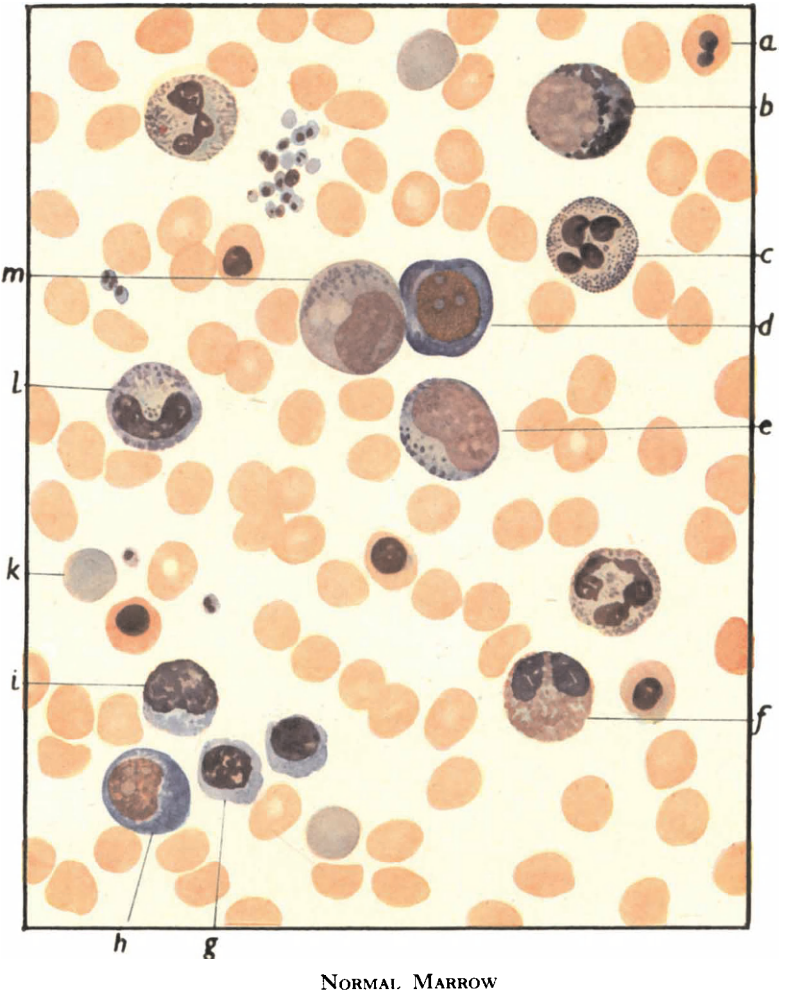
PLATE I



NORMAL MARROW

(*a*) Normoblast.
(*b*) Basophile myelocyte.
(*c*) Polymorph.
(*d*) Myeloblast.
(*e*) Myelocyte.
(*f*) Eosinophile.
(*g*) Basophile normoblasts.
(*h*) Pro-erythroblast.
(*i*) Early normoblast.
(*k*) Polychromatic corpuscle.
(*l*) Metamyelocyte.
(*m*) Premyelocyte.

[*Frontispiece.*

STERNAL PUNCTURE

A METHOD OF CLINICAL AND CYTOLOGICAL INVESTIGATION

By

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WITH A FOREWORD BY

The Rt. Hon. LORD HORDER, M.D., F.R.C.P.

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FOREWORD TO THE FIRST EDITION

STERNAL PUNCTURE is the generally accepted means by which myeloid tissue may be studied during life. It is one of the most modern of the techniques included under the term " clinical pathology." A little hesitant at first, it has now developed into a full-blown method of investigation.

By the help of sternal puncture three things are being accom plished: exact study of the life-history of the blood is being furthered, problems in hæmatology are being clarified and the reactions of the formative elements of the blood to infection and to other abnormal states of the body are being observed. All these aspects of the subjects are covered in Dr. Piney's present monograph.

The limits of usefulness of the new technique cannot yet be stated. They must await much more exploitation of the method than has so far been undertaken.

In connection with the third of the three functions just mentioned, it may well happen that the myelogram will prove to be a valuable adjunct to diagnosis in certain morbid states of an otherwise indeterminate kind; pathological changes in bones and enlargements of the liver occur as possible examples.

The authors have avoided dogmatism and have wisely kept such knowledge as we have in this field in a fluid state, where it should be allowed to remain whilst the technique is in its early history and the body of ascertained facts relatively small. Equal care has been shown in handling the nomenclature.

The book is timely because it summarises the present position of the subject and acts as a manual for investigators, whether their approach is on the pathological or on the clinical side.

The popularity which has been achieved by Dr. Piney's previous books in hæmatology may safely be predicted for this new work also.

HORDER.

PREFACE

THAT a second edition of this book should have been called for within two years shows that, in spite of its defects, the first edition met a need. It has now been possible to embody some of the suggestions made by reviewers, and also to add considerably to the number of pictures. To the professional hæmatologist, illustrations of blood and marrow cells may be supererogatory, but the occasional microscopist seems to be helped by drawings. We have to thank Miss Cruse for the excellent work she has done in preparing drawings that are as lifelike as any we have seen.

No attempt has been made to review the whole of the literature relating to marrow structure; to have done so would have been an enormous labour, which would not have offered the reader any recompense for the inevitable dullness of reading the heavily documented text. All we have done is to call attention to such writings as illuminate special points of interest, or which describe conditions of which we have had little or no experience. And, by selecting papers which themselves have ample bibliographies, we have given the enthusiast the opportunity of delving into the history of the subject.

It cannot be said that examination of marrow films has caused any revolution in hæmatology in the past two years; the procedure is so new that it is still in the stage when detailed description is all that is possible.

Plate 11 was drawn from preparations lent by Mr. R. J. Bromfield, Chief Technician, Redhill County Hospital, Edgware, to whom we are greatly indebted.

A. PINEY.
J. L. HAMILTON-PATERSON.

September 1943.

LIST OF PLATES

INTRODUCTION

BLOOD examination, which has become an important adjuvant to both diagnosis and prognosis, has always suffered from the weakness of depending for its value on the inferences drawn from it. Knowledge of the concomitant changes in the formative tissues have been scanty. Attempts have often been made to correlate blood changes with those in the marrow, but the difficulties have been great. First, the blood has been examined by the film method, whereas the marrow has been examined in sections when the cells look very different. Secondly, marrow sections can usually only be obtained from post-mortem material and will then reveal only the terminal state.

Marrow puncture is a convenient method of examining the formative tissue during life, and it can, without difficulty, be performed several times on the same patient. There can be no doubt that it has led to a much deeper knowledge of the factors on which the characters of the blood picture depend.

The blood is, of course, not a tissue in the usual sense of that word : it is a product of a number of organs of which the bone marrow is the most important, and yet the blood is not a secretion of the hæmopoietic tissue in the same sense that urine is a secretion of the kidneys. It does not consist of constituents that have been abstracted from the body as a whole but of portions of the parent tissue itself. It is for this reason that it is convenient to conceive of the whole mass of circulating cells together with those in the formative tissues as forming an organ. Thus Boycott spoke of the erythron as the organ composed of the whole collection of red cells and their precursors in the body. A similar concept, the leucon, is applicable to the white cells.

These organs are not totally independent of one another because the primitive parent cells of both the red and the white elements are probably identical ; in other words, at the earliest stage, the erythron and the leucon meet. For this reason we must make use of the conception of the hæmaton, which is then the whole of the blood and the blood forming system. Thus, examination of the marrow is so important because it reveals something more than the characters of the parent tissue of the blood ; it shows us an integral part of the hæmaton.

It was the improper abstractive separation of the circulating blood from the formative tissues that led in the past to a deep and fundamental cleavage in the hæmatological world. Thus, there were two main schools of thought—the monophyletic and the polyphyletic. The former contended that all the different types of

blood cells were derived from a common ancestral form, which was variously known as the lymphoidocyte, the hæmocytoblast, and about eighty other synonyms. The polyphyletists, on the other hand, asserted that there were several stem cells, each one with irreversibly determined potentialities. The number of such elements was a matter of discussion among the several groups of this school, but all were agreed that the monophyletists were wrong.

This controversy, which, in the past, filled thousands of pages, is no longer acute because our knowledge of the marrow has increased. In the past, most observations on the blood-forming organs were carried out by professional histologists, who naturally made use of their customary technique, that is to say, fixation, embedding in paraffin or celloidin, section cutting and staining, and most, if not all the histologists were adherents of the monophyletic view. The clinical hæmatological workers, on the other hand, based their conclusions on observations made on stained films, and very many of them were polyphyletists.

The divergence of views was to a very great extent dependent upon these differences of technique, because cells which appear large and full of cytoplasmic and nuclear detail, when seen in films, are small and difficult to recognise in fixed sections. Marrow puncture has enabled us to use the same technique for marrow tissue as for blood, and, as a result, the old controversy has almost died down.

Various methods of obtaining marrow during life were used in the past. Thus the femur was trephined and marrow extracted, or the tibia was similarly treated, and from both these methods a good deal of information was obtained. There are two main objections to such procedures. They are in the nature of major surgical operations and cannot be repeated frequently on the same patient. Secondly, in adult life the red marrow in the limbs is confined to small areas at the upper ends of the humeri and femora, the more distal parts of these bones and the more distal bones of the limbs containing only fat. In diseases, in which there is excessive demand for cells, formative marrow may spread into all the bones. Thus, in advanced leukæmia and pernicious anæmia, valuable information could be obtained from the tibial bone marrow, but in health or in more acute maladies only fatty tissue was present. Obviously then, it would be a great advantage to obtain the specimen from a bone that is always filled with active marrow, such as the sternum. Since 1929, when Arinkin (1) introduced the method of sternal puncture, this has been the universal technique.

STERNAL PUNCTURE

CHAPTER I

THE MYELOGRAM

THE recognition of the various types of cells in blood films needs a good deal of practice ; and it is quite impossible to interpret the appearances in marrow films without a sound knowledge of the cytology of the peripheral blood. This is, of course, not surprising, because the marrow cells are either blood cells or are their precursors ; and, for this reason, we can use a similar terminology to that used in ordinary hæmatology.

Schilling coined the name, *hæmogram*, to designate the qualitative blood picture ; and this is a much sounder concept than is the ordinary term " differential count." The term, *myelogram*, can equally reasonably be applied to the total marrow picture. Just as experience has given us knowledge of the normal percentages of the different types of cells in the blood in health and in a great variety of diseases, observation has led to a knowledge of the marrow picture, the myelogram, in health and disease.

The hæmogram is naturally not an absolutely accurate picture of the cellular composition of the peripheral blood, because it is based on random sampling, both of the whole blood and even of the specimen taken for examination. The myelogram is subject to the same sources of error, but is also less accurate than the hæmogram for another reason : a varying proportion of the cells in the marrow cannot be classified, either because of pathological changes in their structure, or, even in health, by being in a more or less dedifferentiated state, prior to, during or immediately after mitosis. Even so, the myelogram is of great value, but has to be recorded in a more detailed manner than the hæmogram, because there are more classes of cells in the marrow than in the blood. Then again, another difficulty is that marrow films cannot be evenly spread ; there is admixture of a certain amount of fat, and the cells are not floating free, as in blood, but are more or less aggregated into clumps.

Both for purely scientific and for clinical purposes, the myelogram and the hæmogram should be investigated more or less at the same time ; and completeness would, of course, require examination of puncture fluid from the other hæmopoietic organs, spleen and lym-

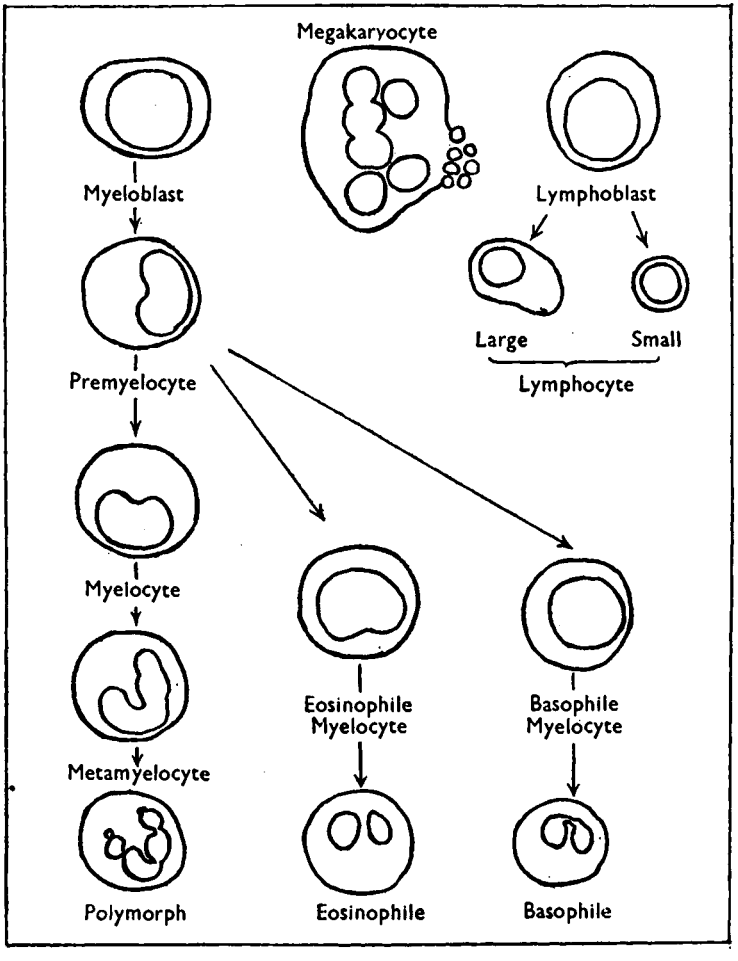
phatic glands, also. Fortunately, clinical investigation rarely, if ever, demands so violent an onslaught on the unfortunate patient. The taking of blood for determination of the hæmogram is a very slight discomfort, and even sternal puncture need not be very painful ; but the discomfort can be mitigated by not doing both on the same day. After all, the state of the blood at any given moment does not depend upon the condition of the bone marrow at the same time, but upon its composition and activity some time before ; and, as that can no longer be determined, exact correlation between the " grams " is impossible. All that is necessary is that the interval between blood and marrow examinations should not be too long.

From the academic aspect, marrow puncture has given access to a wealth of cytological material. Marrow films, even in health, contain innumerable immature forms of granulocytes and red cells. Even in chronic myeloid leukæmia, where the blood is flooded with young cells, where is a probability that many are abnormal as well as immature ; and inferences about cell relationships, based on the study of leukæmic blood, are very liable to error. It is for this reason that study of marrow from healthy persons has so greatly illuminated our knowledge of the genealogy of the cells of the peripheral blood.

The various features, cellular and otherwise, of marrow films must be described in some detail, because all the rest of our study depends upon an appreciation of these basic facts.

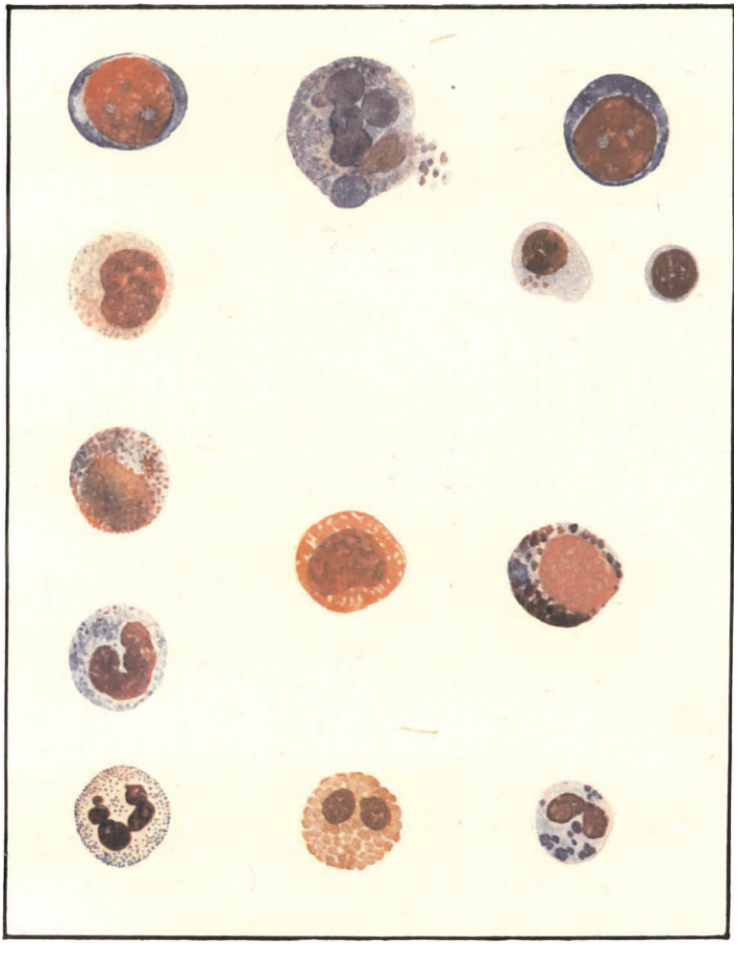
Films of marrow, unlike those of blood, present a background which must not be ignored, because in it lie parts of cells, naked nuclei, and cell granules. Some or even all these may lie free on account of the inevitable roughness in preparing the slides, but there is sufficient regularity about the appearances to permit of inferences being drawn. At least, some idea of the relative fragility of the various elements can be obtained. Further, marrow cells are held together by a semi-solid matrix : they do not float freely as do the cells in the circulation. And it is, therefore, well that marrow films should not be made too thin, because, if they are, the grouping that characterises many conditions is lost. In other words, films should, if possible, be so made that histological arrangements, as well as cytological structure, are preserved.

The determination of the percentages of the different types of cells in marrow films must be based on examination of a far larger number of cells than is needed for a differential blood-count, partly because the total number of cells in the marrow is so great, and,


Megakaryocyte
Myeloblast
Lymphoblast
Large
Small
Lymphocyte
Premyelocyte
Myelocyte
Eosinophile Myelocyte
Basophile Myelocyte
Metamyelocyte
Polymorph
Eosinophile
Basophile

Lymphoblast
Small
Large
Lymphocyte
Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Polymorph
Basophile Myelocyte
Eosinophile Myelocyte
Basophile
Eosinophile

PLATE 2



DEVELOPMENT OF WHITE CELLS

[*To face p.* 2.

partly, because they are so unevenly distributed in the films. At least 1,000 consecutive cells should be examined (remembering to have a separate heading for the elements that cannot be identified), and, if possible, the whole examination should not be done on a single film. Of course, the relative proportions of red and white cells must be determined, just as in the case of the peripheral blood ; but, for comparison with the differential leucocyte-count, a separate myelogram of the white cells alone is required. Whatever precautions are taken, it is clear that the myelogram is a rather rough guide to the state of the marrow, and little significance can be attributed to small changes in the percentages of the different forms of cells.

Hæmohistioblast. Ferrata described a primitive cell which was so little differentiated that it might develop into connective tissue or into blood. He stated that such elements are large (15 to 30 microns), with irregular, slightly basophilic cytoplasm of very tenuous character. The nucleus is round or oval, of fine, delicately reticulated structure, in which lie one or more distinct nucleoli (Plate 3).

In marrow films, these elements usually have to be recognised by the characters of their nuclei : the cytoplasm is so delicate, that it usually ruptures. Indeed, it is not at all clear that hæmohistioblasts are anything more than compressed and ruptured hæmocytoblasts, myeloblasts, or very immature premyelocytes. Nevertheless, it must, we think, be admitted that an occasional intact specimen is seen in narrow films, and that these elements must, therefore, be admitted to the ranks of very primitive blood-cells.

It is interesting and significant that the number of " hæmohistioblasts " in the marrow and the blood, in cases of chronic myeloid leukæmia, increases after X-ray treatment, perhaps because of damage to the cell structure by this physical agent.

Hæmocytoblast. The exact status of this element in the hierarchy of the blood cells is still disputed ; and we need do little more than describe its structure. The cytoplasm, which is devoid of granules, is distinctly basophilic, and varies considerably in amount. The nucleus is a good deal denser than that of the hæmohistioblast, but has the same type of reticular structure, being composed of interlacing fibrils of basichromatin, in which one or more nucleoli can easily be seen (Plate 3).

The followers of Ferrata regard this element as being the first, the most primitive, cell that has been irreversibly fixed in the direc-

tion of hæmopoiesis : unlike its alleged ancestor, the hæmohistioblast, it can no longer produce connective tissue. If this be so, the hæmocytoblast is the real stem-cell of the whole hæmopoietic process. Certainly, in marrow that is hyperplastic from any cause, these cells are increased in number. Thus, in the megaloblastic marrow of pernicious anæmia, in ordinary normoblastic marrow-hyperplasia, in leucoblastic, and even in leukæmic marrow they are increased in number. This fact seems to justify the inference that they are fixed in their general hæmopoietic potency, but are still multi-potent, inasmuch as they can give rise to either red or white cells.

On the other hand, Naegeli contended that the hæmocytoblast is not a multipotent stem-cell, but that it is identical with the myeloblast : the parent of the granular leucocytes only. But his view fails to supply any explanation of the increase of these cells in all types of hyperplastic marrow ; if he were right, it would be surprising to find these elements increased in erythroblastic reactions.

As long as we are clear in our minds about the structure of these elements, so that we can recognise them, their exact status can be left to further research ; but the name, hæmocytoblast, is now so widely used that we may well adopt it.

Myeloblast. The supporters of the view that the haemocytoblast is multipotent, distinguish it from the myeloblast (Plate 2), which they consider to be an element irreversibly determined in the direction of granulocyte-formation ; that is to say, they regard it as a more differentiated cell. It is, unfortunately, difficult to decide on what morphological criteria myeloblasts are to be distinguished from hæmocytoblasts. It is said that the nuclei of the former are rather less tenuous, and that the cytoplasm is less basophilic. But it is, of course, not to be expected that exact distinctions can be made, because we are dealing with a continuous process of development, not with isolated cell-types.

