a new generation of schizonts. A part of the schizonts develop into male forms (microgametocytes) and part into female forms (macrogametocytes).



## PLASMODIUM VIVAX

This species is the most common. An immature schizont corresponds to the signet-ring form already described. In the course of maturation, the parasite increases in size. Owing to its great motility, the schizont acquires numerous and varied shapes. Simultaneously, the erythrocyte increases in size and, losing its haemoglobin, becomes more and more pale. At this stage, the erythrocyte demonstrates the presence of minute granula (Schüffner's stippling), a phenomenon specific for tertian malaria. The mature schizont is polygonal and divides into from 14 to 24 regular segments, each consisting of a part of the cytoplasm and chromatin of the parental form. During the division, the erythrocyte undergoes destruction and merozoites are released into the blood.

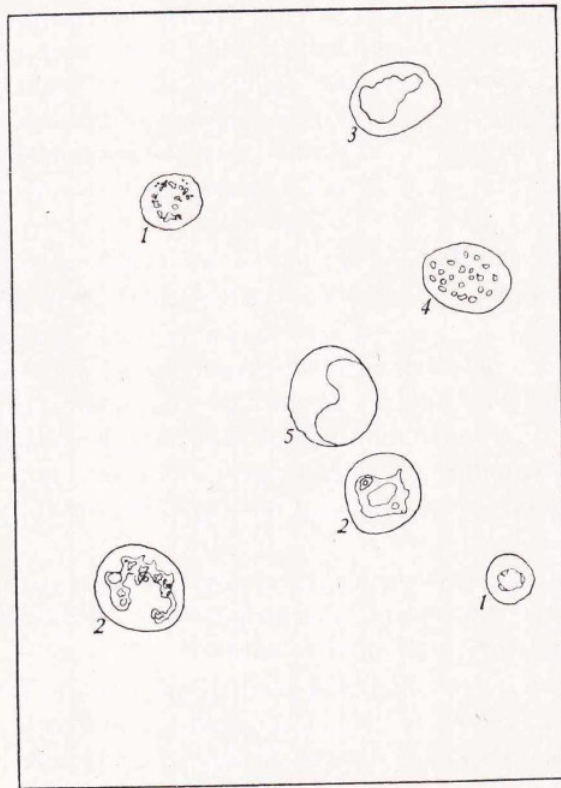


Plate 98. — Tertian malaria (*Plasmodium vivax*). Peripheral blood: 1. Young schizont (ring-form). 2. More mature schizont. The red cell is enlarged and contains typical Schüffner's granulation. 3. Schizont showing further maturation. 4. Presegmenting schizont. The parasite becomes rounded and divides into regular segments. Each of them contains a part of the cytoplasm and of chromatin. These segments correspond to a new generation of merozoites, from 14 to 24 (18—20 average) in number. 5. Monocyte. 6. Lymphocyte.