Premyelocyte. The premyelocyte (Plate 2) is intermediate between the myeloblast and the myelocyte, that is to say, there is no doubt that it is definitely fixed in its potencies, and its structural characters lie, as it were, midway between the two types of cell. There seem to be two grades of premyelocytes. First there are those in which the nucleus is only a little denser than that of a myeloblast and still contains nucleoli, but the cytoplasm is less basophilic and contains azurophilic granules. Most of these cells have minute granules all of the same size (*preneutrophilic premyelocytes*). Some have large rather scanty granules, also all of

the same size (*pre-eosinophilic premyelocyte*); and finally, there are a few elements with large granules of irregular size (*prebasophilic premyelocytes*). From these arise neutrophilic, eosinophilic and basophilic premyelocytes in which the nucleus has become a good deal denser and the nucleoli are obscured, while the granules have reached their definitive staining reactions.

Myelocytes. These cells are the stock from which most of the mature granulocytes arise in health. In the marrow they vary greatly in size, some being little larger than lymphocytes, while a few are as much as 26 microns in diameter. This variation in size is even greater than that seen in the blood in chronic myeloid leukæmia, although in the past it was supposed that the variations in that disease were due to a morbid process. The nucleus of the myelocyte is circular but the edge may be slightly indented by pressure of the granules that fill the cytoplasm. The chromatin still has a reticular arrangement resembling that of the myeloblast, but condensations and small nodules can be seen in the skein. The cytoplasm is slightly basophilic but is difficult to see because it is almost filled with granules, which, at this stage, have, of course, reached their definitive staining reactions (Plate 2). The eosinophil myelocytes present difficulty, because, although many of them contain mature eosinophil granules, others present a mixture of granules, some being basophilic and others eosinophilic.

A variable number of such elements is always found in the marrow, but the exact steps in their origin from premyelocytes are unknown. It is possible that some of the cells in the marrow, which contain only basophilic granules, are really the very earliest stage of eosinophils. If this is so, it would account for the fact that the marrow always contains more basophils than one would expect from the appearances of the peripheral blood.

Metamyelocytes. These are intermediate between myelocytes and mature polymorphs and can be divided into juvenile forms, with very slight indentation of the nucleus, and staff forms in which the nucleus is deeply indented, rather like a band (Plate 2). It is at the stage of the staff form in the marrow that abnormalities of structure occur in pernicious anæmia, some of the cells being as much as 30 microns in diameter.

Polymorphonuclears. As seen in films of normal marrow, these cells are identical with those found in the blood (Plate 2), but, even when the blood polymorphs appear normal, atypical forms may be seen in the marrow. Thus, in chronic myeloid leukæmia, very

large ones may be seen, and in pernicious anæmia equally gigantic forms, mainly neutrophilic, with most complicated nuclei, are common in untreated cases.

Lymphocytes. These are not part of the myeloid tissue proper, but there seems to be no doubt that the marrow is the seat of production of a small proportion of the lymphocytes in the peripheral blood. Certainly, even in adults, it contains more large lymphocytes, which are commonly supposed to be younger forms. In rickets small aggregations of lymphocytes, sometimes even with a germ centre, may be found. It is unusual to find immature cells of the lymphocyte series in normal marrow, but in glandular fever and in lymphatic leukæmia they may be numerous.

The typical immature cell of this series is the *lymphoblast* (Plate 2), which is, so to speak, equal in its developmental potencies to the myeloblast, *i.e.*, it is irreversibly determined in the direction of lymphocyte formation. It is about the same size as the myeloblast but with a relatively larger nucleus, which is more coarsely reticulated than that of the myeloblast. It contains nucleoli around which the chromatin is somewhat condensed as it is also at the edge of the nucleus where it forms an almost distinct membrane.

Monocytes. Monocytes are scanty in normal marrow but appear to arise from the hæmohistioblast. Structurally they differ in no way from those seen in the blood.

Plasma Cells. Plasma cells are scanty but invariable constituents of the marrow even in health. They increase slightly in hypoplastic conditions and a great deal in myeloma. There is still much controversy as to the origin of these cells, and it remains uncertain whether those of the blood and marrow are identical with those found in the tissues. In the marrow most of these cells are quite large (15 to 20 microns), have an excentric nucleus, in which the basichromatin has a "cart-wheel" arrangement, extensive basophilic cytoplasm, and usually a pale area round the nucleus. Similar cells, in which the nucleus is in the middle (Türk cells), are also found, and, like the plasma cells, their origin is uncertain.

There is a good deal of evidence that any type of non-granular blood or marrow cell may, for reasons as yet unknown, assume plasmacytoid characters so that it is probable that lymphocytic, lymphoblastic, myeloblastic and even erythroblastic plasma and Türk cells exist. It has been asserted that plasma cells have a distinct line of development from plasmoblasts, and Mœschlin's work on these cells in rubella seems to support a similar view,

although he gives no criteria by which one can distinguish plasmoblasts from lymphoblasts.

Megakaryocytes. Megakaryocytes are important components of the marrow and, in spite of their great size and irregular shape, they, or parts of them, are usually recognisable in films. As they are rarely numerous, it is best to seek for them with low magnifications, and are most likely to be found intact ouly near the edges and ends of films. They are from 30 to 90 microns in diameter with faintly basophilic cytoplasm in which small groups of azurophilic granules are present, mainly near the edges (Plate 2).

The nuclei present most complicated lobulation, but there does not seem to be any distinctive distribution of the basichromatin, which is mainly disposed in the form of large masses. It is possible that many of the structures, which in marrow films seem to be only parts of megakaryocytes, are really complete but immature elements which lie on the line of development from the histiocyte to the fully formed megakaryocyte. The relative simplicity of the nuclei of these *megakaryoblasts* seems to support this view.

Red Cells. It is now almost universally assumed that all red cells, both normoblasts and megaloblasts, arise from a common ancestor—the hæmocytoblast. We need not enter into the time-honoured discussion of the genealogical history of the red cells in general, but, as the morphological differences between normoblasts and megaloblasts are considerable, they are best described separately. Their supposed relationships are shown on Plate 3.

The Normoblastic Series starts at the haemocytoblastic stage as does every other blood cell, and its culmination is the development of the ordinary red corpuscle. In marrow films it is relatively easy to trace out every stage in this process.

The least mature element that is recognisable as definitely belonging to the red cell series is the *pro-erythroblast*, which is a large cell slightly resembling the hæmocytoblast but possessing a distinctive nuclear arrangement.

The basichromatin is no longer purely reticular, as in the hæmocytoblast, and shows a tendency to be aggregated into almost triangular masses with a roughly radial arrangement. Pale areas, which are perhaps nucleoli, can be detected, but the cells in which these can be seen are scanty. The cytoplasm has become less intensely basophilic, and when no trace of nucleoli can be seen we have reached the next clear-cut stage—the *basophilic erythroblast*. In this the radial arrangement of the chromatin is quite distinct, and

in the rather more mature forms the cytoplasm may show small areas of eosinophilia. These cells are about 10 to 12 microns (*macro-normoblasts*).

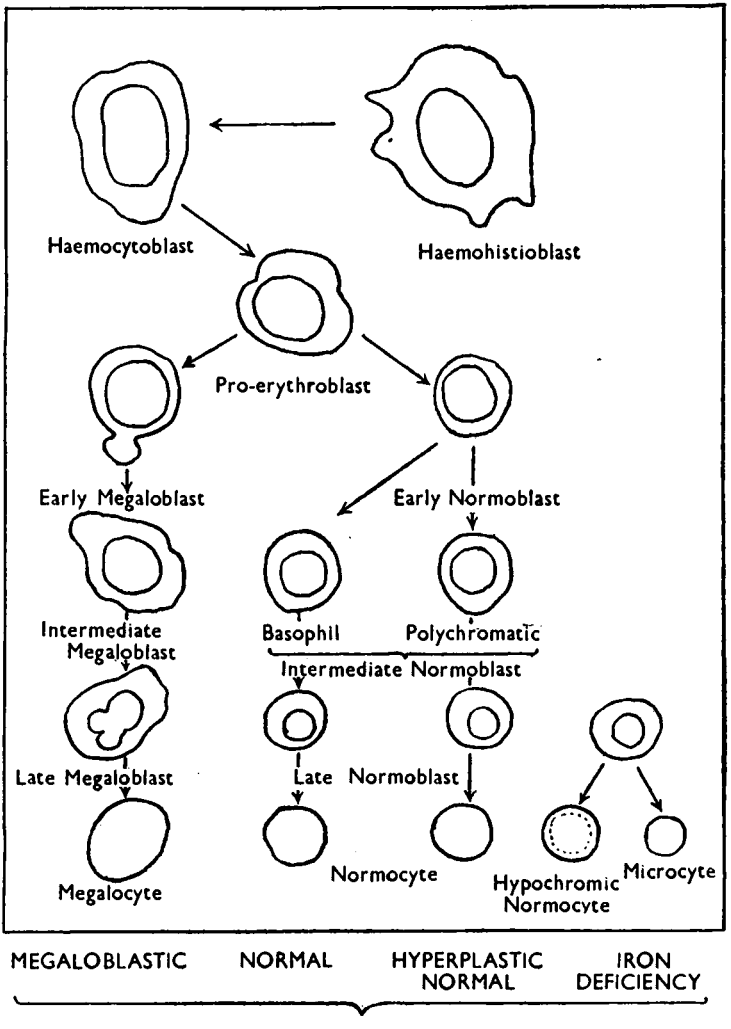
From this stage onwards the whole cell gradually decreases in size while more and more hæmoglobin develops in the cytoplasm. This is the ordinary *normoblast* with a " cart-wheel " nucleus. Then, without further decrease in the size of the cell as a whole, the nucleus becomes denser and smaller until it ultimately disappears. It is during this stage that the staining reaction of the cytoplasm shows polychromasia, which vital staining reveals in the form of a basophilic reticulum (*reticulocyte*). Then, when eosinophilia develops, we have the normal red corpuscle.

The Megaloblastic Series is still the subject of much discussion. Most writers now regard it as arising from the hæmocytoblast and as being capable of giving rise to normal red corpuscles, if a sufficiency of the hæmopoietic factor is present in the body. It will suffice if we describe the morphological characters of these elements without committing ourselves to any particular view of their nature.

The earliest cell that is recognisable as belonging to this series is the *promegaloblast*. This resembles the hæmocytoblast inasmuch as the nucleus is reticular and contains nucleoli, but the cytoplasm is distinctly more basophilic. As maturation proceeds, the nucleoli disappear, but, for a time at least, the rest of the nuclear structure and the basophilia of the cytoplasm remain unchanged (*basophilic megaloblast*). In this and later stages of development, the chromatin never shows the regular " cart-wheel " arrangement seen in normoblasts ; it is present in masses of greatly varying size with a most irregular distribution. Then as hæmoglobin develops, the cytoplasm becomes polychromatic and ultimately orthochromatic ; the nucleus becomes progressively smaller and denser until finally it is lost, and what is left is a red corpuscle much larger than normal (*megalocyte*).

The series of changes that occur during maturation of megaloblasts is not very constant. Thus, the cytoplasm may ripen before the nucleus does so, or *vice versa*. One therefore gains the impression that this series of cells, at least as one sees it in the marrow of post-natal life, is not a normal one. The developmental history of the megaloblast in the early human embryo is, unfortunately, still unknown.

The Normal Myelogram. It is not possible to make accurate total cell counts on the bone marrow. Segerdahl (3), who tried to do so, found that in health the number varied from 10,000 to 190,000


Haemocytoblast
Haemohistioblast
Pro-erythroblast
Early Megaloblast
Early Normoblast
Intermediate Megaloblast
Basophil
Polychromatic
Intermediate Normoblast
Late Megaloblast
Late Normoblast
Megalocyte
Normocyte
Hypochromic Normocyte
Microcyte
MEGALOBLASTIC
NORMAL
HYPERPLASTIC NORMAL
IRON DEFICIENCY

Haemocytoblast
Haemohistioblast
Pro-erythroblast
Early Megaloblast
Early Normoblast
Intermediate Megaloblast
Basophil
Polychromatic
Intermediate Normoblast
Late Megaloblast
Late Normoblast
Megalocyte
Normocyte
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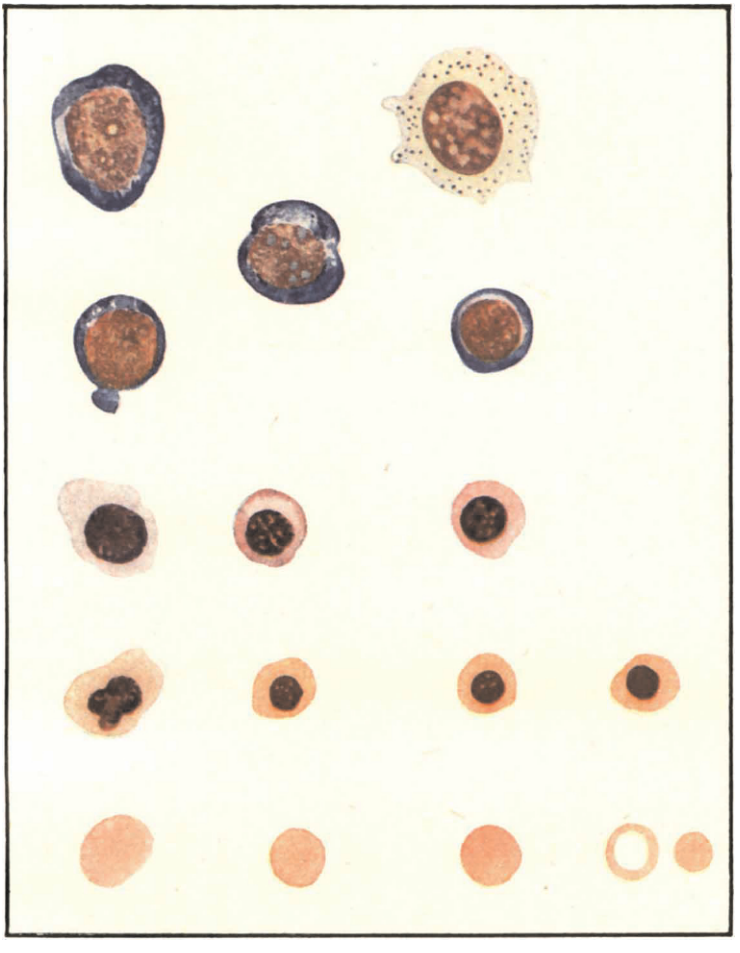
Microcyte
MEGALOBLASTIC
NORMAL
HYPERPLASTIC NORMAL
IRON DEFICIENCY

PLATE 3



DEVELOPMENT OF RED CELLS

[*To face p.* 8.

per cubic millimetre. There can be little doubt that various factors influence the total count quite apart from the structure of the marrow itself. Thus, if only small amounts of marrow are withdrawn by sternal puncture, the total number of cells per unit volume is greater than if a large amount is withdrawn ; and, even more strangely, the proportion of white cells is higher. There seems no doubt that it is only the differential count from which inferences can be drawn, and it is disappointing to find that the marrow obtained by sternal puncture before death gives a very different picture from that obtained by the same procedure within half an hour after death. In other words, the inferences we may draw from the myelogram are much less certain than those which we may draw from the ordinary blood count.

From the figures obtained by examining 500 and 1,000 consecutive cells in marrow films, useful relationships can be revealed : thus, in health, the proportion of white cells of all sorts to nucleated red cells varies from 5–1 to 3–1, but in the anæmias the proportion is much lower and the relationship may even be inverted. Then again, the proportion of granular to non-granular white cells is of some importance. Normally it is about 4–1. Again the numerical relationship of normoblasts to megaloblasts is significant, particularly in the diagnosis of pernicious anæmia.

In the normal bone-marrow the cells multiply by ordinary karyokinetic division, and, in films of the marrow-fluid, mitotic figures are always seen. It is important to arrive at some estimate of the percentage of dividing cells, and in which particular group of cells, *i.e.*, myeloblasts, myelocytes, etc., mitosis is most active, because from this information very useful deductions can be drawn. Japa (4) has given the following figures for normal bone marrow ; in each 1,000 nucleated cells about 15 show mitotic figures ; 40 per cent. of these are in the prophase, 45 per cent. in the metaphase, 10 per cent. in the anaphase, and 5 per cent. in the telophase. The proportion of dividing leucoblasts to erythroblasts is given as 45 : 55 ; of myeloblasts to myelocytes 3 : 97 ; of early normoblasts to late normoblasts 91 : 9.

In simple hyperplasia of the leucoblastic or erythroblastic tissues following acute pyogenic infection or acute hæmorrhage, the number of mitotic figures may double itself, but there will be no significant alteration in the ratios of the dividing cells in the myeloid and erythroid groups respectively. The leukæmias, however, show a very different picture. Here the number of dividing cells also shows

a marked increase, but a larger percentage of the mitotic figures are in the more immature cells. This is well seen in the myelogenous leukæmias ; in the chronic forms there is an increase in the number of dividing premyelocytes, and to a much smaller degree in the myeloblasts ; as the malady becomes more acute the proportion of mitotic myeloblasts rises until, in the true acute form, they are the only dividing cells seen.

NORMAL MYELOGRAM

Neutrophiles :	
Myelocytes	30 –35 per cent.
Metamyelocytes	10 –15 ,, ,,
Polymorphs	24 –30 ,, ,,
Eosinophiles :	
Myelocytes	1 – 2 ,, ,,
Polymorphs	½– 1 ,, ,,
Basophiles :	
Myelocytes	3– 6 ,, ,,
Polymorphs	1 – 2 ,, ,,
Premyelocytes	1 – 2 ,, ,,
Hæmocytoblasts, including myeloblasts	1 – 2 ,, ,,
Hæmohistioblasts	¼– ½ ,, ,,
Lymphocytes :	
Small	6 – 9 ,, ,,
Large	8 –12 ,, ,,
Plasma cells	½– 1 ,, ,,
Pro-erythroblasts	1 – 2 ,, ,,
Normoblasts	15 –20 ,, ,,
Megaloblasts	2 – 3 ,, ,,

Megakaryocytes scanty, but invariable.

It is possible that some, if not all, the cells called megaloblasts in this table are really large normoblasts ; but many writers state that a few genuine megaloblasts are present, even in health.

CHAPTER II

THE MARROW IN LEUKÆMIA

THE marrow changes in the various forms of leukæmia are usually distinctive, and for this reason it is best to start our description with this group of diseases. It is not to be supposed that marrow puncture in the leukæmias is only of academic interest. Admittedly, in many, perhaps most, cases of leukæmia, diagnosis is possible on clinical and ordinary hæmatological grounds. Blood examination usually enables one to discover the type of leukæmia and also gives some indication of the severity of the condition, but the blood is not in the ordinary sense of the word a tissue : it is a mixed secretion from the hæmopoietic organs, and for this reason the marrow naturally gives a clearer and more accurate picture than does the blood. For instance, changes indicative of an approaching relapse will be found in the marrow earlier than in the blood, and, further, as all forms of treatment of leukæmia are directed towards the regulation of marrow function, it is of great value to follow the effects of therapy by repeated sternal punctures.

Chronic Myeloid Leukæmia (Chronic Leukæmic Myelosis) (Plate 4). The blood picture in this disease is usually striking and characteristic, but the changes in the marrow are less easily interpreted. After all the marrow is the main organ in which granular leucocytes are formed, and it is extremely difficult to recognise slight degrees of overgrowth. Fortunately, changes in the proportions of the cells occur early, and may enable one to make a diagnosis. Thus, in health, almost all the polymorphs arise from mitotic divisions of pre-existing myelocytes ; myeloblasts are scanty and play little or no part in the production of fresh myelocytes. In severe infections, where the demand for granular leucocytes is greatly increased, there may be so great a strain that the myeloblasts not only increase in number but also add to the stock of myelocytes.

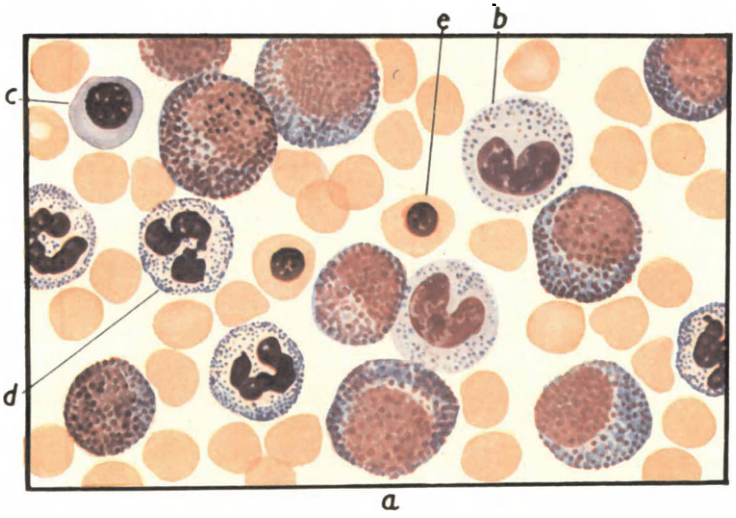
In chronic myeloid leukæmia, even in the stages when there is no increase in the number of cells in the blood, the myeloblasts take part in the formation of myelocytes ; in other words, the whole process of granulopoiesis starts from a more primitive stage. If, therefore, an excess of myeloblasts is found in marrow films, and if infection can be excluded, a diagnosis of leukæmia is very probable. When the leukæmic process is well established, the cellularity of the

marrow is much increased, and this is so extreme that it can be recognised in well-spread marrow-films where one may find as many as 80 cells in each field of the microscope. In the ordinary chronic stage of the disease, this increase depends almost entirely upon the addition to the number of myelocytes, which may form as much as 80 per cent. of the white cells. Most of them are neutrophils, but excessive numbers of eosinophil and basophil myelocytes are also present. Almost all the myelocytes of whatever type are larger than the corresponding cells found in the blood, and many of them are less mature. It is particularly in the marrow that one can easily find myelocytes containing both eosinophilic and basophilic granules.

Mitotic figures are not numerous in chronic cases, but it is very rare to be unable to find a few, usually in myelocytes and much more rarely in non-granular cells, which are presumably myeloblasts or hæmocytoblasts. The number of myeloblasts and of premyelocytes is distinctly greater than in health, and some prognostic inferences can be drawn from the number of these immature elements. The more plentiful they are the worse the outlook. In brief, it can be said that any great increase in the number of cells, less differentiated than myelocytes, is an indication either that a relapse is imminent or that the disease is passing from the chronic into the acute stage. This is, of course, comparable with what has long been known about the changes in the blood, viz. that the greater the percentage of immature cells the more likely is an acute exacerbation, but the marrow changes precede those in the blood and therefore permit of earlier treatment. On the other hand, undue alarm should not be aroused by some myeloblasts in narrow films. Even if none can be found in the blood some will be seen in the marrow in all cases, and, furthermore, they are always more numerous in the marrow than in the blood.

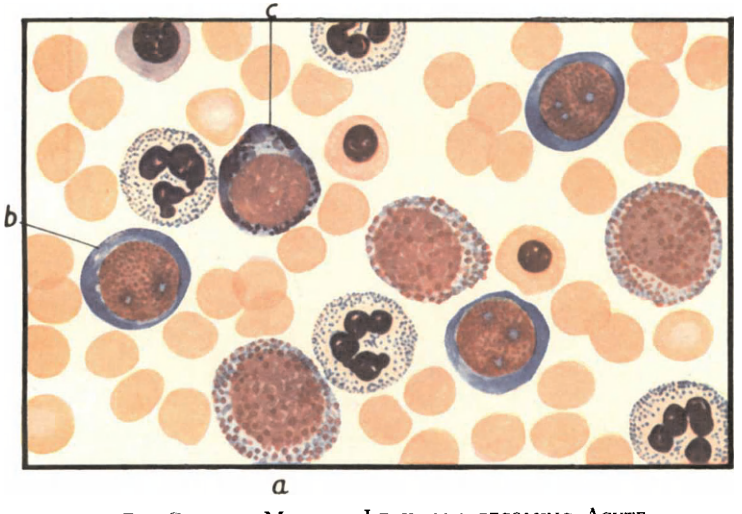
There are two other points of importance which are not easy to determine but which experience has shown to be of importance. The first is that the background of marrow films in chronic myeloid leukæmia is denser and contains more fragments of debris than in health. Recognition of this change naturally depends upon experience of marrow films in general. The second point, which is of great importance and which cannot be detected in films that are too thin or which are made from marrow fluid that is too dilute, is that the cells, or some of them, tend to lie in groups, all of which are composed of a single cell-type. Thus, it is quite common in fairly thick films to find islets of myelocytes, premyelocytes and myelo-

PLATE 4



A. Chronic Myeloid Leukæmia

(*a*) Myelocyte. (*c*) Basophile normoblast.
(*b*) Metamyelocyte. (*d*) Polymorph.
(*e*) Normoblast.



B. Chronic Myeloid Leukæmia becoming Acute

(*a*) Myelocyte. (*b*) Myeloblast. (*c*) Basophile Myelocyte.

[*To face p.* 12.

blasts, indicating that in leukæmic marrow the immature cells have a more focal arrangement than normal. It is in such islets that mitotic figures are most commonly seen. If such figures are present in the myelocyte islets, the outlook is much less grave than it is when the myeloblasts show many mitoses.

Marrow films in chronic myeloid leukæmia are the more pleomorphic because the leucoblastic overgrowth is accompanied both by erythroblastic and megakaryocytic reactions. This, of course, introduces a difficulty in interpretation, because, unless the marrow is very carefully studied, the fact that every type of cell is increased might lead one to suppose that one was in the presence of a simple reactive hyperplasia such as may occur in association with any infection. The recognition of the focal arrangement of the immature white cells and of the increase in the number of myeloblasts is the main safeguard against this false inference.

It cannot be emphasised too strongly or too often that it is essential to correlate the blood picture with the myelogram ; either alone can be most misleading. For instance, there is no peculiarity in the bone marrow that would enable one to recognise the fact that the blood picture was aleukæmic. The amount of hyperplasia is no greater in leukæmic cases than it is in aleukæmic ones, unless, of course, the aleukæmic state is due to treatment. The factor that regulates emigration of cells from the marrow into the blood is unknown. At this point, it may be well to mention that examination of the marrow is of particular importance in those cases in which the hæmogram has shown that the blood picture is aleukæmic. Such a state may be due to partial aplasia of the marrow, either idiopathic or as the result of excessively energetic treatment ; or it may be due to some unknown cause which prevents emigration of cells from the intensely active marrow into the blood. It is obvious that in the former case any method of treatment such as X-rays will induce more rapid and more intense degeneration, but, in the hyperplastic cases, irradiation of the marrow is as safe as in the ordinary leukæmic type of case.

There are other cryptic forms of myeloid leukæmia in which marrow puncture is essential for diagnosis. In the rare type known as *medullary myelosis* there is no enlargement of the spleen, which may appear normal at autopsy and even on histological examination. The blood picture is usually typically leukæmic but may be aleukæmic or even normal. The myelogram, however, is characteristic in these cases. It has been supposed that this is not really a form of leukæmia but that it is a leukæmoid reaction. This view is not

tenable because the blood picture persists and deteriorates until death occurs. Indeed, in prolonged cases, the medullary form of myelosis may develop into the ordinary type of chronic myeloid leukæmia. Early diagnosis by marrow puncture is of importance because radiotherapy causes improvement even at a time when the spleen is still unaffected.

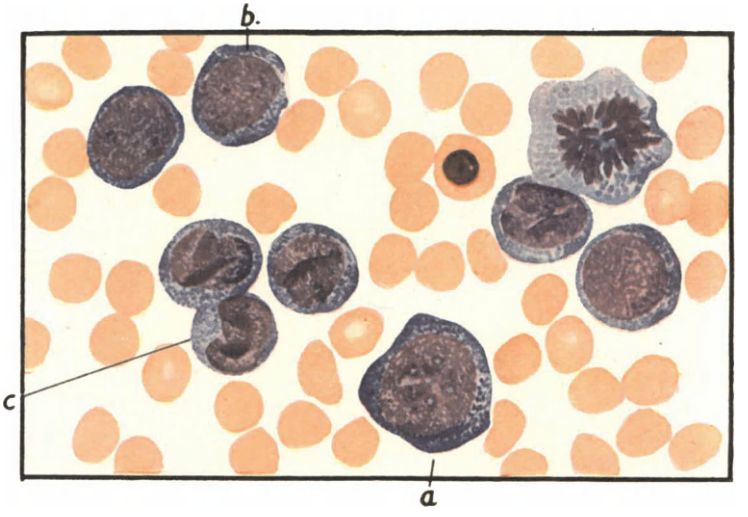
A somewhat similar malady may run an acute or more rarely sub-acute course—the so-called *aleukæmic myelosis*. This runs the clinical course of a rapidly progressive, severe anæmia without any change suggestive of leukæmia in the white cell count, but the marrow has all the characters of the intensely active tissue found in ordinary cases of leukæmia. Strangely enough, one finds that the predominant cells in the marrow are myelocytes, not, as one would expect in an acute case, myeloblasts. If a case of this sort is encountered, it is extremely easy to draw false inferences concerning the acuteness of the disease if one is basing prognosis entirely on the characters of the myelogram.

Chronic Lymphatic Leukæmia (Chronic Lymphadenosis). The myelogram in this disease is usually easy to interpret because lymphocytes are not numerous in normal marrow and never show any signs of mitotic activity. In advanced cases of chronic lymphatic leukæmia, marrow films are as monotonous as blood films. Lymphocytes are found so closely packed together that they almost resemble a tissue which is interrupted here and there by a few small nests of granulocytes and red cells. The background of the marrow films is moderately dense but structureless and unlike that seen in chronic myeloid leukæmia where free cell granules are embedded in it. In almost every case the majority of the cells are small lymphocytes, but a variable number of larger forms can be found : not all of these are lymphoblasts, which, indeed, are usually scanty. They increase in number only if the disease is actively progressing or is about to become acute.

In less advanced cases of chronic lymphatic leukæmia, the lymphocytes can be seen to form small focal masses embedded in hyperplastic marrow tissue. In other words, the arrangement is similar to that in chronic myeloid leukæmia where we have already described foci of myeloblasts, etc. Thus, lymphocyte aggregations in the marrow never possess germ centres, and indeed, it is mainly at their periphery that the less immature forms are seen, and mitotic figures are found.

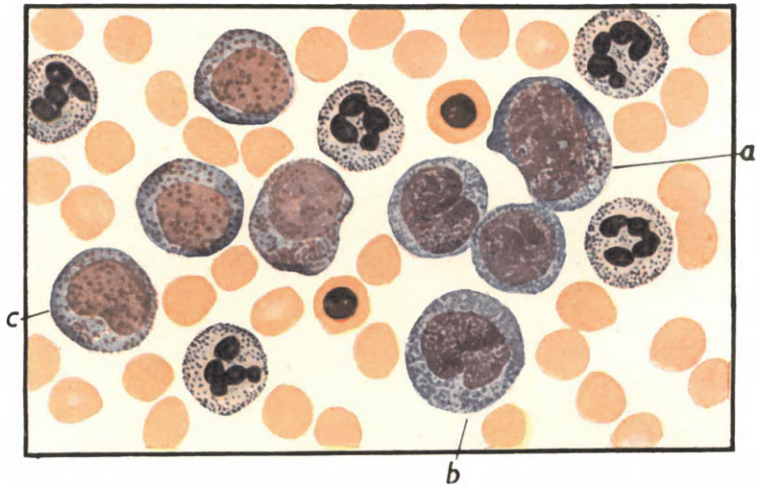
Chronic lymphatic leukæmia is notorious for its tendency to run

PLATE 5



A. Monocytic Leukæmia

(*a*) Monoblast. (*b*) Promonocyte.
(*c*) Monocyte.



B. Monocytic Phase in Myeloid Leukæmia

(*a*) Monoblast. (*b*) Monocyte.
(*c*) Myelocyte.

{*To face p.* 15.

an atypical course. Most cases of so-called crypto-leukæmia are of lymphatic type.

Chronic Aleukæmic Lymphatic Leukæmia. This disease may run the whole of its course without any of the classical, hæmatological, or clinical signs of leukæmia. Quite frequently the picture is that of aplastic anæmia—great reduction of red corpuscles and of hæmoglobin, no signs of blood regeneration, leucopenia, and relative or absolute lymphyocytosis. In the late stages, an occasional lymphocyte of immature type or even a lymphoblast may be found in the blood, but in the past diagnosis was often deferred until autopsy and was sometimes impossible until histological sections were examined. Now, sternal puncture permits of an accurate diagnosis of lymphadenosis, but the myelogram itself gives no indication that the blood picture is aleukæmic. The marrow changes are the same whether the number of lymphocytes in the blood is large or small.

Monocytic Leukæmia (Plate 5). There is no reasonable doubt that this disease exists as a clinical and hæmatological entity, but there seems to be much confusion in the literature as to what shall be called monocytic leukæmia, and what mixed myeloid and monocytic leukæmia. In our opinion the term, monocytic leukæmia (Schilling type, Plate 5A), should be limited to those cases which run an acute or sub-acute course (never chronic), show a leucocytosis due to an increase in monocytes or their precursors, and an aplastic or hypoplastic type of anæmia. The so-called mixed (Naegeli, Plate 5B) types are to be regarded as variants of myelogenous leukæmia. In these there occur transitory increases in the number of monocytic elements in the blood and the marrow; but the general clinical and hæmatological picture, throughout the greater part of the illness, is identical with the myeloid type of leukæmia. This is even more evident in tissues examined histologically after post-mortem; here the reticulo-endothelial system shows the changes characteristic of myeloid leukæmia.

The marrow films in true monocytic leukæmia are quite characteristic. As in all leukæmias there are many cells; monocytes and pro-monocytes predominate and form from 70 to 90 per cent. of all the nucleated cells. The mature monocytes have lobed or rounded nuclei with fine reticular chromatin, and a varying amount of cytoplasm which may or may not contain azurophilic granules. The *pro-monocyte* (or *monoblast*) is a similar cell with 2 or 3 nucleoli and a rather more basophilic cytoplasm. The number of mitotic

figures is striking (many may even be found in the peripheral blood) and these are chiefly in the pro-monocytes. The number of hæmocytoblasts is increased and, indeed, these cells may be difficult to distinguish from the premonocyte if the latter is at all atypical; and in monocytic leukæmia it often is atypical.

Erythropoiesis is markedly depressed. There is a reduction of all the types of normoblast, but particularly of the earlier and more basophilic forms. Megakaryocytes and platelets are scanty. From films it is difficult to say whether the granulocytic cells are affected, but quite often the absolute number of neutrophile polymorphs in the peripheral blood is not much, if at all, reduced.

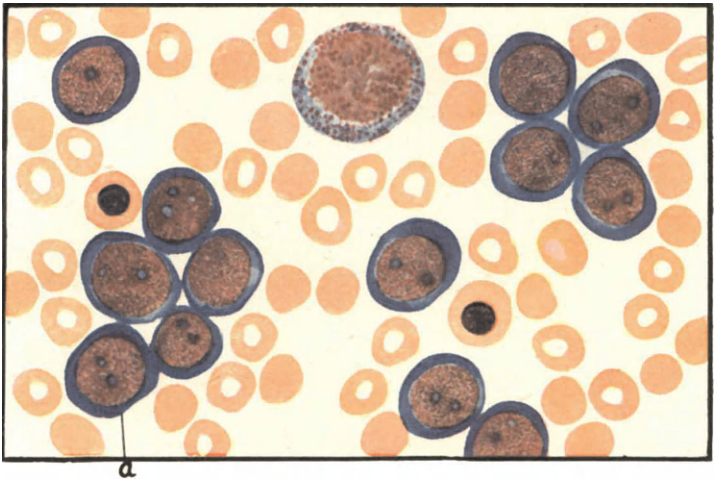
In the monocytic phase of myeloid leukæmia, monocytes and promonocytes appear in varying numbers in the blood and marrow. As this phase always occurs in the chronic form of myelogenous leukæmia, an erythroblastic hyperplasia of the bone marrow is to be expected, unless, of course, the malady is in its terminal stages and aplasia of the erythroblastic tissue has occurred.

Acute Leukæmia (Plate 6). This disease may be primary or it may be the terminal phase in a chronic case. It is usual to distinguish between acute myeloid and acute lymphatic leukæmia, although the differentiation is of purely academic value. The myelogram in this disease is so varied that one might be tempted to suppose that there is an almost infinite variety of types of acute leukæmia. This is really due to the fact that the cells infiltrating the marrow in these acute cases are so extremely undifferentiated that it is impossible to be certain of their nature. When reversion to primitive type is so extreme it is best to speak of *acute leucoblastosis*.

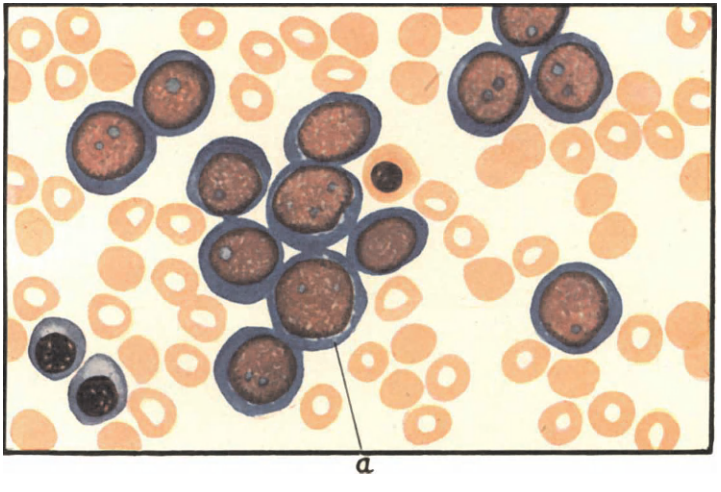
From the practical point of view one can say that a marrow, which is full of immature non-granular cells, the nuclei of which contain nucleoli, is from a case of acute leukæmia, and if mitotic figures are numerous one can be even more certain of this. Odd as it may sound, it is true to say that all cases of acute leukæmia are hæmatologically atypical, and it is impossible to establish a standard myelogram for cases of acute leukæmia in general.

Atypical Leukæmias. It is particularly lymphatic leukæmia that may present itself in clinically unusual forms which used to be classed together under the heading of pseudo-leukæmia. For example, in *splenic lymphadenosis*, there may be a symptomless splenomegaly for four or five years. The blood usually remains normal but occasionally a few immature lymphocytes or lymphoblasts appear. Spleen puncture reveals large numbers of lympho-

PLATE 6



A. Acute Myeloid Leukæmia
(*a*) Myeloblast (*see* p. 4).



B. Acute Lymphatic Leukæmia
(*a*) Lymphoblast (*see* p. 6).

[*To face p.* 16.

blasts but the marrow may remain normal even in the late stages, and this is probably the reason for the blood rarely becoming leukæmic.

Lymphatic proliferation may be confined to the bone marrow (*medullary pseudo-leukæmia*); then the disease runs its clinical course as a severe and progressive anæmia, usually with little sign of blood regeneration. The white cells are at or below the lower limits of normal, and a few immature lymphocytes are present. Marrow puncture reveals an intense lymphocytic and lymphoblastic overgrowth.

Myeloid leukæmia more rarely runs its course in a grossly atypical manner, but in one type—*medullary myelosis*—there is no enlargement of the spleen although the myelogram is characteristic.

There is also an acute form of this purely medullary overgrowth —*acute aleukæmic myelosis*—which clinically resembles the similar lymphatic condition.

The existence of these atypical forms indicates the necessity for marrow puncture in almost every type of blood abnormality.

CHAPTER III

LEUKÆMOID REACTIONS

THROUGHOUT life, the marrow is active in replacing the continuous wastage of cells ; and its power of reacting to the most varied kinds of stimuli is very great. Nevertheless, the number of possible types of reaction is extremely limited : this is the main reason why diagnosis based on blood or marrow examination is difficult and liable to error. If the reaction mainly affects the erythropoietic tissue, there is less difficulty than when the leucoblastic cells are involved. In the latter case, it may be difficult to differentiate simple reactions from leukæmia, and, indeed, blood examination is often more important for diagnosis than is marrow puncture. It is only if the small focal masses of cells typical of leukæmia are found that a definite conclusion can be reached.

Other significant points have to be taken into account. Thus, if the number of myeloblasts is high the condition is more likely to be leukæmia than infective, although admittedly a considerable myeloblastic reaction (leukæmoid reaction) occurs in some severe infections. Even so, the histological arrangement of the cells is different. In infections a definite focal distribution is never found. Then also, the characters of the more mature cells may help to distinguish a leukæmoid reaction from genuine leukæmia ; in the latter they may be abnormal in the sense of being malformed, but they never show the degenerative changes that are so common in infective states.

There can be no doubt that there is still need for much investigation of the marrow in the diseases that show a leukæmoid blood picture, and at the present time we are not in a position to say more about them.

CHAPTER IV

NEOPLASTIC AND ALLIED CONDITIONS OF THE BONE MARROW

IN so far as they directly affect the myelogram, bone-marrow tumours fall into two groups. In the first are *the generalised neoplasms of the hæmopoietic tissue* where sternal puncture may show the marrow to consist almost entirely of tumour cells. In the second, the tumours are *localised*, and unless a nodule of growth is punctured, the myelogram shows only the effects produced by compression and replacement, *i.e.*, leuco-erythroblastosis, which may progress to hypoplasia. In this group, the tumours may be primary in the bone marrow or metastatic from growths elsewhere in the body.

There is a widely held view that the leukæmias are neoplastic in nature, and that they are closely related to the myelomata, which, in turn, have relationships with generalised reticuloses, such as Gaucher's disease. These fall into the first group. In the second group are the localised myelomata and reticuloses as well as the metastatic neoplasms.

It must be borne in mind that it is only when this second type is fairly widespread in the marrow that hæmic erythroblastosis is produced, and that the tumour cells will not be found in marrow films unless a nodule is present at the site of puncture.

In our present state of knowledge, any attempt to classify bone-marrow tumours must be tentative and somewhat controversial. As, in this book, we are concerned only with marrow puncture as a method of clinical and cytological investigation, the discussion here will be devoted to the most helpful methods of elucidating the myelogram, while, of course, not forgetting the origin of the tumours.

Three main groups can be clearly distinguished :—

1. Myelomata.
2. Reticulo-endothelioses.
3. Metastatic tumours.

Myeloma. The term myeloma is properly used only to designate a group of tumour (or tumour-like) masses consisting of cellular elements, which more or less closely resemble specific bone-marrow cells. They may be single or multiple in the bones, and, not uncommonly, in other tissues also : indeed, the skeleton is not

invariably affected. In the usual type, *i.e.*, with osseous involvement, the blood shows no pathognomonic changes ; there may be slight hypochromic anæmia, but nothing else of note.

As a rule diagnosis is possible on clinical grounds alone ; and this can be confirmed radiologically. But by the time that multiple fractures and deformities have developed, it is often possible to detect some more striking changes in the blood : a few plasma cells are a common finding and, occasionally, a frankly leukæmic picture develops.

There are four generally recognised types of myeloma :—

(i) plasma cell ;
(ii) myeloblastic, derived from the primitive myeloid cells ;
(iii) lymphoblastic, derived from the lymphoid cell-series ; and
(iv) erythroblastic, derived from the nucleated red cells.