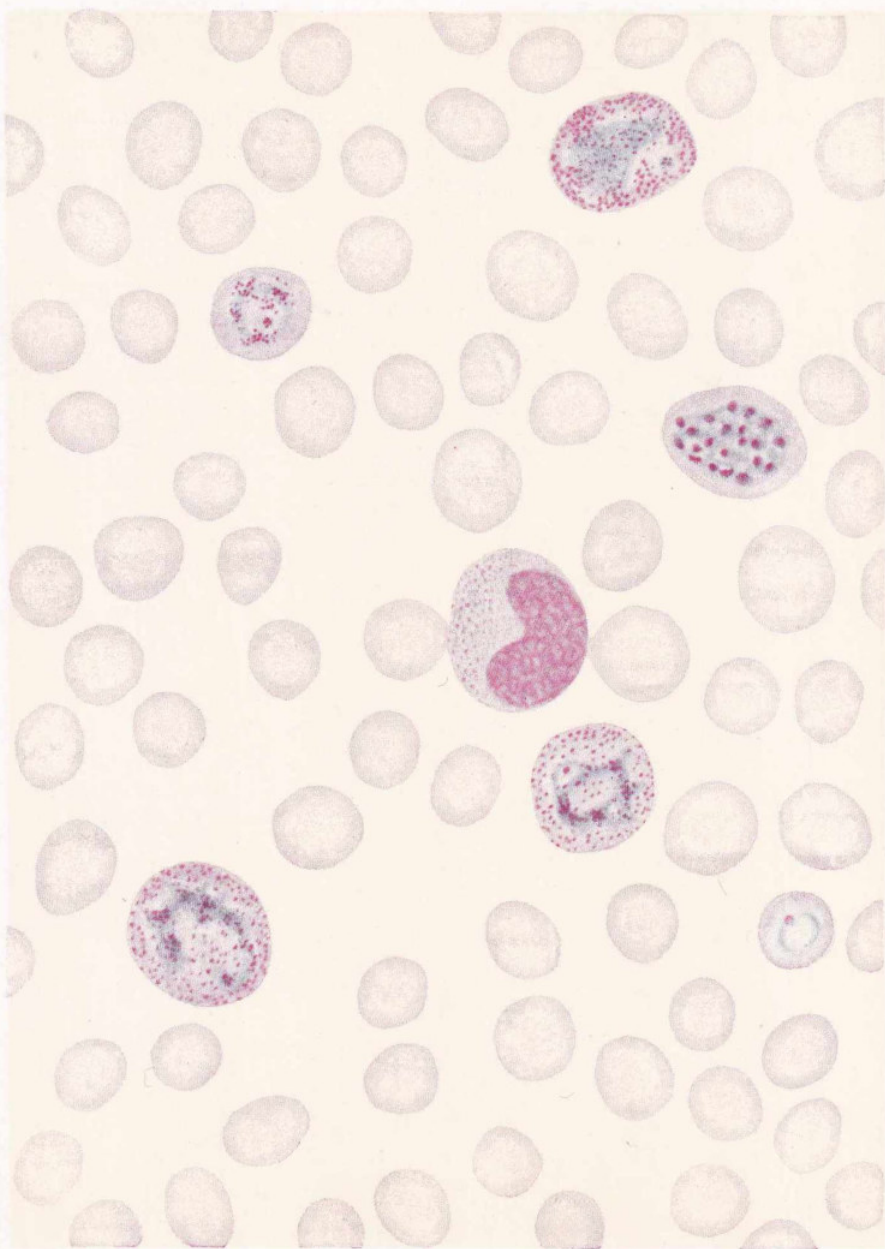


Plate 98.—Tertian malaria (*Plasmodium vivax*). Peripheral blood.



Sexual forms (gametocytes) are also encountered, although less frequently, in blood. They resemble mature schizonts and are round in shape.

The nucleus is larger and excentric. The cytoplasm of macrogametocytes is dark blue, the nucleus red and of loose structure. The cytoplasm of microgametocytes is pale blue, the nucleus large, red and of dense structure (Plate 98).

#### PLASMODIUM MALARIAE

Immature schizonts do not differ from those described above. The development proceeds more slowly, and the parasites do not attain such large dimensions as *Plasmodium vivax*. The host cell does not increase in size and does not contain Schüffner's stippling. The most cha-

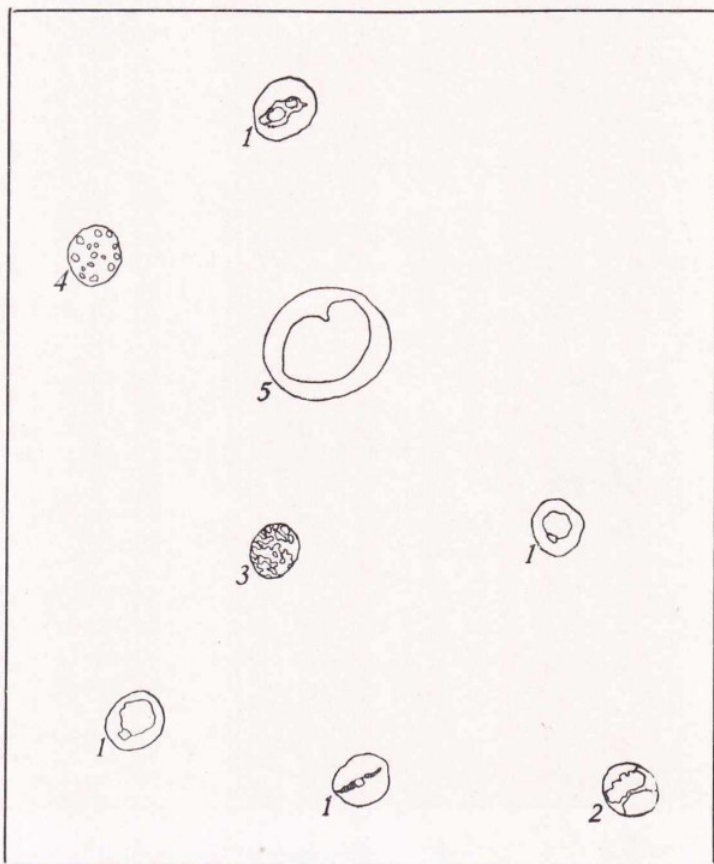


Plate 99. — Quartan malaria (*Plasmodium malariae*). Peripheral blood: 1. Young schizont (ring-form). 2. Mature schizont (band-form). The erythrocyte is not enlarged and does not contain Schüffner's granulation. 3. Schizont before division. 4. Dividing form (resembling a rosette). 5. Monocyte.

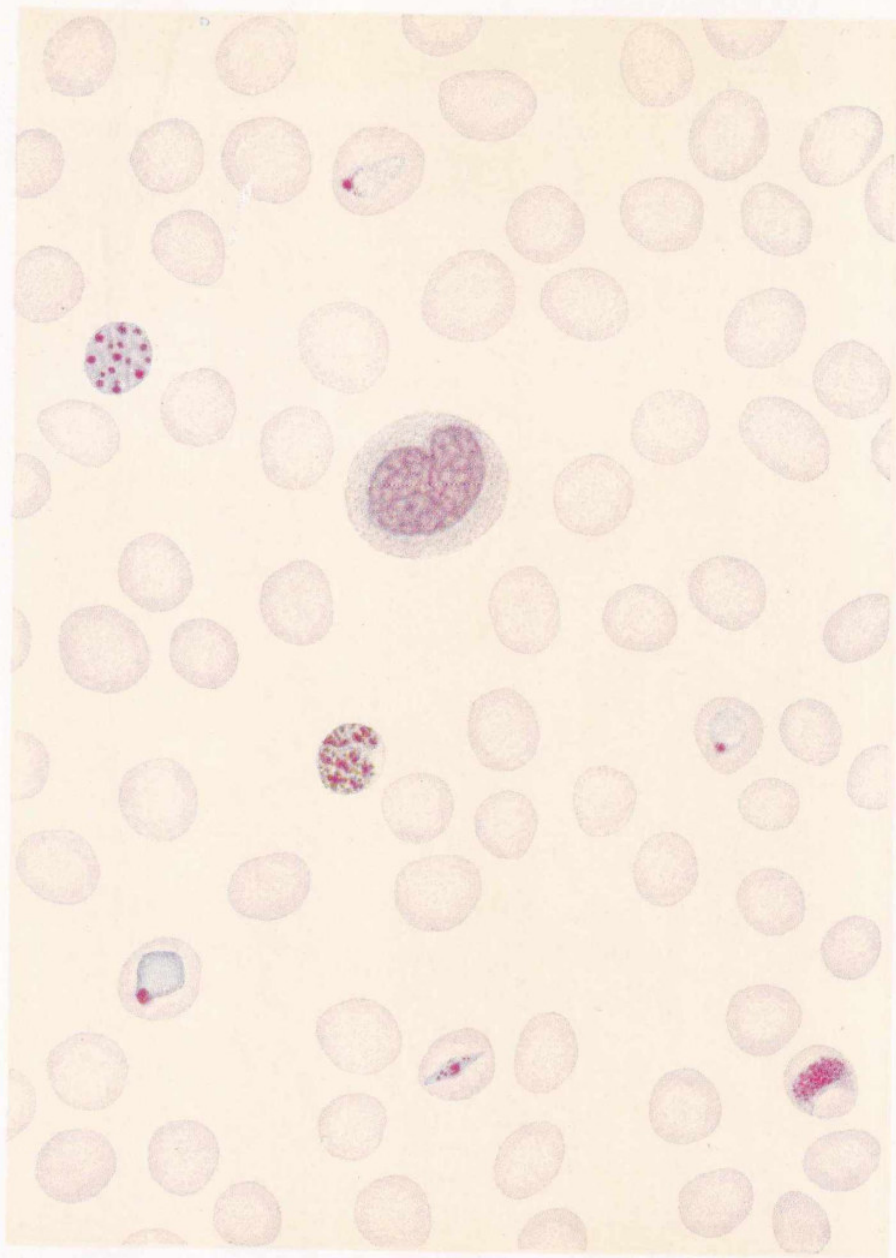


Plate 99. — Quartan malaria (*Plasmodium malariae*). Peripheral blood.



racteristic feature is the band-form schizont, which resembles a blue ribbon stretched across the erythrocyte.

The mature schizont is rounded and fills almost the entire cell. During division, it acquires the shape of a rosette consisting of from 8 to 12 segments. The sexual forms are smaller than those of *Plasmodium vivax*, never attaining a size larger than a red cell. They are difficult to distinguish from *Plasmodium vivax* gametocytes (Plate 99).