A malignant monoblastoma has also been described (Mitchell) (5).

The majority of myelomata are composed of elements resembling plasma cells : indeed, there is little reason for doubting that they are really plasma cells. But, according to one's opinion, these tumours would equally properly be designated as pseudo-plasmacytomata.

We have already seen that the nature of plasma cells is still uncertain (p. 6), though that need not affect our descriptive knowledge of tumours composed of them ; but it must be emphasised that it is often possible to observe what appears to be a continuous series of stages between the cells of the reticulo-endothelium and typical plasma cells. But, of course, these elements, as seen in blood or marrow films, do not look exactly as they do in histological sections, where they appear as round elements, with a circular nucleus, excentrically placed in intensely basophilic cytoplasm, which is vacuolated round the nucleus (*heller Hof*). In films, there is much more variation in size, some of the cells being no larger than small lymphocytes, whilst others are as large as monocytes, and may (in marrow but not in blood) contain more than one nucleus. The basi-chromatin is much more variable in its distribution than it is in the plasma cells of the tissues : it may be " cart-wheel," but it is often quite irregular ; similarly, it may be symmetrically or excentrically placed in the nucleus. The cytoplasm is basophilic, but less intensely so than in the tissues : it may be vacuolated, but not necessarily in the vicinity of the nucleus ; and occasionally it is seen to contain azurophilic granules such as rarely occur in tissue

plasma cells. Mitotic figures are often observable. Azurophilic protein crystals may be found in a few " myeloma cells " (6).

Marrow films, thus, present a very varied appearance, which is extremely suggestive of neoplasia, but which does not always immediately suggest that the predominant element is the plasma cell. But, even so, a little experience allows a diagnosis of myeloma to be made with much greater certainty than is possible even with X-rays (7). Of course, the film does not consist entirely of plasmacytoid elements : it is very rare for all the ordinary marrow cells to be replaced. As a rule about 50 per cent. of the cells are of plasma cell type. But, if the needle has penetrated a nodule of " tumour," it may be found that almost every cell is of this kind. But, from the point of view of diagnosis, these differences are of no special significance.

In the *myeloblastoma*, the cells are, on the whole, larger and may contain few or many neutrophilic granules in the cytoplasm. Many polymorphonuclears and myelocytes are present, and both may be found in the peripheral blood.

In the *erythroblastomas*, the normoblasts are usually well hæmoglobinised, but there may be a large percentage with basophilic cytoplasm. The cells are small, and the nuclei pyknotic. It is still uncertain whether erythroblastic tumours, in which there are no hæmoglobinised elements, really exist.

In some cases, which run the usual course of myeloma, large numbers of small lymphocytes may be present. They have been designated as lymphosarcoma, yet, histologically, they show the features of a malignant infiltrating and metastasising lymphoblastoma.

Malignant monoblastoma is a very rare condition, but that it exists as a pathological entity seems definite from a study of the reported cases (5). Terminally, the blood picture may become that of monocytic leukæmia ; and the cells found in the bone marrow are indistinguishable from those found in monocytic leukæmia (p. 15).

The status of the tumour-like *chloroma* is far from being clear, but we can well mention it here with the myeloma. It seems to be more directly related to leukæmia than are the other myelomas, inasmuch as it is often, perhaps usually, accompanied by a blood picture characteristic of myeloblastic leukæmia. It differs from the " typical " cases of this malady in its tendency to give rise to masses (especially in the orbit), to invade and erode adjacent structures, and to develop a bright green colour (which is, however, far from constant).

As a rule, symptoms and signs suggestive of acute or sub-acute leukæmia precede the development of chloromatous tumours ; but, occasionally, there are no changes in the blood, except perhaps slight hypochromic anæmia, even at a time when quite large masses are present in the orbits. Then, examination of the marrow is likely to be very helpful, especially as in this disease, mitotic activity is remarkably intense. The tumour cells are scattered throughout the marrow, some being isolated, others in groups of six or nine ; and the adjacent myeloid tissue is distinctly hyperplastic. Later, when the tumour has spread, the greater part of the marrow may be replaced by abnormal cells ; and it is then difficult to discover any remnants of normal tissue.

The characteristic cells are of the same type in every case of chloroma. They are large, with a bulky nucleus, which contains distinct nucleoli. The basi-chromatin has an almost reticular distribution, and the cytoplasm, which is moderately basophilic, contains no granules. Cells identical with those in the marrow are only rarely found in the circulation, where the predominant cells are usually myeloblasts or extremely immature premyelocytes. It is this latter fact that suggests that all cases of chloroma are of myeloid nature, although some writers still contend that lymphatic examples do occur. It is, however, more probable that chloroma represents a state intermediate between true acute leukæmia and a neoplastic condition.

Reticulo-Endotheliosis. The pathology of the reticulo-endothelioses has been put on a sound basis by Robb-Smith (8), who has classified them into three groups according to the nature of the reticulum cell from which they arise. Thus, we have the *follicular reticuloses*, the parent cell of which is the reticulum cell of the germinal follicle of a lymph-node ; the *sinus reticuloses*, which arise from the lining cells of the sinuses of the reticulo-endothelial system ; and the *medullary reticuloses*, which take origin from the free reticulum cells or their descendants, lying in the stroma of the reticulo-endothelial tissue between the sinuses. In the last group there are lymphoid, myeloid and monocytic medullary reticuloses, which we commonly call lymphatic, myeloid and monocytic leukæmia respectively. This group also contains the metabolic reticuloses of Gaucher's disease, Niemann-Pick disease, and xanthomatosis. *Fibro-myeloid medullary reticulosis* or Hodgkin's disease is yet another component of this group.

The follicular reticuloses produce no change in the peripheral blood

picture, and it is only when secondary nodules develop in the bone marrow that we get a picture of leucoblastic and erythroblastic hyperplasia, which may eventually develop into aplasia of the marrow if the invasion by tumour becomes more or less complete.

The sinus reticuloses also show no abnormality in the peripheral blood, apart from moderate anæmia and slight thrombocytopenia, but they do cause marked changes in the bone marrow. For this reason, Dameshek (9), who has reviewed most of the recorded cases, refers to them as *aleukœmic sinus reticuloses*. The bone marrow presents a varied picture. Characteristically, there are small collections of large cells, 20 to 30 microns in diameter, each with a rounded or indented nucleus, which occupies more than half of the cell. Sometimes the nuclei appear as if folded on themselves. The basi-chromatin appears as a fine reticulation, and nucleoli may be present. The cytoplasm is faintly basophilic, and about 50 per cent. of the cells show azurophilic granules in the " Hof " of the nucleus. Mitotic figures are frequently seen. The myeloid and erythroid elements are decreased, and megakaryocytes are absent. This probably represents a hypoplastic or destructive condition of the bone marrow, and it is interesting to note that in none of the recorded cases has there been an initial hyperplasia. It seems a reasonable inference that the tumour cells are not merely replacing the normal marrow elements, but that they arise from a primitive cell, which is also the precursor of the blood cells : consequently, on account of this perversion of function, the hæmopoietic tissue is unable to react in the ordinary way.

Of the *medullary reticuloses*, the leukæmias are discussed elsewhere (p. 10).

Gaucher's disease usually affects the spleen more intensely than the bones, although skeletal affection may occasionally predominate. But, even in the clinically splenomegalic cases, some involvement of the bone marrow is common. How usual it is was not realised until sternal puncture became a fairly common procedure. This frequency of skeletal involvement is an important matter, because it would suggest that splenectomy, in such a metabolic disease as this, would accelerate the deposition of the Gaucher substance in the bones, so that the last state might well be worse than the first.

The Gaucher cells in the marrow are large (50 microns or more). The nuclei present no characteristic arrangement of the chromatin, but are always relatively small and dark ; several may be found in one cell. The cytoplasm is bulky, with sharply defined outlines.

Some of the cells are polygonal, others elongated, but few transitional forms are seen. In paraffin sections, the cytoplasm has a foamy appearance, due to solution of the contained lipoid material ; and a faint, diffuse, iron-reaction can often be obtained. There seem to be three fairly distinct stages in the development of these elements. Thus, the least differentiated cells have granular cytoplasm, in which lies a more or less reticular nucleus. Then, there are cells with similar cytoplasm, but dark and almost structureless nuclei ; and lastly, the fully developed Gaucher cell, with vacuolated cytoplasm and small dense nucleus. It is only in the last type that it is possible to detect the presence of fine fibrils, which are best shown by silver-impregnation methods.

The myeloid tissue itself is usually hyperplastic, in contrast to the peripheral leucopenia ; and there is a slight increase of monocytes (up to 10 per cent.) in the myelogram. This is not sufficient by itself to arouse a suspicion of Gaucher's disease, but it does give support to the view that monocytes may arise from the same primitive reticulum cell.

Niemann-Pick disease shows a similar invasion of the marrow with " tumour " cells, and hyperplasia of the myeloid tissue. In contrast to Gaucher's disease, however, there is usually a peripheral leucocytosis. The characteristic cells are large (20 to 80 microns) and contain a reticular nucleus. The cytoplasm is filled with droplets that give rise to a " foamy " appearance very different from the fibrillary appearance of the typical Gaucher cells. As the disease is always fatal by the age of two years, marrow biopsy must be done by tibial trephine, or smears must be made from spleen puncture.

Hodgkin's disease affects lymphatic glands so much more obviously than it does the marrow, that relatively little attention has been paid to the latter tissue. And, although we probably never obtain assistance in diagnosis by examining the bone marrow, there are points of interest and importance in connection with it.

The cases fall into four groups, viz., those with normal marrow ; those with simple reactive hyperplasia of unspecific type ; those with excess of giant cells ; and, rarely, cases with obvious lymphadenomatous infiltration.

In the third group it is not, as a rule, possible to be certain that the giant cells are really lymphadenoma (Reed-Sternberg) cells, as there is no certain method of distinguishing them from megakaryocytes. One may, therefore, suspect that the film with such appear-

ances is from a case of Hodgkin's disease, but such marrow changes are, by themselves, insufficient evidence.

The fourth group, which is very small (10), contains those cases in which there is development of the typical granulomatous tissue in the marrow, and, when this occurs, there seem to be direct irritation of the marrow, which is invariably hyperplastic and may give rise to leuco-erythroblastic anæmia.

The majority of cases of Hodgkin's disease show a progressive anæmia of the hypochromic type, which only becomes severe in the terminal stages. The bone marrow shows the corresponding changes described with this type of anæmia (p. 39).

Ewing's tumour is a clear example of a growth that arises from the reticulo-endothelium of the marrow. It usually, perhaps always, arises at the site of an injury to a long bone in a young person ; and in the early stage is a localised condition, so that sternal puncture will reveal a normal myelogram. Nevertheless, long before there are any clinical or radiographic indications of the presence of secondary deposits, the sternal marrow may be found to contain scattered tumour cells. These are large, sometimes gigantic, elements, which resemble a caricature of histiocytes, with indented nuclei, irregular hyaline cytoplasm, and all the characteristics of what are sometimes called *dysmorphokaryocytes*.

Much work remains to be done on these and allied reticulosarcomas. At present we can make a diagnosis of malignancy or innocence, but of the origin of the characteristic cells we know little.

Metastatic Tumours. Metastatic tumours of the marrow are much commoner than are primary growths ; indeed, one is surprised at the frequency with which tumour cells are found in the sternal marrow, in the absence of clinical or radiographic signs of metastasis (10, 11, 12). It is probable that the use of this procedure as a routine would show that many clinically operable cases have already passed the stage at which cure is possible.

A good deal of experience is required for recognition of metastatic tumour-cells in marrow smears, because they often differ a good deal in character from those found in the primary growth. But they are usually found in groups composed of small and large cells, with a tendency to become much more elongated than epithelial cells elsewhere (*cf.* Gaucher cells in marrow). For this reason alone, it is rarely possible to infer the site of a primary tumour by study of the metastatic cells in the marrow.

Probably the commonest metastatic growth of the bone marrow is a secondary deposit arising from a primary carcinoma of the prostate, breast, stomach, kidney or thyroid, but, of course, sarcomas also freely metastasise to the marrow.

Apart from the presence of carcinoma cells, the marrow shows hyperplasia of the erythroblastic tissue, with hæmoglobinisation taking place in the earlier forms of normoblast. Areas of proliferating myeloblasts and myelocytes may be encountered, the general picture being that of leuco-erythroblastosis.

Such hyperplasia is, of course, a vital and not a purely mechanical response on the part of the marrow and is found in association with other metastatic tumours. It is also sometimes found with follicular reticuloses, Ewing tumour, Gaucher's disease and Hodgkin's disease. It may also occur with primary " tumours " of bone, such as myeloma, and with other overgrowths, for example, myelosclerosis.

CHAPTER V

THE ANÆMIAS

In the anæmias, more than in any other group of diseases, sternal puncture has thrown light on the pathology of the maladies. It might have been supposed that careful clinical examination and the ordinary methods of hæmatology had already given us almost all the information that we need; but study of the bone marrow has illuminated many dark corners, not only of pathogenesis, but of diagnosis also. For instance, we have seen many cases of severe anæmia in elderly people where both the clinical picture and blood investigations have been inconclusive. As in any prolonged or severe anæmia, the bone marrow tends to become aplastic and at this stage the colour index, mean cell volume, and mean cell hæmoglobin concentration may give misleading answers. Iron-deficiency anæmia, in these circumstances, may show a hyperchromic macrocytosis in the peripheral blood and pernicious anæmia may appear normocytic and orthochromic. Sternal puncture in these cases gives an immediate clue to the real type of anæmia, although the myelogram is not absolutely typical of the malady in question.

In view of the striking changes which occur in the marrow in almost any anæmia undergoing successful treatment, it is most essential that any recent medication be taken into account in assessing the myelogram. A patient with a severe anæmia recently examined concealed the fact that, during the week previous to admission, he had received three injections at home. A tentative diagnosis of myelosclerosis was made after a review of the clinical and hæmatological evidence and a sternal puncture. The anæmia began to improve rapidly without treatment, the hæmoglobin rising from under 20 to 40 per cent. within a few days. On further questioning he admitted having had injections, which his doctor later confirmed as anahæmin. Re-examination of the marrow films showed eventually a few hæmoglobinised megaloblasts confirming the anæmia as Addisonian. This case also illustrates the need for a wide search and the counting of many cells in compiling a myelogram. The peculiar grouping of the cells already discussed (p. 3) gives an erroneous picture when only a few fields are examined.

For the purposes of this book the anæmias can first be broadly classified into four chief groups—hæmorrhagic, hæmolytic, toxic and

dyshæmopoietic. In the following chapters the main changes from normal in the myelogram will be discussed in each group. Individual differences in different anæmias will be discussed in the lists given under each heading.

Hæmorrhagic Anæmia. Anæmia following hæmorrhage may be acute or chronic, depending on the amount, frequency, and duration of the bleeding. Acute anæmia is most commonly seen following trauma, bleeding from a peptic ulcer, or childbirth. Providing that treatment is adequate, the blood loss is replaced in about thirty days and this replacement is brought about by an increase in the normal activity of the bone marrow.

The chronic type, usually induced by long continued, frequent and relatively small bleedings, such as occur in menorrhagia and some types of peptic ulcer, produces characteristic changes in the myelogram. A study of these two types of marrow change helps us to interpret the findings in hæmorrhagic anæmias due to abnormalities of the blood or blood vessels, of which thrombocytopenic purpura and hæmophilia are examples. The special findings peculiar to these anæmias will be discussed later. Certainly the myelogram in traumatic anæmias does not help in diagnosis, either of the nature of the anæmia (which is usually obvious on clinical grounds) or of the site of the loss of blood.

Acute hæmorrhagic anæmia. The myelogram here shows increased normal activity. Both the erythroblastic and myeloid elements are affected, the former more than the latter. The hyperplasia of the myeloid marrow is reflected in the peripheral leucocytosis.

The normoblasts are increased in number and show a tendency to become hæmoglobinised at an earlier stage, *i.e.*, the usual basophilic normoblast becomes polychromatic, and the polychromatic one becomes eosinophilic. The number of reticulocytes present is markedly increased. Megakaryocytes are more frequent and may be seen in moderately large numbers in the thick end of the film. In the percentage count the polymorphs are decreased, but this decrease is only apparent because the total number of cells is increased. Mitosis is more frequent, indicating general hyperplasia. The mitoses, both of red and white cells, takes place at the normal level of cell development. The myelogram is as follows :—

Neutrophiles :	
Myelocytes	30–35 per cent.
Metamyelocytes	10–15 ,, ,,

Eosinophiles :	
Myelocytes	1– 2 per cent.
Polymorphs	1– 2 ,, ,,
Basophiles :	
Myelocytes	3– 6 ,, ,,
Polymorphs	1– 2 ,, ,,
Premyelocytes	1– 2 ,, ,,
Hæmocytoblasts (including myeloblasts)	2– 4 ,, ,,
Lymphocytes	6–12 ,, ,,
Pro-erythroblasts	1– 2 ,, ,,
Normoblasts	25–30 ,, ,,
Megakaryocytes	frequent

Chronic Post-hæmorrhagic Anæmia. When the anæmia is but moderate the bone-marrow activity is only slightly increased. In more severe cases there may be a great increase in the amount of red marrow. This hyperplasia may eventually be succeeded by exhaustion, and the marrow then becomes hypoplastic or aplastic. In a fully reacting case, *i.e.*, before marrow exhaustion supervenes, the most striking feature of the myelogram is the great increase in the number of normoblasts, many of which are large and strikingly basophilic. The whole picture is one of active erythropoiesis with numerous mitotic figures of normal type. The myelocytes are little, if at all, reduced in number, and the total number of cells is somewhat increased. Reticulocytes are fairly numerous though *not* as abundant as in acute post-hæmorrhagic anæmia. The erythrocytes tend to be microcytic and hypochromic. If iron therapy is instituted, there is no obvious increase in normoblastosis ; this being an indication that iron is needed for complete maturation of the cells, and not as a stimulus to erythropoiesis itself.

Neutrophiles :	
Myelocytes	20 –30 per cent.
Metamyelocytes	5 – 8 ,, ,,
Polymorphs	8 –12 ,, ,,
Eosinophiles :	
Myelocytes	1 – 2 ,, ,,
Polymorphs	1 – 2 ,, ,,
Basophiles :	
Myelocytes	½– 3 , ,
Polymorphs	½– 3 ,, ,,

Premyelocytes	1 – 3 per cent.
Myeloblasts	0 – 2 ,, ,,
Pro-erythroblasts	3 – 5 ,, ,,
Normoblasts	40 –50 ,, ,,
Megakaryocytes	moderate

Hæmorrhagic Anæmias. The specific marrow changes in *essential thrombocytopenic purpura* will be described in the chapter on aplasia of the bone marrow.

In true *hæmophilia*, apart from the changes described above, the marrow films show a marked increase in the number of megakaryoblasts and megakaryocytes, but the number of platelets is within normal limits. Custer and Krumbhaar (20) have described the appearance of the bone marrow in three fatal cases.

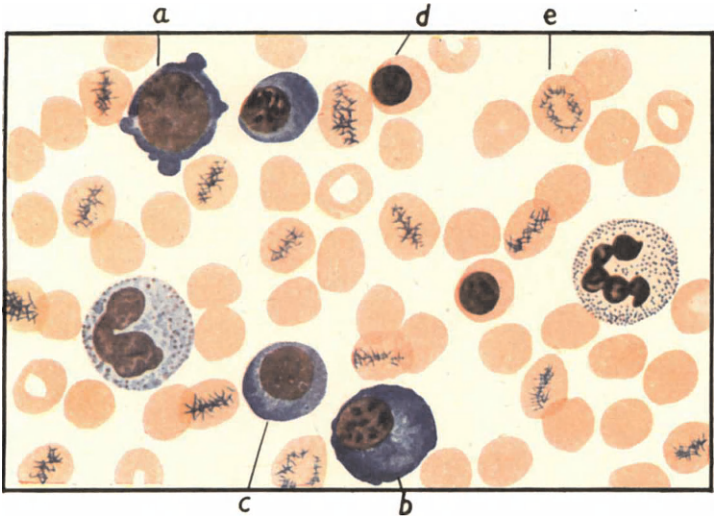
Hæmolytic Anæmia (Plate 7A). In all forms of hæmolytic anæmia the activity of the marrow is at its maximum. There is great and continuous normoblastosis which is presumably induced by loss of corpuscles, due to destruction in the body: but whether any erythropoietic stimulus is liberated by the destroyed cells is still uncertain, although probable.

The clinical state is not clearly, if at all, reflected in the marrow, which is in a condition of extreme activity throughout the course of the malady. About three-quarters of the cells in the marrow are nucleated red-cells in various stages of development; some are mature, but the majority are basophilic and possess large cart-wheel nuclei. Another striking feature is the presence of considerable numbers of cells as immature as the proerythroblast: these, as previously described, differ from megaloblasts but are at least as large, if not larger. In some cases a true megaloblastic reaction may develop as described by Fairley (13) in cases of Bartonella fever. The large number of reticulocytes is another characteristic feature.

The myelogram is of the type shown below :—

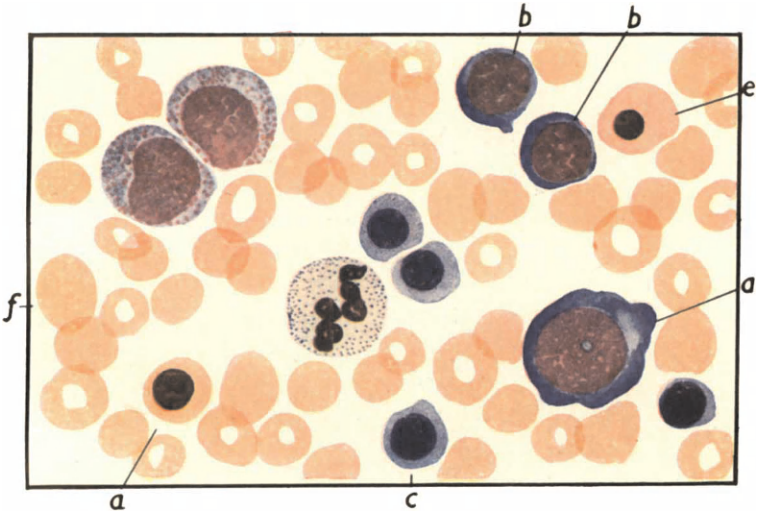
Neutrophiles :	
Myelocytes	10–17 per cent.
Metamyelocytes	2– 5 ,, ,,
Polymorphs	2– 5 ,, ,,
Eosinophiles :	
Myelocytes	0– 2 ,, ,,
Polymorphs	2– 5 ,, ,,
Lymphocytes	1– 2 ,, ,,

PLATE 7



A. HÆMOLYTIC ANÆMIA

(*a*) Pro-erythroblast.
(*b*) Early normoblast.
(*c*) Basophilic normoblast.
(*d*) Normoblast.
(*e*) Reticulocyte.



B. SPRUE

(*a*) Promegaloblast.
(*b*) Early normoblast.
(*c*) Basophilic normoblast.
(*d*) Normoblast.
(*e*) Eosinophilic megaloblast.
(*f*) Megalocyte.

[*To face p.* 30.

Monocytes	1– 2 per cent.
Premyelocytes	5–10 ,, ,,
Hæmohistioblasts	1– 3 ,, ,,
Hæmocytoblasts	1– 4 ,, ,,
Pro-erythroblasts	25–34 ,, ,,
Normoblasts	20–30 ,, ,,
Megaloblasts	0– 2 ,, ,,
Megakaryocytes	not increased

Reticulocytes numerous. May be as high as 80 per cent. of the non-nucleated elements of the films.

In many of the hæmolytic anæmias, the anæmia may be macrocytic in type. In all the groups one or more such cases are described in the literature. Apart from the true megaloblastic reaction in Bartonella fever, however, the characteristic feature of these marrow films is the high percentage of pro-erythroblasts. The macrocytosis is, therefore, probably due to rapid maturation of these cells, the nucleus being extruded rather early in the normoblastic cycle. That it is not due to megaloblastosis is borne out by the fact that liver preparations do not cause improvement or return of normal gastric function.

In the *infective group of hæmolytic anæmias*, the myelogram does not show such a high proportion of pro-erythroblasts, and the regeneration of red cells is not as a rule so intense. The hæmolysis of the blood is probably due to the infecting organism causing a " toxæmia " of the red cells which render them more liable to the normal process of destruction. This toxic action is also exerted on the bone marrow, which cannot then respond so rapidly. Where the infection is prolonged and the toxæmia extreme, the marrow fails to respond at all and becomes aplastic *erythronoclasia*). We have recently seen a case of this type due to tuberculosis, and sections of the bone marrow removed at autopsy showed a few minute tubercles.

Gas gangene infection (*Cl. Welchii*), and sometimes an anærobic hæmolytic streptococcus, produce a hæmolytic anæmia in a different way. They produce a hæmolysin which acts directly on the red cells. In these cases, until the toxæmia becomes severe, the marrow may be expected to react more exuberantly.

Malaria produces a chronic hæmolytic anæmia, the hæmolysis being caused by direct action of the parasite on the red corpuscles. Even in malignant tertian infection, where the hæmolysis may be so intense as to produce blackwater fever, and early death, the marrow

shows a well-marked erythroblastic hyperplasia with large, intensely basophilic, normoblastic cells (see Chapter IX).

Some hæmolytic anæmias are due to poisons : lead, potassium chlorate, benzedrine, phenylhydrazine, phosphorus, T.N.T., dinitrobenzene, etc. The reaction of the bone marrow to all these compounds is as that described by hæmolytic anæmias in general. Aplasia develops rarely, if ever, because the patient dies of some other toxic effect of the poison before the aplastic stage is reached, although a small number of workers with trinitrotoluene have died of aplastic anæmia. The sulphonamide group of drugs, however, may produce a hæmolytic anæmia, and, if still more is given, the marrow may become aplastic. In lead poisoning, of course, the characteristic basophilic stippling of the red cells will be seen in marrow films. The hæmolysis produced by incompatible transfusions and paroxysmal hæmoglobinuria causes no peculiar abnormalities in the bone marrow.

In *acholuric jaundice*, in both the congenital and acquired type, no new information about the pathology of the disease has been acquired by studying bone-marrow films. The spherocytosis can be seen as easily as in the peripheral blood. A constantly high reticulocyte count may be found in the marrow even when the red cell count is nearly normal. The reticulocyte count may be normal during a remission of the disease and the spherocytosis may temporarily disappear. Spherocytosis is not so marked in the acquired form of the disease.

In *sickle cell anæmia*, the latent phase is much more common than the active one. In the latent form, sickling of the cells is not found in the peripheral blood but has been found in the bone marrow of a case in this phase coming to autopsy. The presence of sickle cells is not diagnostic of anæmia, as the sickle cell trait has been found in 5·7 per cent. of all negroes, whether or not they are in good health. The phenomenon of sickling cannot be seen ordinarily in stained films. A fresh preparation sealed under a cover slip should be examined a few hours after it is made. The myelogram does not differ from that of any other form of hæmolytic anæmia except, of course, in the shape of the red corpuscles. Perhaps, however, the number of monocytes containing red corpuscles or pigment is rather greater, and Wintrobe (14) has recorded the presence of long bands of erythrocyte cytoplasm (about 2 microns thick) lying in marrow films.

In *Lederer's anæmia* the marrow picture may be complicated by

the presence of a leukæmoid reaction (Chapter III). This is usually myeloid in type, but a case has been described with the appearances of lymphatic leukæmia in the peripheral blood, including lymphoblasts.

Toxic Anæmias. This group contains the hypoplastic and aplastic anæmias. It is a rather heterogeneous group of maladies, which may be primary or secondary. If of the latter type, the cause may be poisoning of various kinds, infection or toxæmia. But, whatever the cause, the malady is a grave and dangerous one ; and, in all, the marrow picture is similar ; no indication of the cause can be inferred from the myelogram. The conditions are more fully described later with other aplastic conditions of the bone marrow.

Dyshæmopoietic Anæmias. The hæmopoietic bone marrow is a complicated and highly specialised structure which has to supply the formed elements of the blood continuously and rapidly. In order that it may do this efficiently, an adequate and properly balanced supply of nutriment is necessary. This has long been recognised, but our knowledge of the food requirements of the blood-forming tissue has, until the end of the first quarter of this century, been confined to one constituent, iron. Now we know that the hæmopoietic principle, copper and the metals, thyroxin, and vitamin C all play their part. Lack of, or failure to utilise, any of these factors may result in anæmia. As a result of malnutrition of the bone marrow, two main groups of anæmia are produced : (1) hypochromic and microcytic ; (2) hyperchromic and macrocytic. The first group arises with deficiency of iron, internal secretion of thyroid, and vitamin C. The second arises with deficiency of the hæmopoietic principle.

Deficiency of hœmopoietic principle.

Pernicious anæmia (Plates 8 and 9) is, of course, the best known anæmia of this group. An exactly similar condition of the blood and marrow may obtain in other anæmias of the same type, whether the cause be carcinoma of the stomach, complete gastrectomy, or other conditions giving rise to deficiency of the hæmopoietic principle.

The myelogram in pernicious anæmia is striking and characteristic. The constant feature is the presence of megaloblasts ; but it is rather on the large number of these cells than on their simple presence that diagnosis is to be based. As mentioned earlier, some writers aver that a few of these cells can be found, even in health ; it is, therefore, an increase that is significant. But there is another point : even if the megaloblast be not regarded as peculiar to pernicious anæmia,

there is no doubt that the marrow contains a larger number and far more young forms in this disease than are found in any other. It must not, however, be supposed that the whole of the erythropoietic process in this disease is megaloblastic in type; there is great normoblastic activity also: in other words, there is a mixed erythroblastic reaction of an intensity never seen in other maladies.

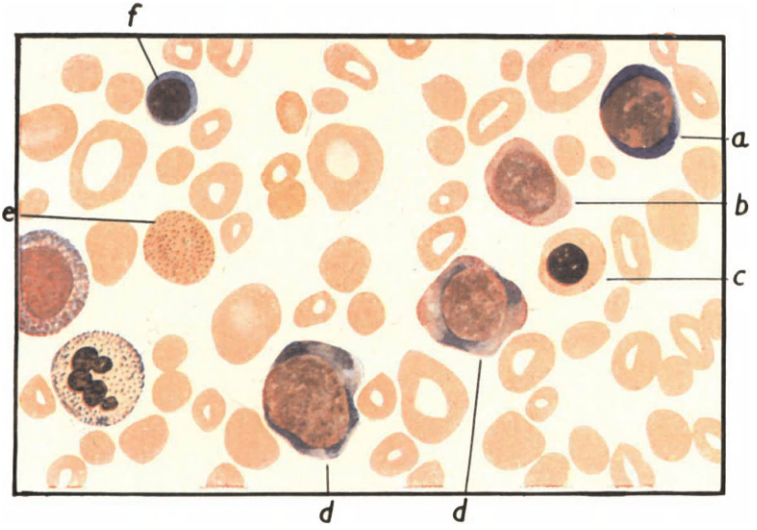
Little is known of the changes in very early cases, but Segerdahl (3) states that the characteristic blood picture can be found before there are any recognisable changes in the marrow. But in definite, and untreated cases, the myelogram is very striking, inasmuch as there are two changes: intense megaloblastosis, which, of course, is pathognomonic, and leucoblastic reaction of a non-specific kind.

It is surprising that the latter change should occur, because at this stage there is leucopenia with relative lymphocytosis in the blood. This may be due to some inhibition of emigration and also of proper maturation of the granular leucocytes. Certainly it is common to be able to detect slight abnormalities of both nuclei and cytoplasm in the neutrophiles, especially in the metamyelocyte stage (p. 5). Further, there is a considerable excess of hæmocytoblasts, which, of course, adds to the appearance of leucoblastic activity. In some long-standing, untreated, cases, there may be some depression of granulopoiesis.

The megaloblastosis is, naturally, the most significant change; and it is by progressive increase that we can recognise deterioration: by diminution, that we see that improvement is occurring: and by disappearance that complete relief is established. The cells are easily distinguished from normoblasts, but too much attention should not be paid to their size, which is very variable. The nuclear characters (p. 8) are the differential feature. These cells are found lying in groups, often surrounded by a clear area, devoid of cells, and they seem to develop more or less independently of all other marrow elements. Promegaloblasts are also present, and their number is some guide to prognosis. It is interesting to find that the number of these cells is closely related to that of the hæmocytoblasts; as the one decreases, so do the others. And it is reasonable to assume that they are closely related, and indeed, probably of the same series.

In an untreated case of moderate or great severity, the striking feature of the marrow film is that there is a general immaturity, and the marrow appears to be both hyperplastic and metaplastic, the latter appearance being due to the presence of so many hæmocytoblasts. Indeed, so many of the marrow cells are immature, that,

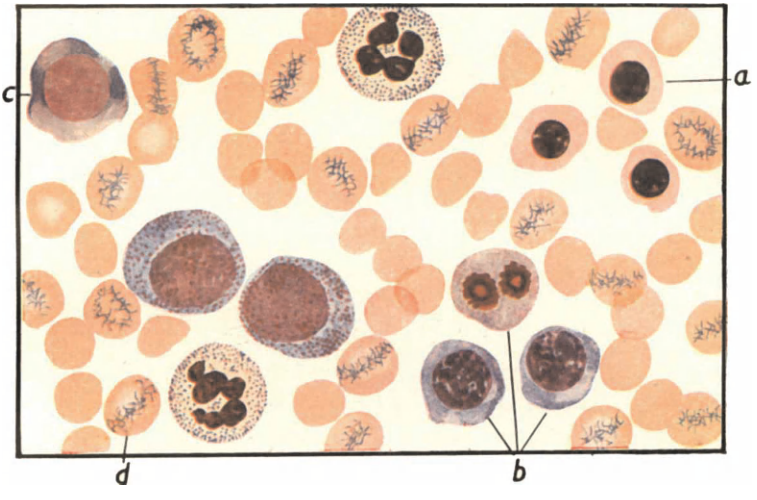
PLATE 8



A. Pernicious Anæmia (Untreated)

(*a*) Early megaloblast.
(*b*) Late megaloblast.
(*c*) Pyknotic megaloblast.
(*d*) Intermediate megaloblast.
(*e*) Stippled megalocyte.
(*f*) Lymphocyte.

Note.—Megalocytosis, poikilocytosis and anisocytosis.



B. Pernicious Anæmia (Early Stage of Treatment)

(*a*) Intermediate normoblast.
(*b*) Early normoblasts.
(*c*) Intermediate megaloblast.
(*d*) Reticulocyte.

Note.—Megalocytosis less marked than in A.

[*To face p.* 34.

with the ordinary hæmatological stains, the films have a strikingly blue colour, which, by itself, is very suggestive of pernicious anæmia. In myeloblastic leukæmia, the films are far less blue, because the myeloblast has a cytoplasm somewhat less basophilic than the hæmocytoblast and the promegaloblast.

Of course, the other signs of pernicious anæmia, to which we attach so much importance in the blood, can also be detected in the marrow: megalocytosis, anisocytosis, Jolly bodies, hyperchromia and polychromasia are always seen. If the marrow-fluid is stained with cresyl blue (see Appendix) before the films are made, there is a distinct though moderate increase in the number of reticulocytes.

Mitotic figures are often numerous, and so far as it is possible to be sure, they occur mainly, or perhaps entirely, in the megaloblasts. But the number of atypical mitoses is always great: some are pyknotic, some multipolar, and others even more grossly irregular. This is pathognomonic of pernicious anæmia; and is never seen in the normoblastic mitoses that may be numerous in post-hæmorrhagic anæmia during the phase of regeneration.

The myelogram is of the following type:—

Neutrophiles:	
Myelocytes	12–20 per cent.
Metamyelocytes	2– ,, ,,
Polymorphs	–12 ,, ,,
Eosinophiles:	
Myelocytes	0– 2 ,, ,,
Polymorphs	0– 2 ,, ,,
Basophiles	0– 1 ,, ,,
Lymphocytes	1– 3 ,, ,,
Monocytes	1– 3 ,, ,,
Hæmohistioblasts	1– 5 ,, ,,
Hæmocytoblasts	–10 ,, ,,
Promegaloblasts	10–15 ,, ,,
Megaloblasts	25–40 ,, ,,
Normoblasts	20–25 ,, ,,
Megakaryocytes	very scanty.
Reticulocytes: from 10 per cent. of the non-nucleated elements.	

In the phases of remission, induced by liver treatment, a series of significant changes can be detected. These start about twenty-four hours after intramuscular injection of a potent liver extract; and

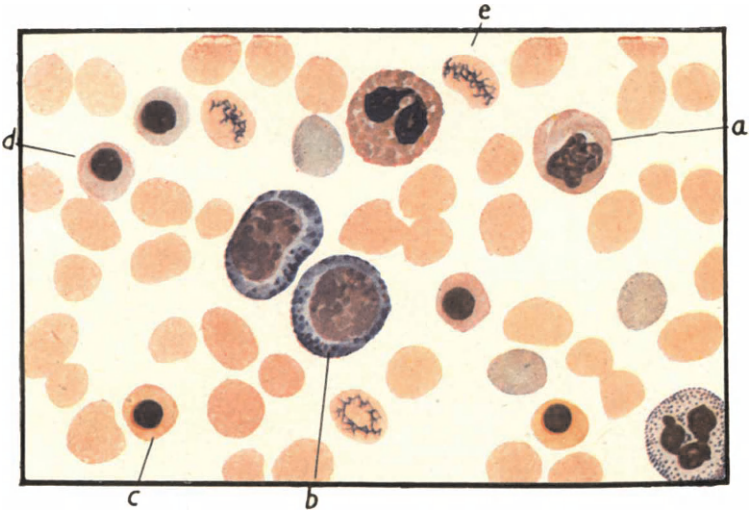
the first change to be detected is increase in the number of reticulocytes. After 4 c.cm. of Neo-Hepatex, we have seen the reticulocytes rise from 8 per cent. of the total non-nucleated elements to 39 per cent. twenty-seven hours after the injection. Within two days there are signs of a change in type of the erythropoiesis : the intense megaloblastosis is beginning to die down and to be replaced by normoblastosis. The percentage of the latter cells may rise from twenty-two to thirty-seven in fifty hours. Even four to five days after treatment has commenced, the marrow picture is still characteristic of pernicious anæmia. About half of the normoblasts are large and have an intensely basophilic cytoplasm : these are of the early and basophil type of normoblast. The remaining half are composed of equal numbers of polychromatic and eosinophil normoblasts of normal size. Mitosis is still intense, but is now confined almost entirely to the large basophil early normoblasts. Promegaloblasts and basophil megaloblasts are still present in moderate numbers and are easily recognised by their nuclear characters. Very few of them show mitosis. Polychromatic and eosinophil megaloblasts are conspicuous only by their absence. A few megakaryocytes can now be found in the thick end of the film. The activity of the myeloid tissue does not show much change, but in some cases there may be a marked increase in the numbers of eosinophile myelocytes and polymorphs. This may be reflected in the peripheral blood picture.

It is not at all clear by what means the change in erythropoiesis comes about, but it is probable that it is due to a stimulus to normal maturation of young normoblasts, rather than to fresh formation of cells. The intense karyokinesis seen in these cells supports this supposition, and the failure of hæmoglobinisation of the megaloblastic cells shows that they are no longer required as oxygen carriers.

The myelogram is of the following type :—

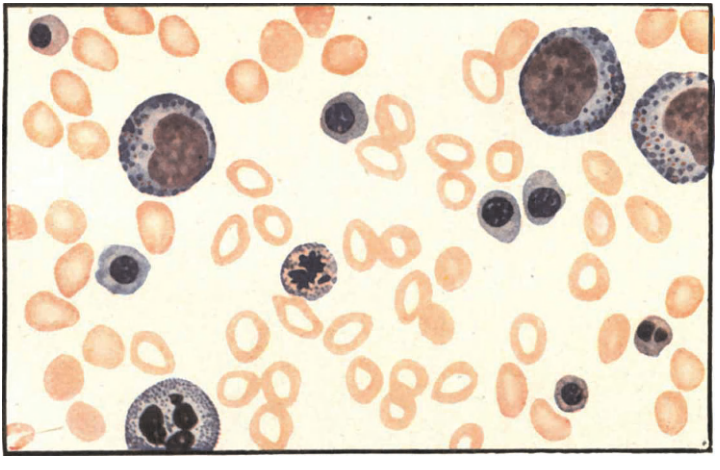
Neutrophiles :	
Myelocytes	10–20 per cent.
Metamyelocytes	2– ,, ,,
Polymorphs	5–15 ,, ,,
Eosinophiles :	
Myelocytes	2–15 ,, ,,
Polymorphs	2–15 ,, ,,
Basophiles	0– 1 ,, ,,

PLATE 9



A. Pernicious Anæmia (Late Stage in Treatment)

(*a*) Late megaloblast. (*c*) Normoblast.
(*b*) Myelocyte. (*d*) Polychromatic normoblast.
(*e*) Reticulocyte.



B. Idiopathic Hypochromic Anæmia

Note.—Numerous basophilic normoblasts, and hypochromic microcytosis.

[*To face p.* 36.

Lymphocytes	1– 3 per cent.
Monocytes	1– 3 ,, ,,
Hæmohistioblasts	0– 2 ,, ,,
Hæmocytoblasts	2– 5 ,, ,,
Promegaloblasts	5– 8 ,, ,,
Basophilic megaloblasts	10–20 ,, ,,
Macronormoblasts (basophilic)	20–40 ,, ,,
Normoblasts (late)	20–25 ,, ,,

Megakaryocytes—moderate numbers at ends of film.
Reticulocytes : up to 50 per cent. of the non-nucleated elements.
Erythrocytes : still show megalocytosis, some hyperchromia, anisocytosis and poikilocytosis.

In the late stages of treatment and in the stage of remission during continued treatment, the marrow film is rather uninteresting (Plate 9, A). The megaloblastosis has disappeared, although a very occasional megaloblast may be found before the anæmia has completely disappeared. The myeloid elements form a normal proportion of the film. Normoblasts are present in normal proportions, but there is a tendency for them to become hæmoglobinised earlier, and for the nucleus to be discarded while the cell is still large. This phenomenon probably accounts for the macrocytosis, which still persists in treated cases of this disease. We have already seen that this mechanism may account for some of the macrocytic hæmolytic anæmias, and it may be that there is still some further factor concerned with normoblastic development with which we are not yet acquainted. Reticulocytes in fully treated cases number about 3 per cent. in bone-marrow films.

The practical purpose of sternal puncture in pernicious anæmia is not usually that of early diagnosis ; however, in cases which have become hypoplastic, and have an atypical blood picture, marrow puncture is diagnostic. As has been said, in ordinary cases, blood changes precede detectable alterations in the marrow, but signs of relapse occur in the marrow before they can be appreciated in the blood ; they naturally take the form of megaloblastic increase at the expense of the normoblasts. Nevertheless, the most important purpose of marrow examination in this malady is the understanding of the morphological changes that characterise the disease ; no other method has cast so much light into the dark places that surround the erythropoietic process as a whole.

There are three other diseases in which an anæmia indistinguish-

able from pernicious anæmia may occur. These are intestinal infestation with *diphyllobothrium latum ; tropical nutritional anæmia*, due to deficiencies in the diet ; and the *macrocytic anæmia of pregnancy*. In this latter condition the anæmia is probably dependent on two factors ; partial failure of production of hæmopoietic principle owing to temporary derangement of the gastric function, and the necessity of supplying the principle to the fœtal liver. The macrocytic anæmias associated with disease of the liver are of the same type as pernicious anæmia, but are rarely severe and will react to liver therapy. If cirrhosis of the liver gives rise to large gastric hæmorrhage, the marrow picture will be complicated by a normoblastosis and leucoblastosis as in the hæmorrhagic anæmias.