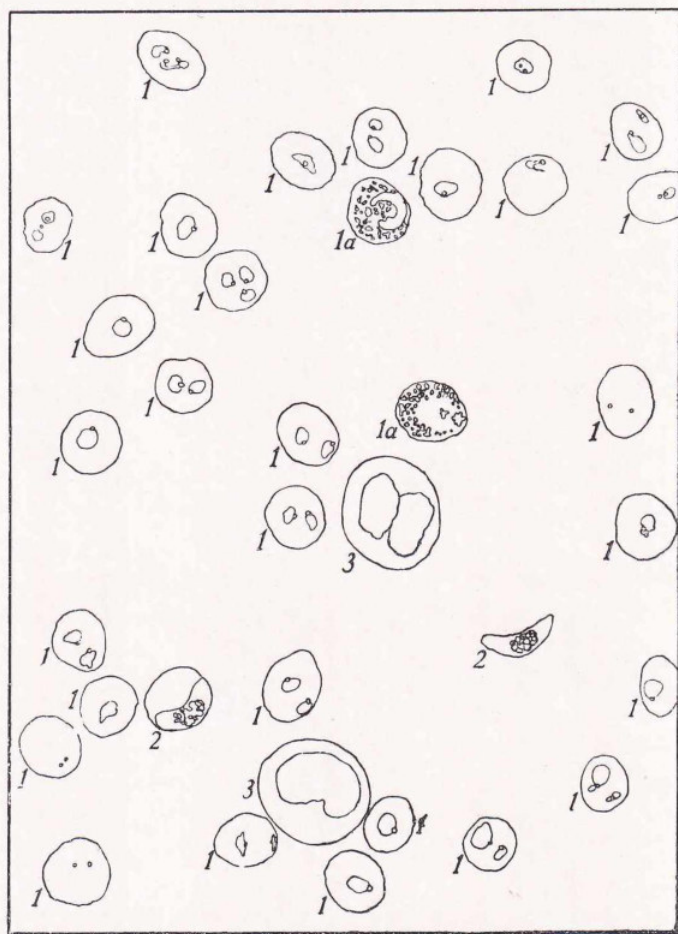


Plate 100. — Tropical malaria (*Plasmodium falciparum* vel *immaculatum*). Peripheral blood: 1. Young schizonts (ring-forms). Several *Plasmodium falciparum* schizonts are present in a single erythrocyte. The rings are distinctly smaller than those of *P. vivax* or *P. malariae*. Certain schizonts contain two chromatin aggregates. The cells infested are not enlarged. 1a. — schizont at an more advanced stage of maturation. Coarse Maurer's dots are to be seen in the infested erythrocyte. More mature stages and forms in division are usually absent from peripheral blood. 2. Macrogametocyte. 3. Monocyte.