Anæmia associated with lesions of the alimentary canal is rarely due solely to lack of hæmopoietic principle. The conditions here fall into the following groups :—

(1) Steatorrhœa (including sprue) (Plate 7, B).
(2) Diarrhœa (chronic bacillary dysentery and pellagra).
(3) Carcinoma of the stomach.
(4) Gastric operations.
(5) Intestinal stenosis.

In all the conditions, anæmia may be due to lack of hæmopoietic principle or iron, or commonly a combination of both. With the exception of carcinoma of the stomach and the operation of gastrectomy, the deficiency of both factors is due to failure of absorption. In these two conditions, a megalocytic anæmia, if it develops, is due to lack of the intrinsic factor ; the amount of hæmopoietic principle available depends, of course, on the site of the carcinoma or the part of the stomach removed. The most common anæmia with alimentary disease is certainly a hypochromic microcytic one associated with failure to absorb iron. The myelogram will be fully discussed later. In the combined deficiency, the marrow-megaloblastosis is present with the changes due to lack of iron. In these cases marrow puncture may give indications of great therapeutic value in showing that both iron and liver are necessary for adequate treatment. Quite often the peripheral blood shows only the macrocytosis, which is treated with liver ; only when the hæmoglobin becomes constant at a relatively low figure with a low colour index does the need for iron become apparent. Both therapeutic agents could have been given at once if the need for them had been recognised at first.

Anæmia in Gastric Carcinoma. Currie (15) states that the marrow condition may be : megaloblastic, as in pernicious anæmia ; normoblastic, as in any ordinary hypochromic anæmia ; and erythroblastic.

The last-named has not been clearly described before. It appears that the marrow is very cellular and active, with many mitoses. The predominant cells are macroblasts, *i.e.*, large, basophilic normoblasts. They may be as large as megaloblasts, but have a definitely " cart-wheel " arrangement of the nuclear chromatin. The anæmia in patients with this type of marrow is more severe than in those with a megaloblastic reaction.

Currie is of the opinion that the type of marrow reaction is not related to the severity of the gastric lesion ; and he also says that there is no obvious correlation between the apparent degree of deficiency of specific hæmopoietic substance and the type of reaction of the erythron. " Deficiency of specific hæmopoietic substance *per se* does not appear to result in a megaloblastic marrow reaction and a megalocytic anæmia."

Deficiency of Iron. This results in chiefly the malady known as idiopathic hypochromic anæmia (Plate 9, B), so clearly depicted by Witts, the nutritional anæmias associated with infancy, the anæmia associated with alimentary disease, and some anæmias of pregnancy. Where the anæmia is solely due to deficiency of iron, the blood shows no spectacular changes : and there are never very many normoblasts. The marrow, however, is characterised by a great preponderance of normoblasts, many of which are large, immature, and strikingly basophilic. The whole picture is one of very active erythropoiesis, and mitotic figures are numerous : these are unlike those seen in the megaloblasts of pernicious anæmia, inasmuch as they are all typical : abnormal karyokinetic figures are never seen.

Neutrophiles :	
Myelocytes	17–22 per cent.
Metamyelocytes	5– 8 ,, ,,
Polymorphs	8–12 ,, ,,
Eosinophiles :	
Myelocytes	2– 4 ,, ,,
Polymorphs	2– 4 ,, ,,
Premyelocytes	1– 3 ,, ,,
Myeloblasts	0– 2 ,, ,,
Pro-erythroblasts	8–10 ,, ,,

Normoblasts (basophilic) . . .	20–30 per cent.
Normoblasts (eosinophilic) . . .	20–30 „ „
Megakaryocytes	scanty.

Reticulocytes : up to about 6 per cent. of the non-nucleated elements.

The activity of the myeloid tissue is somewhat reduced, with a consequent reduction in the number of myelocytes : this in marked distinction to the microcytic hypochromic anæmia produced by hæmorrhage. The eosinophiles, however, remain normal in numbers or may show a slight increase.

The myelogram has the characters shown above, which, of course, vary considerably as the result of treatment : and as so many patients have been given some iron before they are properly investigated, characteristic pictures are uncommon.

During, and even after, successful treatment with iron, the eosinophiles in the marrow increase considerably, although there may be no noteworthy excess in the blood. In pernicious anæmia, as we have seen, eosinophilia may develop in the marrow and in the blood, but this is usually a sign of overdosage with liver (which is, of course, quite innocuous) : but it is interesting to note that eosinophilia is a condition that develops during recovery from several kinds of anæmia.

As in pernicious anæmia, one of the most striking features of the marrow during the phase of recovery is the increase in the number of reticulocytes. These may increase to form 20–30 per cent. of the total non-nucleated elements. There is, however, one difference between the two diseases. In pernicious anæmia the reticulocyte response is high if the red cell count is low, and low if the red cell count is high ; in hypochromic microcytic anæmia the reticulocyte response depends more on the hæmoglobin level. If this is moderately high, the response is poor, and *vice versa*. In both maladies, the reticulocyte response to therapy is extremely rapid.

Other changes in the marrow during the period of recovery are a gradual restoration of the normal number of neutrophiles and a steady drop in the number of normoblasts : the percentage of basophil normoblasts drops more rapidly, till, when the anæmia is abolished, they and the eosinophil normoblast have reached a normal numerical relationship.

The iron deficiency anæmias of pregnancy are similar to idiopathic hypochromic anæmia in blood and marrow. The nutritional

anæmias of infancy show the same changes in the peripheral blood, but here, of course, sternal puncture is not possible.

Deficiency of Vitamin C. Anæmia is not a constant finding in scurvy, but is commoner in adults than in children. The anæmia is normochromic or hypochromic and is cured by the administration of vitamin C, whereas iron has no effect on it. In view of the findings in the bone marrow, vitamin C is now regarded as a general stimulus for hæmopoiesis. No one element in the marrow seems to be particularly affected, but all the elements undergo a slow degeneration which may eventually result in complete aplasia. The proportions of the different marrow constituents remain unaltered and only the total number of nucleated elements becomes smaller. During treatment with vitamin C, reticulocytosis develops.

If there has been much hæmorrhage into the tissues with the disease, the peripheral blood may show a microcytic anæmia. The marrow, however, is unable to respond in the usual way, and the myelogram of hæmorrhagic anæmia does not develop until vitamin C has been given. A few cases of macrocytic anæmia with scurvy have been reported, but the sternal marrow findings are not recorded.

Deficiency of Thyroxin. The exact relationship of thyroxin to the hæmopoietic tissues is not yet worked out, but it is probable that it acts as a general stimulant, like vitamin C, rather than on any particular hæmopoietic tissue.

In myxœdema, if uncomplicated by deficiency of iron and of hæmopoietic principle, the bone marrow shows simple hypoplasia. In thyrotoxicosis there is a general hyperplasia of the bone marrow which may affect the myeloid elements to a greater degree than the erythroblastic tissue.

CHAPTER VI

ERYTHRÆMIA AND ALLIED STATES

THIS is a particularly difficult group of diseases to describe from the point of view of marrow changes, because there are no clearly defined syndromes. All that is certain is that the most significant and most constant changes are those in the bone marrow. Enlargement of the spleen and liver may or may not occur, and, even when it does, the increase in size of these organs is mainly due to increased hæmolytic activity, not to blood formation.

In every type of erythrocytosis or erythræmia the marrow shows increase in the number of cells and signs of exaggerated activity affecting both the white and the red elements. Usually there is only little alteration in the percentage composition of the myelogram, the only striking change being absence of lymphocytes and an unexplained excess of megakaryocytes, which are usually mature in structure.

The group of cases that has been called erythro-leukæmia is particularly difficult, because it seems certain that this is not a malady *sui generis* but represents a whole group of rather heterogeneous conditions. Characteristically, the blood shows considerable increase in the number of red corpuscles, together with leucocytosis, and in an advanced stage the blood picture may become frankly leukæmic. At that stage, the erythrocyte increase is usually replaced by anæmia, and the predominant white cell is usually the myeloblast.

The pathogenesis of this disease is extremely obscure. It seems certain that the marrow is not necessarily, or indeed we think commonly, the site of the original hæmatopoietic overgrowth. Changes in the liver and spleen usually precede those in the marrow, and indeed, remain the most striking throughout the course of the disease.

The spleen, which is always enlarged, often to a very great size, shows evidence of myeloid metaplasia, in which the red cells greatly outnumber the leucocytes. In spite of this, the appearance is totally unlike that found in infective conditions, mainly because there is a great increase in the number of megakaryocytes, which may form 3 or 4 per cent. of the nucleated elements. The liver is also enlarged but never becomes enormous. In it there is always

myeloid metaplasia, but the appearances and the percentage composition are different from those in the spleen. In the latter, the predominant type of red cell is small and normoblastic, but in the liver there are large numbers of big red cells, which some writers regard as being genuine megaloblasts but which may well be macroblasts or even pro-erythroblasts. There is also some leucopoiesis in the liver, but this is not intimately mixed with the immature red cells.

The sternal bone-marrow may appear normal during the greater part of the disease, only becoming involved in the terminal stage. It is important to remember this, for it shows very clearly that it is never safe to assume that, because the marrow is normal, we are not dealing with a disease of the hæmopoietic tissues. It has long been known that the blood may still appear healthy at a time when the formative organs are already abnormal, but it is less widely recognised that the bone marrow may appear healthy at a time when the blood already shows signs of disorder.

What little evidence is available seems to suggest that erythroleukæmia is a special mode of reaction to some unknown noxious agent, that is to say, it is a form of leukæmoid reaction, but there is no doubt that it usually ends as a typical acute leukæmia.

The myelogram is of the following type :—

Neutrophiles :	
Myelocytes	10–18 per cent.
Metamyelocytes	5– 7 ,, ,,
Polymorphs	35–42 ,, ,,
Eosinophiles	2– 4 ,, ,,
Lymphocytes	1– 2 ,, ,,
Monocytes	8–12 ,, ,,
Premyelocytes	0– 1 ,, ,,
Hæmocytoblasts	1– 3 ,, ,,
Normoblasts	12–18 ,, ,,
Megaloblasts	1– 3 ,, ,,

CHAPTER VII

INFECTIVE DISEASES

LEUCOCYTE counts have been of considerable value in medicine and surgery as well as in more purely academic studies of immunity. The blood changes in infective diseases are relatively well known, but the marrow findings have not yet been investigated in much detail.

The types of changes that may occur can be classified as follows :—

(1) Normal marrow with no alteration in the percentage composition.

(2) Hyperplastic marrow with predominance of mature and almost mature neutrophiles.

(3) Hyperplastic marrow with many immature neutrophiles, mainly myelocytes.

(4) Extremely immature marrow in which premyelocytes are numerous or even prominent.

(5) Myeloblastic marrow.

An alternative classification suggested by Barta (16) was :—

(1) Moderate reaction with plentiful cells.

(2) Intense reaction with immature cells including many myelocytes.

(3) Extreme reaction with many premyelocytes or even myeloblasts, and

(4) Failure of reaction with decrease of granular leucocytes.

No rigid classification is possible because there is a continuous series of gradations between the different types of marrow. All that can profitably be done is to call attention to the outstanding characters of the myelogram.

As in the blood, so in the marrow, the picture is not pathognomonic of a particular infection. The myelogram, like the hæmogram, gives an indication of the intensity of reaction but not of the underlying cause. In a few infections it is alleged that there are pathognomonic changes ; for example, in both lobar and bronchopneumonia there is a greater degree of variation in the size of the granulocytes than in health, but this anisocytosis is not visible in the blood, and further observation has shown that it occurs in other infections also.

Scarlatina. In this disease there is hæmic leucocytosis, and, except in the most severe cases, eosinophilia. The marrow shows a corresponding activity in the production of both neutrophils and eosinophils, but the latter are always more prominent in the myelogram than in the blood. During the first fortnight of the illness, erythropoiesis is depressed. The neutrophilia in the bone marrow soon disappears after the fever ceases, and the eosinophiles also fall, but the latter elements increase again about the twenty-second day. This secondary rise is not peculiar to scarlatina but is the underlying cause of that post-infective eosinophilia which occurs in so many maladies. At about the same time as the secondary marrow-eosinophilia, there is an erythroblastic reaction which soon restores the number of corpuscles in the blood to normal.

German Measles. In the peripheral blood there is a considerable number of plasma and Türk cells. Details of these will be found in Moeschlin's paper. He states that, in rubella, punctures of lymphatic glands show the presence of considerable numbers of large primitive cells with reticular nuclei, in which nucleoli are present. He calls these elements plasmoblasts, and states that a complete transitional series between them and the typical plasma cells of the blood can be found. The highest number of plasmoblasts is found on the second day of the illness, but they reach their maximum in the blood about the fifth day. The marrow shows no significant increase of plasmoblasts or plasma cells.

Pneumonia. The essential feature is hyperplasia, mainly due to neutrophiles in all stages of development. As in the neutrophiles of the blood, those of the marrow may show degenerative changes such as nuclear pyknosis, absence of granules, and vacuolation of the cytoplasm, and there seems to be a direct relation between the intensity of such changes in the narrow cells and the severity of the illness.

In very severe cases, partial failure of reaction is common, but even more striking than this is proliferation of reticulo-endothelial cells in the marrow and the appearance of many elements resembling myeloblasts.

Glandular Fever. The clinical and hæmatological features of glandular fever (infectious mononucleosis) are now fairly well known, but cases of this kind are still sometimes mistaken for acute leukæmia. The Paul-Bunnell test is available, but a negative result does not exclude the diagnosis, and the blood picture may be extraordinarily difficult to interpret. For this reason, cases with

high fever and much enlargement of glands and spleen may arouse fear of acute leukæmia.

The myelogram is of a peculiar type inasmuch as there is little or no hyperplasia of the tissue as a whole ; indeed, it may be slightly hypoplastic. There is no real resemblance to leukæmic marrow. The picture is not greatly disturbed, but plasma cells and monocytoid elements are seen in every field. These latter cells are even less like monocytes than are the glandular-fever cells found in the blood in this disease. In the marrow they may exhibit every kind of cytoplasm from the most intensely basophilic to that which has no affinity for any dyes at all ; and the nuclear characters also vary very greatly from the most immature, with nucleoli, to those with the most bizarre and extraordinary convolutions.

Marrow puncture can thus easily exclude acute leukæmia.

In some cases of glandular fever, a cutaneous eruption, resembling that of german measles, is seen, but here again marrow puncture will prevent confusion. We have already seen that in german measles there is great excess of plasma cells in the lymphatic glands, whereas the sternal marrow contains few. In glandular fever the reverse is the case, for the marrow contains large numbers of plasma cells or, at least, elements resembling them, while the glands contain many monocytoid elements but few plasma cells. This is one of the reasons on which Moeschlin (2) bases his view that gland and marrow plasma cells are totally distinct from one another both in structure and in origin.

A typical myelogram is of the following type :—

Neutrophiles :	
Myelocytes	1– 2 per cent.
Metamyelocytes	1– 2 ,, ,,
Polymorphs	10–15 ,, ,,
Lymphocytes :	
Small	6–10 ,, ,,
Large	5– 8 ,, ,,
Monocytes	15–20 ,, ,,
Plasma cells	3– 6 ,, ,,
Turk cells	2– 4 ,, ,,
Pro-erythroblasts	2– 4 ,, ,,
Normoblasts	25–35 ,, ,,
Megaloblasts	1– 3 ,, ,,
Megakaryocytes	scanty, but always present.

CHAPTER VIII

HYPOPLASIA AND APLASIA OF THE BONE MARROW

APLASIA of the bone marrow may arise from a variety of causes, ranging from exhaustion, to poisoning or to lack of specific nutritional factors. A heterogeneous group of diseases is involved including anæmia, agranulocytosis, and purpura, or a combination of all three, according to which part of the hæmopoietic tissue is involved. Sternal puncture has been of great use in elucidating the pathology of these conditions and has shown that the peripheral blood may give a very inaccurate reflection of the conditions in the hæmopoietic bone marrow. This tissue may be truly aplastic with failure of division of the primitive cells (primary aplasia), or there may be normal division of the precursor cells resulting in a very cellular marrow, which, owing to lack of some maturation factor, is unable to furnish finished elements to the blood stream (maturation type).

Whitby and Britton (17) have made an excellent classification of these diseases based on the marrow findings and the separate or in combination affection of the erythroblastic, leucoblastic, and thromboblastic tissues :—

1. Aplasia of the erythroblastic tissue.
(*a*) Primary—pure red cell anæmia. The authors have found only five cases in the literature.
(*b*) Maturation defect—dyshæmopoietic anæmias may become aplastic if the maturation factor is never supplied.

2. Aplasia of the leucoblastic tissue.
(*a*) Primary—agranulocytic angina.
(*b*) Maturation defect—agranulocytic angina with marked myeloid reaction in the marrow. These cases respond more readily to pentnucleotide therapy, which possibly supplies some maturation factor.

3. Aplasia of the thromboblastic tissue.
(*a*) Primary—essential thrombocytopenic purpura. An acute form in which there is no rise of platelets after splenectomy.
(*b*) Maturation defect—essential thrombocytopenia. Splenectomy is probably beneficial in this type by removing a factor which inhibits platelet maturation.

4. In combination. Aplastic anæmia.

(*a*) Primary—produced by the action of sepsis, toxins, irradiation, or poisons on the hæmopoietic tissue. May be idiopathic.

(*b*) Maturation defect—the aplastic stage of anæmias due to maturation deficiencies. Two of the hæmopoietic issues only may be involved as in benzol poisoning.

APLASTIC ANÆMIA. The sternal (marrow) films may not be very revealing in this disease. At first the marrow of the long bones is affected, the red marrow being replaced by yellow fatty marrow. The blood picture may show marked changes by this time, but as the bone marrow of the vertebræ, ribs and sternum, is only partly, if at all, hypoplastic, the myelogram may be normal; however, this normality coupled with a normocytic anæmia may suggest the correct diagnosis.

When changes are present, the most obvious is a reduction in the number of nucleated elements. These may be so few as to suggest that the film is one of peripheral blood and not marrow at all. Usually either the erythropoietic or leucopoietic tissue appears to be mainly affected, and megakaryocytes are absent. These variations may be reflected in the clinical picture where purpura and angina may accompany the anæmia. Many of the normoblasts, sometimes the majority, are small and of unusual shape, and the nuclei tend to be more pyknotic and pleomorphic. Aplasia of the myeloid tissue is shown by the degenerate and aged appearance of the polymorphonuclears and the paucity of myelocytes. There is an increase in the percentage of lymphocytes, although this may be only apparent and not absolute. Reticulocytes may be found after careful search, but usually number less than 0·5 per cent. If, as sometimes occurs, particularly in the idiopathic form, there is some spontaneous remission, this is preceded by changes in the myelogram, which then more closely resembles that of the actively regenerating marrow found in other anæmias. It is, however, important not to base a good prognosis on such marrow changes, because they are very likely to regress: and indeed, no idea of the ultimate prognosis can be obtained from the myelogram at any stage of the malady.

The myelogram is of the following kind :—

Neutrophiles :	
Myelocytes	8–12 per cent.
Metamyelocytes	10–15 ,, ,,
Polymorphs	10–15 ,, ,,

Eosinophiles :	
Myelocytes	0– ½ per cent.
Polymorphs	0– 2 ,, ,,
Lymphocytes :	
Small	9–14 ,, ,,
Large	30–35 ,, ,,
Monocytes	2– 5 ,, ,,
Plasma cells	1– 3 ,, ,,
Normoblasts	10–12 ,, ,,
Megaloblasts	0– 1 ,, ,,
Megakaryocytes	absent.

Reticulocytes : less than ½ per cent. of the non-nucleated elements.

A remarkable and quite unexplained finding is that of an excess of plasma cells in many of these cases ; but there is neither diagnostic nor significance to be attached to them. Lymphocytes are the predominant cells in most cases of this type of aplastic anæmia ; and there is also a considerable excess of monocytoid elements, many of which are obviously phagocytic. But it is always difficult, and sometimes impossible, to classify accurately lymphocytes, monocytes and pathologically altered myelocytes. Davidson calls these unclassifiable elements " Q " cells. If myeloblasts are present they are always abnormal, commonly they have indented nuclei which may even be lobulated (Rieder type).

Any of the agents that can cause aplastic anæmia, *e.g*, benzol, arsenobenzene, thorium, the more modern sulpha compounds, X-rays, etc., may in smaller concentrations (or after less prolonged exposure) cause a less severe hypoplastic anæmia. It is unfortunate that, although such stages are, at first at least, less grave, they tend to deteriorate, and recovery is very uncommon. All these substances tend to attack the leucoblastic tissues first and the erythroblastic tissues later. X-rays behave in the same way. With sulphapyridine the effect on the leucoblastic tissue may be so severe that death ensues before any anæmia makes itself apparent, and these cases are often classed as agranulocytosis. Benzol commonly attacks the leucoblastic tissue first, but cases have been recorded in which the platelets were first affected. Even when there is a marked leucopenia, due to benzol, there may be some excess of eosinophiles both in the blood and in the marrow.

It is important to realise that the myelogram can give us very early information in cases of suspected poisoning of the bone marrow with

these compounds. The number of people working with benzol has increased enormously during the years of the war, and we have had the opportunity of examining large numbers of these. In some workers, showing the typical clinical syndrome of benzol poisoning, the hæmatological findings have proved negative. Sternal puncture, however, may show definite alteration in the hæmopoietic tissue. The myelogram quoted below was found in a female benzol worker, aged thirty-three, who had complained of dizziness, lassitude and menorrhagia for some four weeks. Her blood count showed a moderate microcytic hypochromic anæmia (Hb. 68 per cent., R.B.C. 4,000,000, C.I. 0·85). The white blood count was 5,500 per cubic centimetre and the differential-polymorphs 61 per cent, lymphocytes 32 per cent, monocytes 4 per cent., eosinophiles 3 per cent. The myelogram was as follows :—

Neutrophiles :	
Myelocytes	14 per cent.
Metamyelocytes	10 ,, ,,
Polymorphs	23 ,, ,,
Eosinophiles :	
Myelocytes	1·5 ,, ,,
Polymorphs	1·0 ,, ,,
Lymphocytes	2·5 ,, ,,
Monocytes	4·5 ,, ,,
Myeloblasts	2·5 ,, ,,
Normoblasts	17 ,, ,,
Megakaryocytes	very scanty.

Reticulocytes : 1·5 per cent of the non-nucleated elements.

Here we see at once some depression in the myeloid tissue. Normoblasts are present in normal numbers, but not in such large quantities as one would expect if the anæmia were due to iron deficiency. The low reticulocyte count is striking. The number of lymphocytes is raised, and this point brings one to an interesting speculation. We know that it is not possible at the moment to determine the activity of the bone marrow by doing cell counts on the sternal marrow-fluid ; but we may assume that if there is no absolute lymphocytosis or lymphopenia in the peripheral blood, that an increase in the percentage of lymphocytes in the bone marrow is only a relative increase, and that there is no alteration in their absolute number. Then, an increase in the percentage of lympho-

cytes can be used as an index of the decrease in the amount of the other elements of the marrow. In the case quoted above, the absolute number of lymphocytes in the peripheral blood is within normal limits (1,820); in the marrow, however, the lymphocytes are increased one-and-a-half to two times. It would therefore seem reasonable to assume that the myeloid and erythroblastic elements are reduced to the same extent, as there is no valid reason to account for an absolute increase of lymphocytes in the bone marrow. Whether this inference is admissible, the other observation that this marrow showed a general depression compatible with benzol poisoning, before any typical changes in the peripheral blood were noted, is of diagnostic value.

One further case may be quoted where changes in the sternal marrow were detected before they appeared in the blood. A male, aged forty-eight, suffering from lobar pneumonia, was being treated with sulphapyridine. His blood count was done after he had had 15 gms. and showed Hb. 85 per cent., R.B.C. 4,240,000, C.I. 1·0, W.B.C. 1,000. A differential white-cell count was not done, but no polymorphonuclears were seen in the film. Sternal puncture was performed with the object of showing the leucopenia to be either of the primary or maturation type: if the latter, it was hoped that pentnucleotide therapy would be of value. The myelogram corresponded to the one already described, except that the normoblasts numbered only 12 per cent. The lymphocytes were 25 per cent. The polymorphs were very degenerate, and there were only 10 per cent. of myelocytes. Here we had a primary aplasia of both the leucoblastic and erythroblastic tissue, the latter not yet being apparent in the blood. Two days later the hæmoglobin and the red cell counts began to fall and the patient died in spite of repeated transfusions with fresh blood.

One more point remains to be mentioned in connection with this group of marrow poisons. It would seem that only susceptible persons are affected. Most cases of fatal poisoning with benzol have had no relation to the length of exposure or the total amount absorbed. Similarly, poisoning with sulphapyridine and the allied drugs occurs mostly with people who have not had a full dose.

When marrow hypoplasia is due to infection, there may be a picture identical with that seen in cases due to poisoning; but more often there is excessive leucopoietic activity associated with the defect of red cell formation. Of course, typhoid fever, which is always accompanied by leucopenia and relative lymphocytosis is

here an interesting subject of study. But, strangely enough, the myelogram is scarcely that of hypoplasia, and the red cells are affected more than the leucocytes. It is also strange that eosinophiles are present, sometimes in excessive numbers, in the marrow, although they have disappeared from the blood. Of course, during convalescence there is intense normoblastic activity ; but at no time is there a myelogram that is suggestive of typhoid fever.

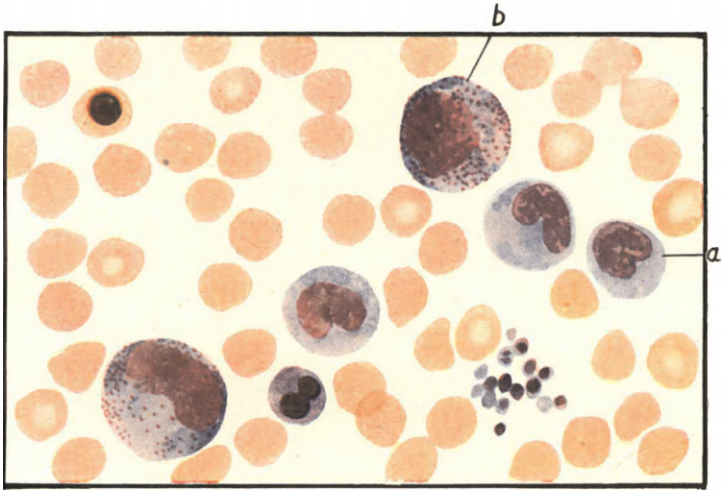
In the aplasias so far discussed, all the tissues of the marrow have been to some extent affected. It now remains to describe those conditions in which only one of these elements is affected.

Pure erythrocytic anæmia (aplastic) is a rare condition, and very few cases are recorded. Sternal puncture does not appear to have been done, but the changes in the bone marrow at post-mortem are described and will, of course, be comparable with the sternal puncture findings during life. Characteristically, there are no erythroblastic elements, but the myeloid and thromboblastic tissue is present in roughly normal amounts.

Agranulocytosis. This condition may occur in a primary aplastic form or as a " maturation " type. Both of these conditions may be idiopathic or secondary. The ætiology in the idiopathic cases remains uncertain, although it is unlikely that it depends on an intrinsic defect in the marrow itself. In the secondary cases, amidopyrine can be incriminated in a considerable number of cases, and gold, sulphonamides, and other poisons may account for others. Occasionally, infections, such as diphtheria and typhoid, may initiate the changes ; and even X-rays and radium may induce it. Many of these ætiological factors are such as can, in stronger concentrations, produce aplastic anæmia : the only one which induces agranulocytosis only is, so far as we are aware, amidopyrine. If there be general aplasia of the marrow, the name agranulocytosis should not be applied to the malady ; one is then dealing with aplastic anæmia. Perhaps the changes of agranulocytosis pass over into those of marrow aplasia ; but when this happens, it is no longer justifiable to speak of the disease by the name that Schultze reserved for the malady he described.

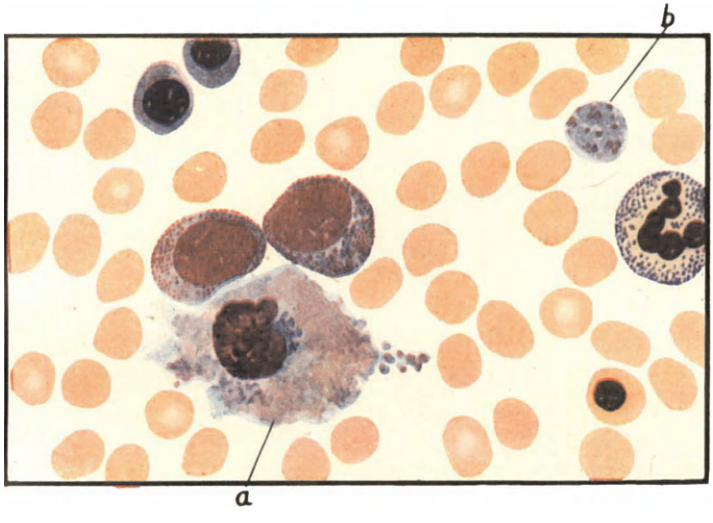
In the majority of well-developed cases of aplastic agranulocytosis the marrow shows almost complete disappearance of the granulocytes and their precursors ; but, at the same time, there may be some diminution in the intensity of erythropoiesis. The percentage of lymphocytes is somewhat increased. The first cells to disappear from the marrow are commonly the myeloblasts and the myelocytes.

PLATE 10



A. AGRANULOCYTOSIS (MATURATION TYPE)

Note.—Absence of polymorphs ; non-granularity of metamyelocytes (*a*) nuclear degeneration in myelocytes (*b*)



B. THROMBOCYTOPENIA (MATURATION TYPE)

Note.—Abnormal megakaryocyte (*a*) and giant platelet (*b*).

[*To face p.* 52.

The neutrophile polymorphs may persist for a little longer, but that these are old cells is shown by the excessive lobulation of the nucleus and granular degeneration. These cells rupture very easily in the preparation of the film and may be seen as pale nuclei with no cytoplasm, but surrounded by granules. This picture suggests that primary divisions of the reticulo-endothelial cells are at fault. When the catastrophe happens, such myeloblasts and myelocytes as are present keep on dividing and produce mature polymorphonuclears, but no more precursor cells appear. The myelogram in such an early case is as follows :—

Neutrophiles :	
Myelocytes	5–10 per cent.
Metamyelocytes	10–12 ,, ,,
Polymorphs	15–20 ,, ,,
Eosinophiles :	
Myelocytes	0– $\frac{1}{2}$,, ,,
Polymorphs	0– $\frac{1}{2}$,, ,,
Basophiles	0– $\frac{1}{2}$,, ,,
Premyelocytes	0– $\frac{1}{2}$,, ,,
Hæmocytoblasts	1– 2 ,, ,,
Lymphocytes	15–20 ,, ,,
Normoblasts	15–25 ,, ,,
Megakaryocytes	scanty.

Some of the polymorphs show degenerative changes in the nucleus and cytoplasmic granules.

In the " maturation " type of the disease, the marrow contains large numbers (perhaps normal numbers) of the less mature forms of granulocytes, but practically no polymorphonuclears, and the peripheral blood shows the typical picture of extreme leucopenia, with relative lymphocytosis. Of the granulocyte precursors, Jaffé (18) has found that the myeloblasts show no morphological abnormality, but the myelocytes show degenerative changes in the nucleus which eventually lead to the death of the cell (Plate 10, A). The nuclear changes are preceded by some splitting and degeneration of the cytoplasmic granules. Jaffe emphasises these changes as the specific pathological features of the malady, and it would seem that they depend upon the absence of some specific factor that is normally required for the proper differentiation of the myelocytes : if this factor can be supplied, the prognosis would be good. The available evidence suggests that part, if not all, of this material can be supplied

by pentnucleotide, and certainly the intramuscular injection of such substances as sodium pentose nucleotide is often followed by cure. But in the aplastic form, there is little evidence that treatment on these lines is of any value at all. The myelogram in the " maturation " type of agranulocytosis is of the following type :—

Neutrophiles :	
Myelocytes	30–35 per cent.
Polymorphs	1– 5 ,, ,,
Eosinophiles :	
Myelocytes	1– 2 ,, ,,
Premyelocytes	1– 2 ,, ,,
Hæmocytoblasts (including myeloblasts)	1– 2 ,, ,,
Lymphocytes	20–35 ,, ,,
Normoblasts	15–20 ,, ,,
Megakaryocytes	scanty.

The myelocytes show degenerative changes in the nucleus and specific cytoplasmic granules.

Whether the aplastic type of agranulocytosis is an advanced stage of the " maturation " type is still unknown ; but it is obvious that, in spite of this large gap in our knowledge, sternal puncture is of the utmost prognostic value. By it we can determine whether there is any hope that treatment will do good, or whether, with the means available, death is inevitable.

Essential Thrombocytopenia. In this malady, as with agranulocytosis, there would seem to be a primary aplastic form as well as a " maturation " type resulting from failure of the megakaryocytes to develop properly. Clinically the aplastic form is an acute illness, and splenectomy is attended by a 70–80 per cent. mortality. In the chronic recurring type of the malady the marrow often shows a maturation defect, and in these cases splenectomy practically always alleviates the condition. This has led to the supposition that the spleen in these cases produces an inhibitory factor which acts on the bone marrow.

In the primary aplastic form of the disease, the significant change is the complete absence of megakaryocytes and the paucity of platelets. Careful search of even the ends of the films fails to reveal any fragments of megakaryocytes, and such platelets as there are may be deformed. If any anæmia has resulted from extensive purpuric bleeding, the myelogram will show a normoblastic hyperplasia, but

this is to be regarded as a reaction to the loss of blood and not as a part of the malady itself.

In the " maturation " type, megakaryocytes are present in normal numbers and show some alteration in structure. Platelets are deficient, however, in number and quality ; giant forms are much more common than in normal marrow (Plate 10, B). The megakaryocytes, although as large as fully differentiated forms, present much less nuclear lobulation and contortion. The cytoplasm is often vacuolated and may be completely free from granules ; but whether this is a sign of degeneration, as some writers have contended, is quite unknown. The one point that suggests the megakaryocytes are, indeed, the seat of degenerative changes, is that the number of platelets, both in the marrow and in the blood, is greatly reduced ; and it is reasonable to suppose that this may be due to loss of functional activity of the parent forms.

We may well consider here, although a hypoplastic or aplastic condition of the bone marrow is not involved, other forms of purpura. Sternal puncture is valuable here, because there are so many conditions that can give rise to purpura ; and their accurate differentiation is of great therapeutic importance. Thus we may find a myeloblastic overgrowth of the marrow which demonstrates that we are dealing with a leukæmia ; or, we may discover a marrow that is entirely devoid of erythroblastic and granulocytic elements, and we realise that the malady is some form of aplastic anæmia.

In the purpuras associated with infection, or with the action of poisons, there may be a transient increase in the number of megakaryocytes ; but quite soon these elements become scanty. The myelogram is of the following type :—

Neutrophiles :	
Myelocytes	10–15 per cent.
Metamyelocytes	4– 6 ,, ,,
Polymorphs	18–25 ,, ,,
Eosinophile polymorphs	2– 4 ,, ,,
Premyelocytes	2– 4 ,, ,,
Lymphocytes	1– 2 ,, ,,
Monocytes	1– 3 ,, ,,
Plasma cells	1– 3 ,, ,,
Pro-erythroblasts	3– 5 ,, ,,
Normoblasts	30–45 ,, ,,
Megaloblasts	2– 4 ,, ,,
Megakaryocytes	1– 4 ,, ,,

CHAPTER IX

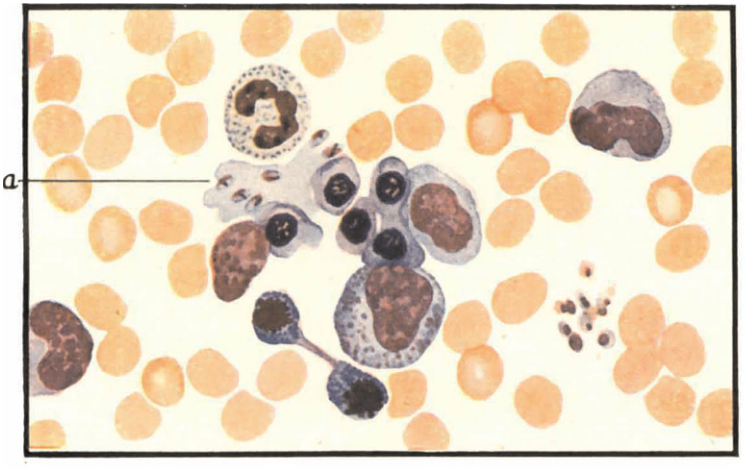
SOME PROTOZOAL DISEASES

IT would seem that with these diseases there is an extensive and promising field of work in connection with sternal puncture, as many of the parasites make their home in the reticulo-endothelial system, and so one would expect to find them frequently in the bone marrow. Also the literature on the subject is scanty. The enlargement and softening of the spleen, which so often accompany these diseases, may make spleen puncture an operation of some consequence because of the possibility of persistent bleeding. Liver puncture, though safe, does not usually give very satisfactory material for examination. Sternal puncture, however, is rapid, safe, and provides material which not only may contain the parasites in large numbers, but at the same time provides an opportunity of explaining the more or less characteristic blood changes.

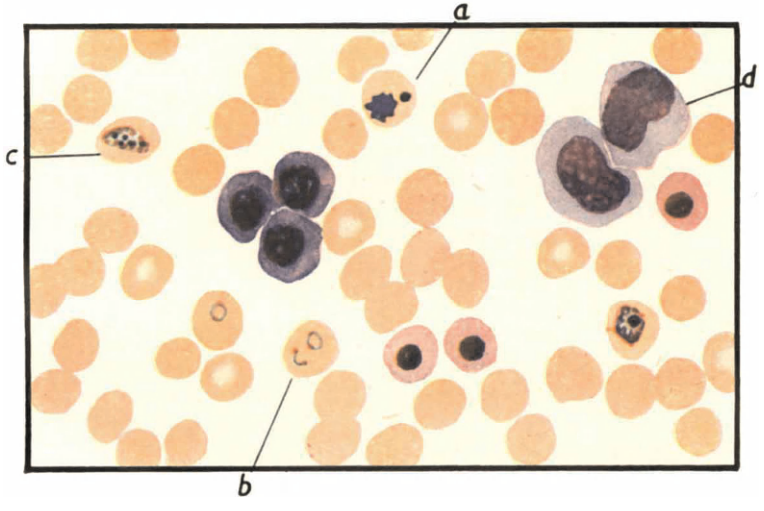
Kala Azar (Plate XIA). Let it not be supposed that this disease is confined to the tropics; many cases from the margins of the Mediterranean basin and from the Sudan have been recorded; and one is tempted to presume that even more have been overlooked.

The myelogram has remarkable characters, inasmuch as there is lymphocytosis, increase of monocytes, intense reticulo-endothelial hyperplasia, patchy deposition of platelets, and, of course, the pathogenic agent, the Leishmania itself. The last is found lying inside monocytes, but some lie free and rather closely resemble platelets, with which they may easily be confused. Close examination shows that the Leishmania has a far more complicated structure than have platelets, and in good films the differentiation from platelets is quite simple. The extracellular position of the protozoa in marrow films is probably an artefact due to rupture of the cells during spreading of the film. In the films we have seen, where a group of Leishmania is encountered, they are always entangled in a faintly basophilic web, probably representing the remains of the monocyte cytoplasm. Quite often a degenerate nucleus is found in the close vicinity. Extracellular forms must occur in the passage from cell to cell, but these are rare and probably occur singly. In histological preparations, where the cells remain intact, the parasites are almost invariably intracellular. In films also they may sometimes be seen superimposed on a red cell, and they have been thought to lie within

PLATE II



A. KALA AZAR
(*a*) Leishmania in ruptured monocyte.



B. MALARIA (MALIGNANT TERTIAN)
(*a*) Schizonts.
(*b*) 2 ring-forms in 1 corpuscle.
(*c*) Young gametocyte.
(*d*) Monocytes.

[*To face p.* 56.

them, as with the malarial parasite. This is an artefact. The organisms may be found in peripheral blood films but always within either the monocytes or the polymorphonuclears.

In the human body the parasite is in a non-flagellate stage. It is circular, oval, or cigar-shaped in outline and is 2 to 4 microns long and 1 to 3 microns wide. The cytoplasm, faintly basophilic, is contained in a tenuous membrane and contains two characteristic structures, which are essential to its recognition. The one, the nucleus, is larger than the other, and is usually spherical. Its diameter is about one-third to one-half of the shortest diameter of the organism. It usually lies against the membrane and is flattened on this side. The flattening may be extreme so that the nucleus appears as a thick line, and this appearance may be accentuated by the presence of vacuoles within the cytoplasm. With Jenner-Giemsa stain the nucleus appears as a bright red granular mass. The second, the blepharoblast, is a short rod-shaped structure with one end pointing to the nucleus. It may appear as a dot if its long axis is perpendicular to the slide. The blepharoblast stains purple with Jenner-Giemsa (Plate 11, A).

Quite apart from the presence of Leishmania, the myelogram is characteristic, and may remain so for many months after suitable treatment has caused the disappearance of these organisms.

Neutrophiles :	
Myelocytes	18–24 per cent.
Metamyelocytes	0– 1 ,, ,,
Polymorphs	8–10 ,, ,,
Eosinophiles	0– 1 ,, ,,
Lymphocytes :	
Small	18–22 ,, ,,
Large	18–22 ,, ,,
Monocytes	14–18 ,, ,,
Plasma cells	1– 3 ,, ,,
Hæmohistioblasts	0– 1 ,, ,,
Hæmocytoblasts	0– 1 ,, ,,
Normoblasts	3– 5 ,, ,,
Megaloblasts	1– 3 ,, ,,

In Leishmania tropica infections (oriental sore), the lesions are usually confined to the skin, sometimes appearing in the mouth also. Enlargement of the glands may occur if lymphadenitis is present and the organism has been found in them. Very rarely

it has been demonstrated in the peripheral blood, but further investigation may show it to occur more frequently in the bone marrow.

Malaria (Plate XIB). It is not common to be able to discover the parasite in the sternal marrow, but, even so, sternal puncture can render useful service in determining the intensity of reactive response to the anæmia that so often complicates severe and chronic malarial infection. This response is of the normoblastic type seen with hæmolytic anæmias.

In benign tertian and quartan infections the asexual life-cycle of the parasite takes place in the peripheral blood. In malignant tertian fever, the cycle takes place in the tissues. It would appear, from sternal puncture films of a case of cerebral malignant tertian malaria, that this schizogony occurs, in part at least in the bone marrow. All the changes from the signet-ring stage to the rosette stage could be traced, while peripheral blood films showed only a few signet-ring and gametocyte forms. The number of infected corpuscles in the marrow exceeded by far those in the bloodstream. Plate 11, B was prepared from this film.