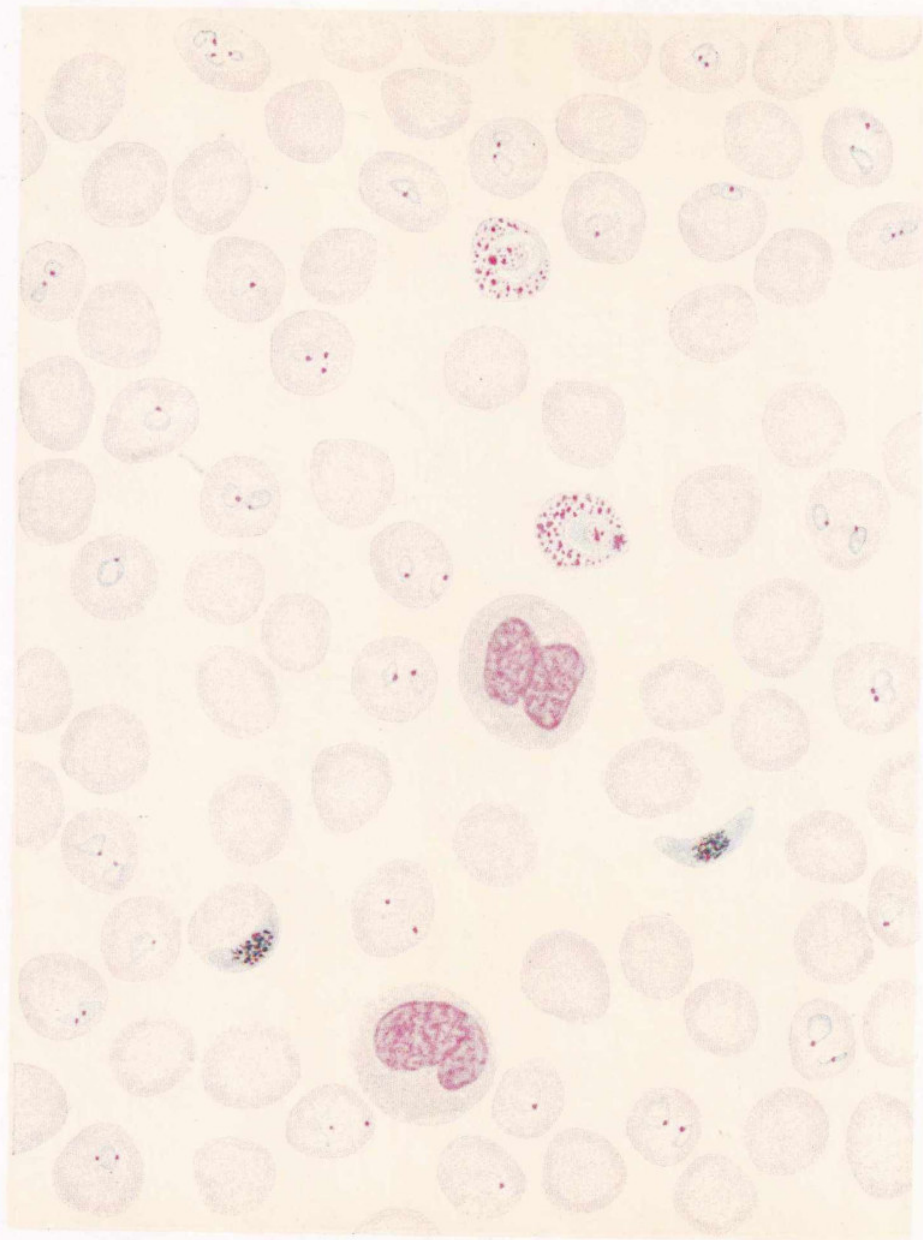


Plate 100. — Tropical malaria (*Plasmodium falciparum* vel *immaculatum*).  
Peripheral blood.



## PLASMODIUM FALCIPARUM VEL IMMACULATUM

Immature schizonts resemble minute rings containing from 1 to 2 chromatin aggregates. The chromatin stains faintly. A single erythrocyte often contains several parasites. In the course of maturation, the schizont increases in size, though remaining smaller than *Plasmodium vivax* schizonts. Erythrocytes containing more advanced schizont stages show coarse Maurer's dots. Mature schizonts cannot be demonstrated by peripheral

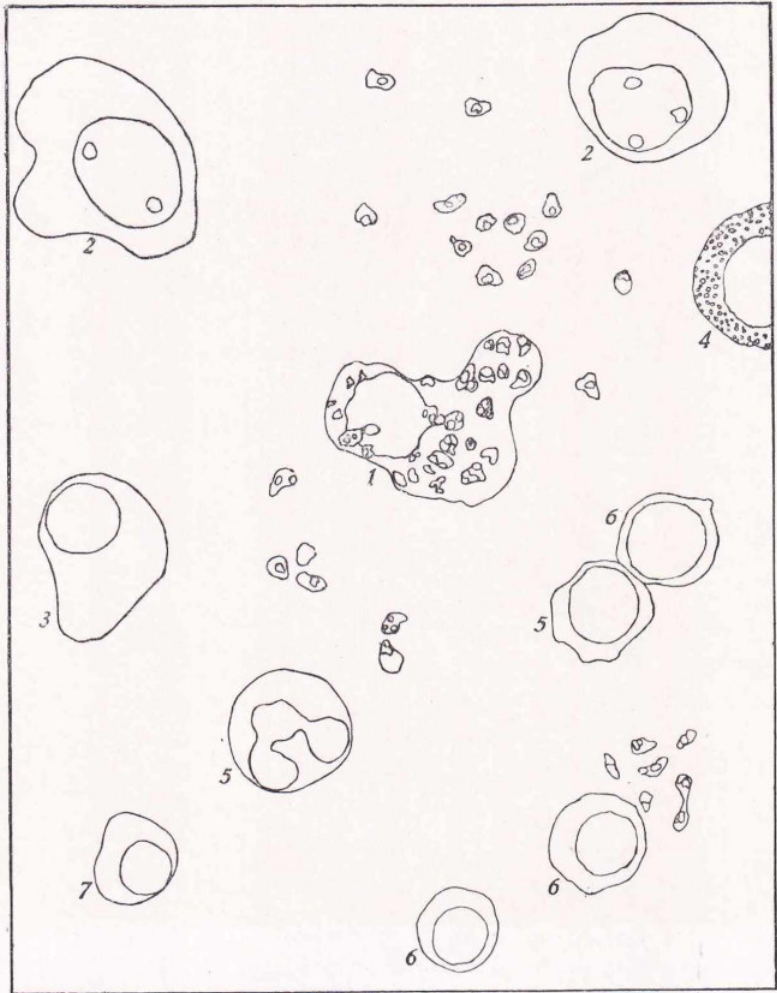
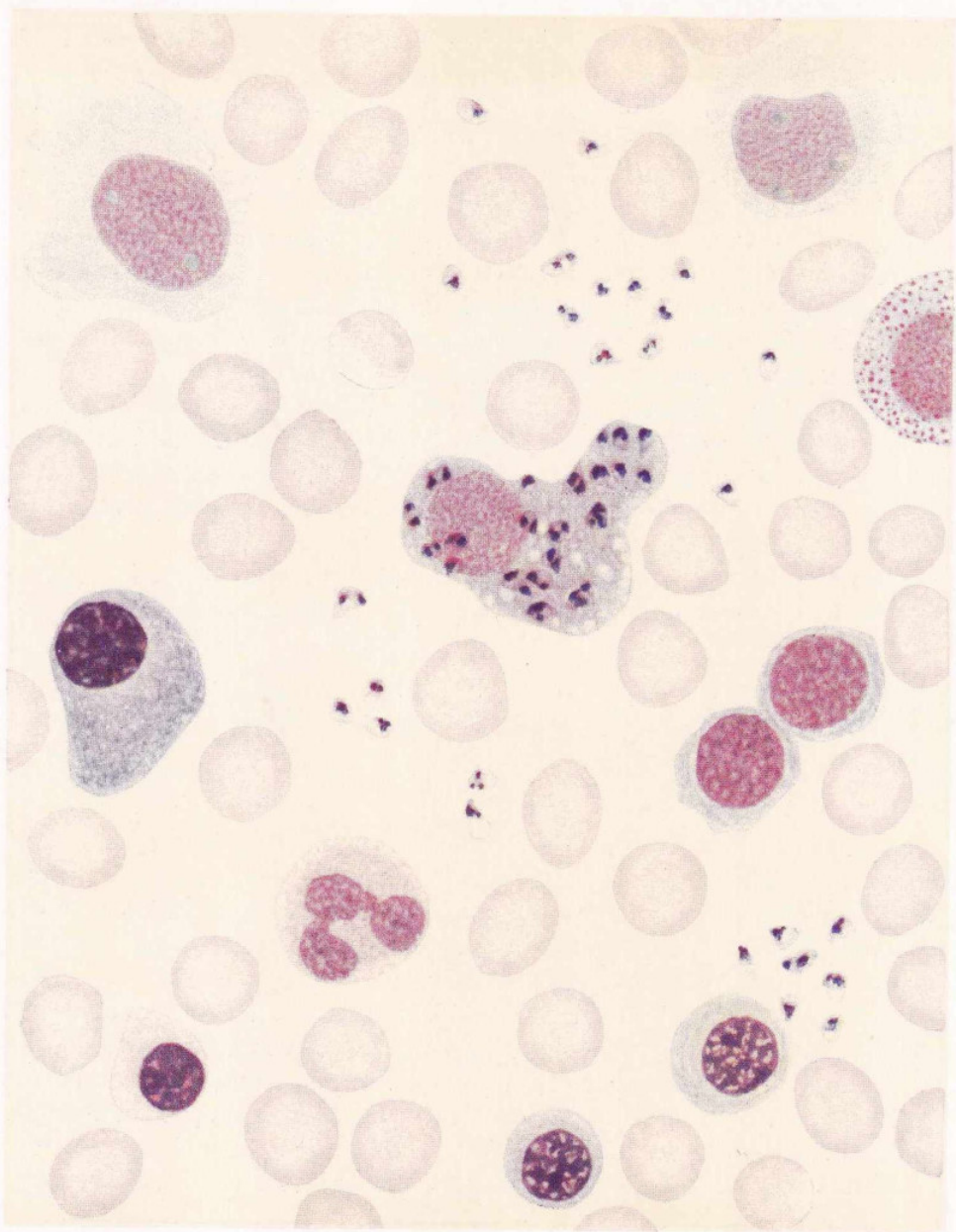