In response to the hæmolysis of red corpuscles by the malarial parasite, the marrow response is essentially normoblastic and monocytic—changes that do not disappear for many months after recovery Monocytosis, which is considerable in chronic cases, is less marked in acute ones. The following myelogram is of a fairly characteristic type :—

Neutrophiles :	
Myelocytes	5– 7 per cent.
Metamyelocytes	0– 2 ,, ,,
Polymorphs	9–12 ,, ,,
Eosinophiles	0– 2 ,, ,,
Monocytes	10–14 ,, ,,
Premyelocytes	0– 2 ,, ,,
Hæmocytoblasts	0– 1 ,, ,,
Normoblasts	40–50 ,, ,,
Macronormoblasts	12–1 ,, ,,

CHAPTER X

THE TECHNIQUE OF STERNAL PUNCTURE

Anatomy. The cartilage of the second rib joins the side of the sternum at the junction of the manubrium and the body. This junction can be felt, and often seen, as a transverse ridge on the front of the chest, as the two bones are slightly thickened at their point of union. The ridge can usually be felt, in the adult male, about 2 inches below the jugular notch. In the female, the manubrium is longer and the ridge consequently lower. Until late adult life the two bones are joined by cartilage, which then undergoes ossification. The sternum is composed of highly vascular spongy bone enclosed in thin compact bone. This compact layer is thinnest on the lower surface of the manubrium which adjoins the body of the sternum.

The centre of ossification in the manubrium appears during the fifth month of fœtal life, and at three years of age the bone contains a moderate amount of red marrow. Despite this, sternal puncture is an unsatisfactory procedure if performed much before the age of seven to eight years. The needle enters the manubrium very easily, but it is difficult to obtain any marrow at all.

Technique of Sternal Puncture. This is a comparatively simple procedure, but in order to cause as little pain as possible, a few details must be adhered to.

The type of needle to be used is of some importance, because it needs to be thick and strong, so as neither to bend nor break in piercing the bone. Something in the nature of a very short French lumbar puncture needle, with an obturator stilette, is best, and a movable guard on the needle itself is useful to avoid the danger, admittedly slight, of penetrating the bone too deeply. The needles designed by Witts and Sahlah fulfil every requirement.

A good syringe, with a well-fitting plunger, is needed for sucking out the marrow substance, and in order to be able to obtain enough suction it is well to use a large syringe, *e.g.*, about 20 or 25 c.c.

It is probably not of much importance where the bone is punctured, but as has been said, the thinnest spot lies on the under (caudal) surface of the manubrium—that is, in the upper surface of the joint that forms the angle of Luis. And this is the easiest and least painful site for the purpose.

Now the procedure is as follows: the patient is placed on his

back, with a pillow under the shoulders, so as to throw the head backwards. Then novocaine is injected into the site of election, taking care that the deeper tissues, including the sternal periosteum, are infiltrated.

STERNAL PUNCTURE NEEDLE (WITT'S TYPE)

The needle resembles a short, thick lumbar puncture needle, with obturating stilette and movable guard. The guard is so adjusted that there is no possibility of piercing the posterior wall of the sternum. If, however, it is found that the guard has been placed so near to the point that the anterior wall is not pierced, it can be moved after the needle is in the tissues.

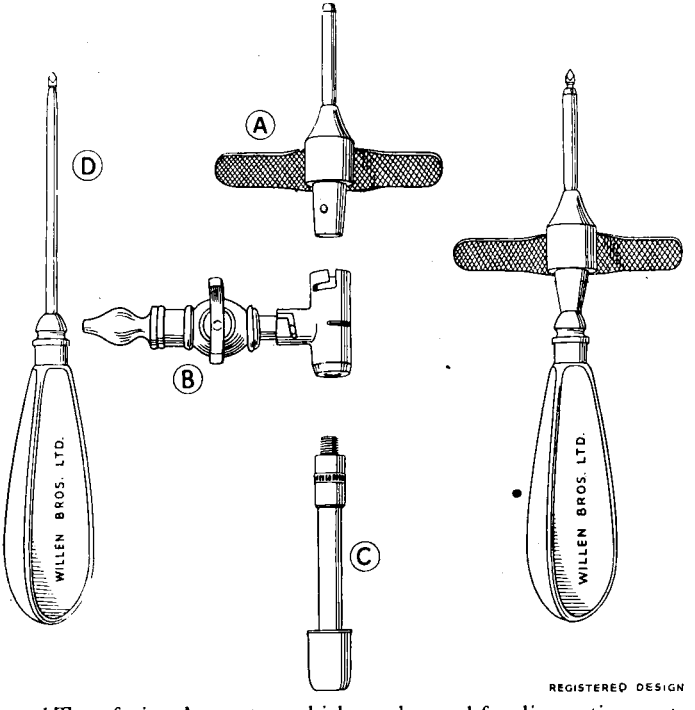
The needle is plunged in just below the angle of Luis, and when the point is felt to reach this it is pushed a little further until it penetrates the small piece of cartilage in the joint. When it seems to be in about the middle of this the butt of the needle is lowered until it is almost touching the chest, and then it is pushed straight upwards until the bone is pierced. The manœuvre is sometimes accomplished more easily if a rotatory motion is imparted to the needle as in using a bradawl. The sensation obtained when the outer layer of compact bone is penetrated is rather like that of a lumbar puncture when the point of the needle enters the theca. Now the stilette is withdrawn and the needle pushed onwards, thus collecting narrow tissue in the lumen.

A good deal of information can be obtained, after some experience, by the different sensations obtained as the needle moves onwards. Thus the hard push needed in osteosclerosis is very different from that required by the soft marrow of a leukæmia.

The syringe, either thoroughly dry or rinsed out with normal saline, is now attached to the needle and the plunger pulled out. However well the tissues have been anæsthetised, there is now always an unpleasant sensation, which some patients allege amounts to pain, but it is quite transient. Thick blood-like material should enter the barrel of the syringe, but some marrows are too dense for this to occur : even then a sausage of

myeloid tissue will usually be found, often mixed with spicules or cancellous bone, lying in the lumen of the needle. Suction is now stopped, and after removing the needle the puncture wound is sealed with collodion.

It is best to expel the whole of the contents of the syringe into a watch glass, as it may then be possible to find fragments of marrow



Sternal Transfusion Apparatus, which can be used for diagnostic puncture, also middle figures A B C. A. Cannula. B. Union for rubber tube. C. Glass viewing tube. D. Trocar. Only A. and D. are needed for marrow puncture.

that are large enough for histological examination. If so, they should be removed and fixed in Helly-Zenker solution. More commonly it is impossible to find any fragments, and then films must be made from the thick fluid. This is often difficult, as all but the most hyperplastic marrows contain globules of fat, which render the preparation of really good films difficult, and, of course, the spicules of cancellous bone make it even more so. For this reason a large number of films should be prepared, and among them good ones will be found. The films should be made as quickly as possible

because marrow clots as rapidly as blood ; but no attempt should be made to make the films as thin as those of blood ; if this is tried, far too many cells will be ruptured in the attempt.

Ordinary films can be stained by whatever method is desired, but as a rule it is, of course, sensible to use the same method as is used for blood ; in this way comparison is considerably facilitated. On the whole, Jenner's solution is not satisfactory, because the nuclear detail is poorly displayed, and because any azurophilic granules remain unstained. Leishman's solution or the excellent Jenner-Giemsa method is very suitable. In combined staining May-Grünwald may be used instead of Jenner, and Panchrom stain or Kardos mixture in place of Giemsa. Different effects can be obtained by using different combinations ; for instance, May-Grünwald and Kardos mixture gives extremely brilliant granule staining.

Vital Staining. (a) Reticulocytes. A clean slide is flooded with an alcoholic solution of cresyl blue. This film is allowed to dry, and any tendency to roughness on the surface of the dye film is smoothed by gently polishing with a piece of soft paper. One small drop of marrow fluid is put on the slide and gently stirred with a glass rod, taking care not to spread the marrow over too large an area so that drying takes place. As the marrow fluid mixed with cresyl blue does not clot for some time, it is allowed to stand for two or three minutes so that maximum staining of the reticulum is obtained. Films are then made from this drop and allowed to dry in air. They can be examined directly for reticulocytes or counterstained for making permanent preparations. Counterstaining, however, tends to hide the reticulation in cells which have only a little of the vitally stainable basophilic substance.

(*b*) Fresh preparations. The most commonly used dyes for this purpose are neutral red chloride and Janus green. The preparations are made in the usual way. Equally good results, however, can be obtained by dark ground illumination of fresh preparations as described below.

Dark Ground Illumination (Plate 12). A good account of this technique was given by Whitby and Hynes (19). Thoroughly clean and degreased slides and coverslips must be used. A small drop of marrow fluid is placed on a slide and covered with a cover slip ; the preparation is immediately sealed with wax and kept warm in the incubator. The slides should be covered so that light is excluded. If good results are to be obtained, the marrow films must be as thin

as possible ; the smaller the drop of marrow, the thinner the final preparation, and as a rule, the drop should be of such a size that when the cover slip has settled, the film does not quite reach its edges. In this way one attains the maximum capillary attraction between the slide and the cover, whereas if there is enough marrow fluid to reach the edges of the coverslip, this tends to float up and make a thick preparation.

After half an hour in the incubator, the preparation is examined with dark ground illumination, and if possible, some form of warm stage. Under proper conditions the movements of the motile cells are easily seen, as are also the mitochondria and other elements seen in any vitally stained preparations. Dark ground illumination has further advantages in that the cell and nuclear membranes are easily visible and that nuclear structure is also quite clear. The motility of cells is rapidly slowed down by light, so the preparation should only be illuminated for a few minutes at a time, and then allowed to recover in the dark. For observing cell structure the films last quite well for four to six hours without any protection or warming.

The characters of the cells are as follows (see Plate 12).

(1) GRANULOCYTE SERIES. (A) *Myeloblast*. A rounded cell 8 to 10 microns in diameter. Thin defined cytoplasmic rim ; nuclear membrane equally well defined and somewhat thicker. Nucleus rounded and appears filled with a fine dust ; nucleoli appear as circular outlines where the dust-like particles are thicker. Cytoplasm contains mitochondria, which appear as faint dots. Cell non-motile.

(B) *Premyelocyte*. Ten to 20 microns in diameter and rounded ; cytoplasmic rim, nuclear membrane, and nucleus as in the myeloblast. The mitochrondria are now grouped round the nucleus and appear as thin, wavy rods, sometimes showing Brownian movement. Cell non-motile.

(C and D) *Myelocyte*. Ten to 20 microns in diameter. The specific granules appear in the cytoplasm of the premyelocyte in increasing numbers, first among the mitochondria. Eventually they fill the whole of the cytoplasmic space and may partly obliterate the nucleus, which has now no nucleolar structures. The specific granules of each of the neutrophile, eosinophile, and basophile myelocytes are described under the mature polymorphonuclear cell.

(E) *Neutrophile Polymorphonuclear*. An active motile cell, 12 to 20 microns in diameter when at rest. The nucleus, now lobed, has

still the same fine dust-like structure. The cytoplasm is filled with coarse bright granules. While moving, the leading end of each pseudopodium shows a small space free of granules.

(F) *Eosinophile Polymorphonuclear*. Less actively motile than the neutrophile cell, and of about the same size. The granules are readily distinguished as they are larger, appear as rings of light, and may be oval.

(G) *Basophile Polymorphonuclear*. A smaller cell, 7 to 10 microns in diameter. The granules are of the same type as the eosinophile granules, but are much fewer in number and may appear even larger.

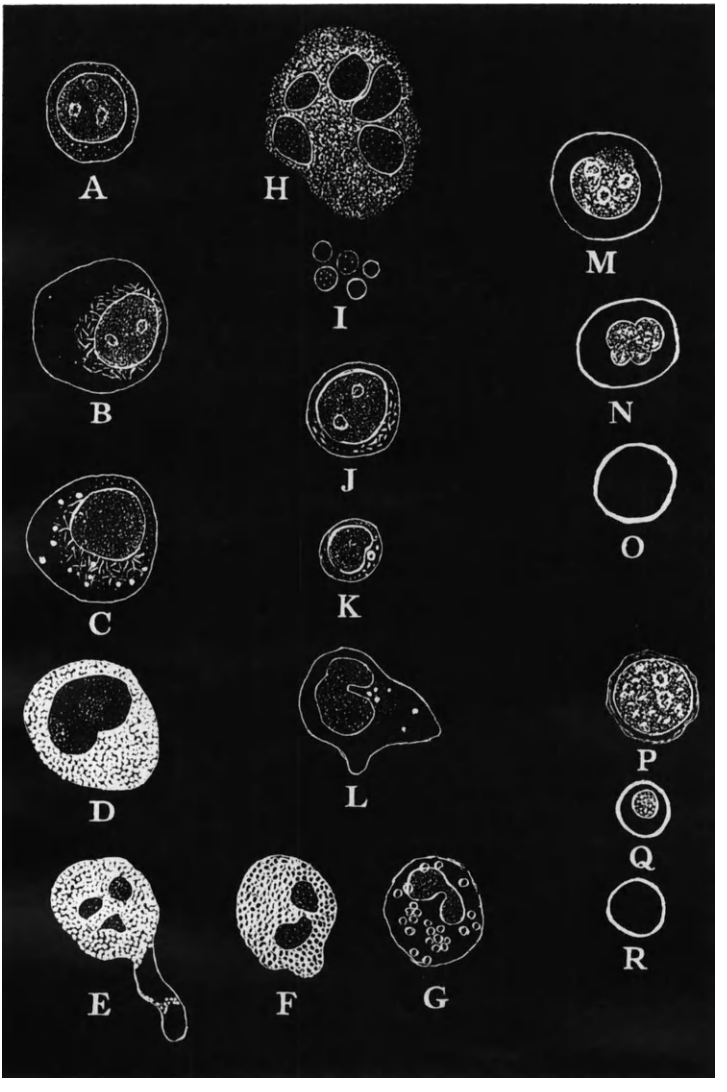
(2) LYMPHOID SERIES. (J) *Lymphoblast*. A rounded cell, 8 to 10 microns in diameter. The nucleus is indistinguishable from that of the myeloblast. The two cells, however, are easily separated by the appearances of the mitochondria. These in the lymphoblast are larger, less numerous, and definitely rod shaped. Cell non-motile.

(K) *Lymphocyte*. Rounded, may occasionally be motile and vary in size from 10 to 15 microns. The nucleus is round or indented and filled with fine dust-like granules. The mitochondria are grouped to one side of the nucleus and appear as fine round oval dots. Two, three, four or five large bright granules are present. " Gall's mahogany granule " shows as a refractile ring of light.

(3) THROMBOBLASTIC SERIES. (H) *Megakaryocyte*. A large cell up to 40 microns in diameter. The nucleus usually appears as a group of separate lobes, each of which has a definite nuclear membrane, and is filled with fine dust-like particles. The cytoplasm has no definite rim and is filled with small moderately bright granules.

(I) *Platelets*. These appear most as small circles of light, 2 to 5 microns in diameter. The larger number appear to be empty, but some contain five or six small bright granules, which show active Brownian movement, apparently bouncing from side to side of the cell.

(4) ERYTHROBLASTIC SERIES. (M) *Promegaloblast*. A non-motile cell about 10 microns in diameter. The cytoplasmic rim is thick and well defined. This cell shows marked pleomorphism. The cytoplasm appears completely free of granules and other structures although there may be a group of small rounded mitochondria adherent to one side of the nucleus ; when this occurs, the nuclear membrane is absent at the point of contact. This membrane is otherwise well defined and thin. The nucleus is filled with fine dust-like particles which are more closely packed and appear much



DARK GROUND ILLUMINATION OF MARROW CELLS

(*a*) Myeloblast.
(*b*) Premyelocyte.
(*c*), (*d*) Myelocyte.
(*e*) Neutrophile.
(*f*) Eosinophile.
(*g*) Basophile.
(*h*) Megakaryocyte.
(*i*) Platelets.
(*j*) Lymphoblast.
(*k*) Lymphocyte.
(*l*) Monocyte.
(*m*) Promegaloblast.
(*n*) Megaloblast.
(*o*) Megalocyte.
(*p*) Early normoblast.
(*q*) Late normoblast.
(*r*) Normocyte.

For explanation, *see* p. 63.

[*To face p.* 64

brighter than those of the leucoblastic cells. Also the nuclear particles show some small areas of condensation which appear as brighter granules. The nucleoli, two or three in number, are outlined by a ring of such condensation.

(N) *Megaloblast*. Very variable in size and shape. There is a thick cytoplasmic ring and clear cytoplasm without mitochondria. The nucleus may appear lobed and shows areas of localised condensation.

(P) *Early Normoblast*. The nuclear structure is the same as in the megaloblast, and the nucleus is of much the same size. The cytoplasmic ring is not well marked, and the cytoplasm is scanty and may contain a few dust-like mitochondria. The cell is easily recognised by its nuclear structure.

(Q) *Late Normoblast*. Seven to 8 microns in diameter, with a thick refractile rim. The small granular nucleus is easily visible.

(R) *Normocyte*. This appears as a bright ring of light with a thick cytoplasmic rim. The megalocyte (O), though larger, has the same appearance.

Cell Propagation. Mitosis. While mitotic figures are easily recognisable, it must be remembered that the whole process is a continuous one, and that the early changes in the nucleus, between the interphase and the prophase, are very easily overlooked. These changes may completely alter the apparent structure of the nucleus and cause some confusion as to the actual nature of the cell. The same remarks apply to the final transition from the telophase to the interphase, except that the presence of two daughter cells, lying in apposition, should give a clue to the real condition. Failure to appreciate these changes, especially in histological material where there is much cell shrinkage, has led to much of the confusion which has existed over the morphological characters of blood cells and their apparent relationships.

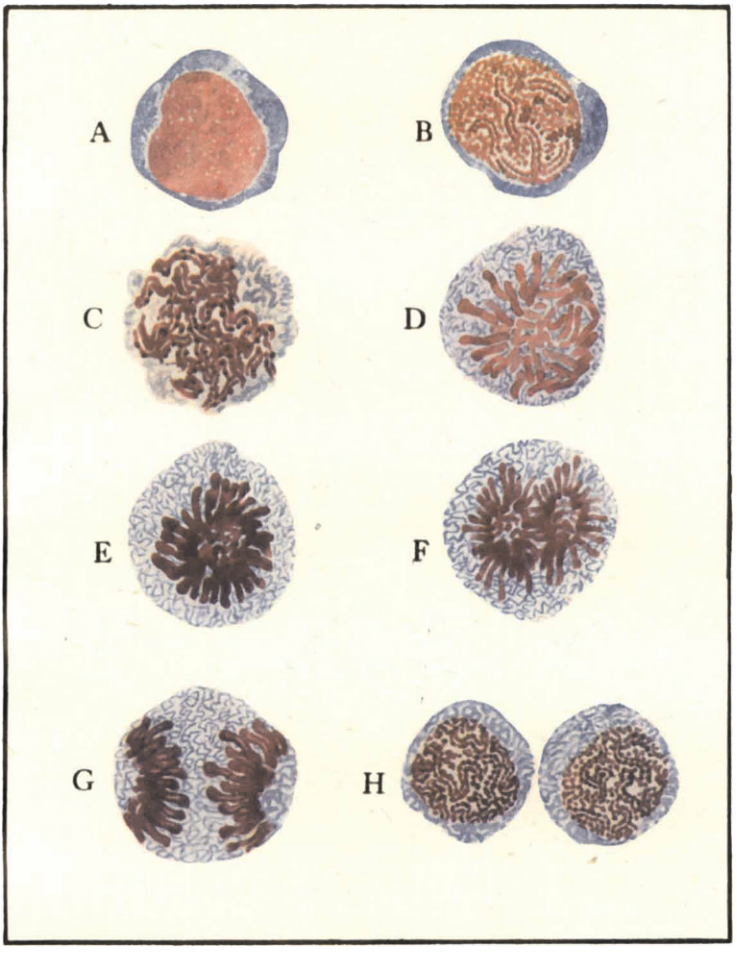
The advent of sternal puncture has made it possible to study these changes much more closely, and they are illustated on Plate 13. The cells depicted here are promonocytes from a case of monocytic leukæmia. Care has been taken to choose typical figures such as will be seen in normal hæmopoiesis. One of the characteristics of a leukæmia is, of course, the presence of atypical mitosis; tri- and even tetrapolar figures can be seen.

In the resting cell (A, Plate 13) the nucleus is rounded and the chromatin shows a fine, rather irregular reticulation. The cytoplasm has a ground glass appearance. In the earliest stage of the prophase

the pattern of the nucleus becomes more distinct owing to the disappearance of small anastomosing links, leaving the permanent chromatic elements of the chromosomes, the chromonema (B, Plate 13). It is at this stage that the greatest care is required in labelling a cell; a lymphoblast in this stage of development might easily be mistaken for a myeloblast. Some authors have described such changes in monocytes, and used them to suggest that the cell was derived from a lymphocyte. In the later stage of the prophase (C, Plate 13) the chromosomes are beginning to appear as separate entities. Each consists of two chromonemata lying parallel and fixed in an achromatic matrix. During the metaphase this matrix stains intensely and the internal structure of the chromosome is no longer visible. The arrangement of the chromosomes in the metaphase appears to be constant for all the cells of the hæmopoietic tissue. The long chromosomes are arranged radially at the periphery, and the short ones lie irregularly in the centre (E, Plate 13). In the anaphase, the chromonema from each chromosome move to opposite poles so that each daughter nucleus will have a representative part of the parent chromosome. The anaphase is shown in Fig. F, Plate 13. In the telophase the matrix of each daughter nucleus becomes achromatic again and the chromosome structure is again visible (G, Plate 13). Further changes in the new nuclei are the same as in the prophase, only occurring in the reverse order (H, Plate 13).

The cytoplasm divides by furrowing. It undergoes quite definite changes during the nuclear division, changing from a fine homogeneous structure to a mass of fine interwoven threads (Plate 13). The cytoplasm of dividing erythroblasts and normoblasts appears to be granular rather than thread-like.

PLATE 13



MITOTIC DIVISION (× 2,000)
(*See* p. 65.)

(*To face p.* 66.

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