Plate 101. — Kala-azar (bone marrow): 1. Reticulum cell containing parasites in the cytoplasm. 2. Proper reticulum cells. 3. Plasmocyte. 4. Staff cell. 5. Polymorphonuclear neutrophils. 6. Myeloblast. 7. Basophilic erythroblast. 8. Polychromatic erythroblast. 9. Orthochromatic erythroblast.



*Plate 101.* — Kala-azar (bone marrow).



blood examination, because further development and division takes place in the capillaries of internal organs.

The gametocytes, which often occur in peripheral blood, are characteristic. They are markedly larger than erythrocytes and semilunar in shape. The nucleus of the macrogametocyte is small, the cytoplasm abundant, containing a few granules of brownish pigment. On the other hand, the nucleus of microgametocytes occupies almost the whole cell, the cytoplasm containing numerous pigment granules.

Faultlessly executed and stained smears are necessary for the demonstration of *Plasmodia*. Smears in which the nuclei of leukocytes are pale are valueless. May-Grünwald-Giemsa stain is generally used. In cases in which only few parasites are present, the thick-drop technique proves to be fruitful. A drop of blood is posed on the slide, allowed to dry on air, and without previous fixation is stained in Giemsa solution for 30 or 60 minutes. The red cells haemolyse; leukocytes, thrombocytes and *Plasmodia* are clearly seen.

In doubtful cases, where peripheral blood examination yields negative results, bone-marrow biopsy or spleen puncture is recommended.

## KALA-AZAR

*Splenomegalia Tropica* — *Leishmaniosis Interna* — *Febris Dum-Dum* — *Panos*

Leishmanias are intracellular protozoan parasites belonging to the *Flagellatae*. They are small, from 2 to 4  $\mu$  in size, oval or round. The intracellular form demonstrates a clearly visible nucleus and centrozome. In culture, they possess a flagella and a blepharoplast, in appearance resembling trypanosomes.

Leishmanias multiply in the infected organism in the bone marrow, the spleen and lymph nodes. They propagate from cell to cell by cytoplasmatic processes. They may also be released from destroyed cells and infect healthy ones. Extracellular parasites are readily taken up by leukocytes and reticulo-endothelial cells.

Leishmanias are typical parasites of the reticulo-endothelial system. Peripheral blood examination is therefore usually negative.

The diagnosis depends on identification of the organisms in myelograms, splenograms or lymphadenograms. In preparations stained by the May-Grünwald-Giemsa technique, the parasites are present as intracellular inclusions or lie in clusters near destroyed reticulum elements. They are round or oval and contain two violet chromatin aggregates, of unequal size. The cytoplasm is blue or pale violet. Among haematological

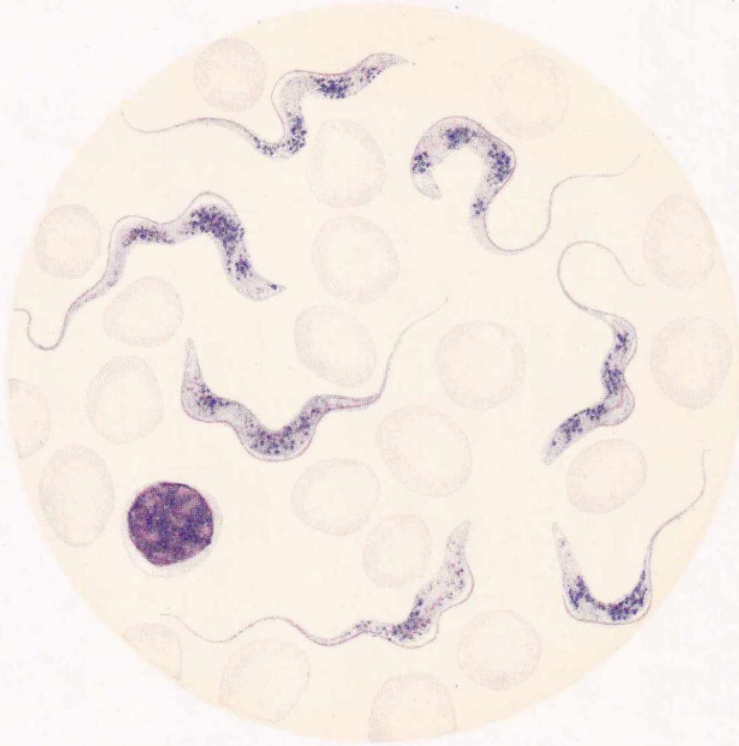


Plate 102. — Trypanosomiasis (*Trypanosoma gambiense*).  
Guinea-pig blood smear.



symptoms, hypochromic anaemia, granulocytopenia (with relative lymphocytosis) and thrombocytopenia should be mentioned.

Bone-marrow examination demonstrates a marked hyperplasia of the reticulo-endothelial system (Plate 101).

### TRYPANOSOMA

Trypanosomas are *Protozoa* belonging to the *Flagellatae*. They are spindle-shaped, from 20 to 35  $\mu$  in length, and from 1.5 to 3  $\mu$  in breadth. Their structure is readily revealed in Giemsa-stained preparations. The organism is equipped with a nucleus, a blepharoplast, a long flagella, which originates from the blepharoplast, and runs along the undulating membrane. The free end of the flagella reaches beyond the body of the protozoon.

Trypanosoma may be encountered in the blood of all vertebrates but in certain species they are not pathogenic. The trypanosomas are usually transmitted by insect bites. Pathogenic in man are:

1. *Trypanosoma gambiense*;
2. *Trypanosoma rhodesiense*;
3. *Trypanosoma cruzi*.

The two first named are transmitted by *tse-tse* fly (*Glossina palpalis*, *Glossina morsitans*) and cause African sleeping sickness. The third variety occurs in Central and South America and is transmitted by "kissing bugs" (*Panstrongylus*). *Trypanosoma cruzi* causes an acute disease (in rare instances the course may be chronic) characterised by anaemia, spleen and lymph-node enlargement, diarrhoea and neurological symptoms (Chagas disease).

Infestation with *Trypanosoma* is usually accompanied by anaemia and monocytosis. The demonstration of the organisms in peripheral blood may, on account of the paucity in numbers, be difficult. Thick smear or thick-drop technique is therefore recommended. Better results are obtained by bone marrow or lymph-node biopsy. In doubtful cases, the administration of from 2 to 5 cu.cm. of the patient's blood to a guinea-pig may establish the diagnosis (Plate 102).

## CHAPTER X

### TUMOUR METASTASES

Bone-marrow biopsy is often helpful in the detection of tumour metastases. Although several authors claim the superiority of histological sections, nevertheless cytological examination of smears may serve to establish the presence of metastases. Neoplastic cells, being elements foreign to the bone marrow, are readily recognisable, particularly when occurring in aggregates. Such clusters of metastatic cells are best searched for under low magnification. The recognition of single neoplastic cells may be difficult or even impossible.

Most common are carcinoma or sarcoma metastases. Reliable criteria for differentiation between cells of epithelial and mesenchymal origin are, however, lacking. It is also impossible, by the morphological features of the metastatic cell, to determine the primary site of the tumour.

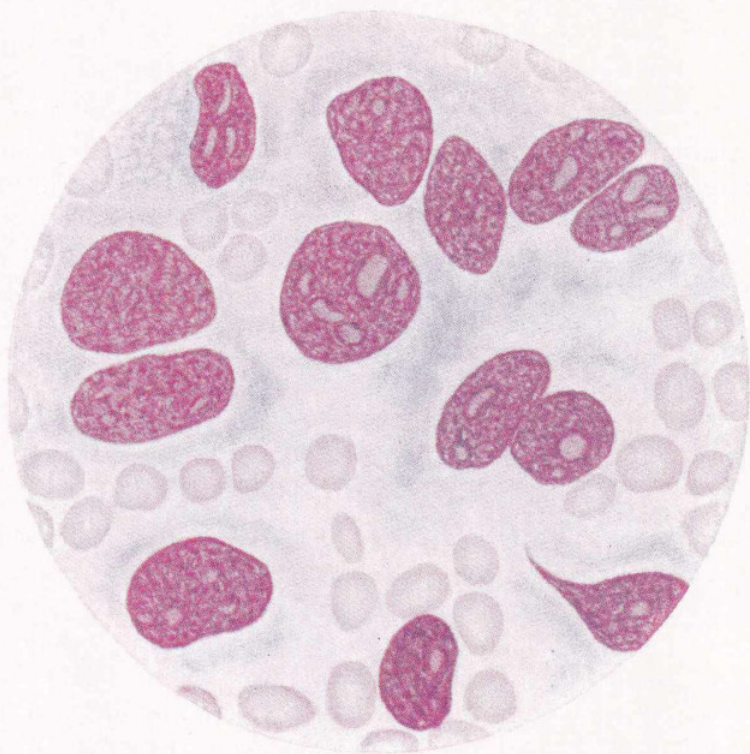
Neoplastic cells are characterised by a marked polymorphism, which makes classification difficult. Such attempts have, however, been made; Kienle divides metastatic cells into six categories. In the present authors opinion, most important for the clinician, nevertheless, is the fact that these cells are present and all his endeavours should be directed to differentiating metastatic cells and normal bone-marrow constituents. This is not always easy, metastatic cells often resembling reticulum elements.

It should be emphasised that the size of the cell cannot be regarded as a differential feature. Neoplastic cells exceeding  $30\ \mu$  in diameter are encountered, together with small cells similar in size to small lymphoidal cells.

In recognition of metastatic cells, the following features may be helpful:

1. Polymorphism of the cells;
2. Nucleo-cytoplasmatic ratio; the nucleus is often unusually large as compared to the narrow rim of the cytoplasm;
3. Atypical shape and structure of the nuclei; binucleated cells, cells with three or more nuclei, resembling syncytial cells; atypical mitoses;





*Plate 103.* — Metastatic cells in bone marrow (*Carcinoma*).

4. Large nucleoli: the nucleio-nucleolar index may be as high as 0.4; in normal cells, the index amounts usually to approximately 0.2;
5. Basophilia of the cytoplasm; irregular cell outlines;
6. Vacuolation or fatty degeneration;
7. Phagocytosis of small elements by large cells, "cannibalism" of neoplastic cells (Vadász after Libánsky);
8. Neoplastic cells occur in clusters — "nests", which are readily demonstrable under low magnification (Aleksandrowicz; Libánsky, Rohr, Stahel).

Metastatic cells of epithelial origin are usually larger and more varied in appearance than sarcoma cells. These are smaller, often spindle-shaped, and form dense clusters (Tischendorf). These features may be misleading and do not constitute reliable differential indications.

Plate 103 illustrates several metastatic cells lying close together in a bone-marrow smear. The fact that the cell borders are invisible suggests a syncytium. The nuclei are of different sizes. The nuclear structure bears signs of immaturity of the cells, and the nucleoli are prominent. The cytoplasm is agranular and basophilic. One of cells contains vacuoles.

Manubrium of the sternum is among the common sites of tumour metastases. If neoplastic cells are searched for, several smears should be thoroughly examined under low magnification. Negative results constitute an indication to essay the puncture of other bones, such as the ribs, spinal processes of vertebrae or the iliac crest. X-ray examination of the skeletal system should precede selection of site for puncture.





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ANATOMICAL ATLAS  
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AND SPINAL CORD

by

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