A THESIS

THE VALUE OF THE EXAMINATION OF THE MARROW IN THE DISEASES OF THE BLOOD FORMING ORGANS.

by

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C O N T E N T S

F O R E W O R D

During the past twenty years sternal marrow examination has become increasingly important in the study of diseases of the blood forming organs. Many reviews of the importance of this method of investigation have appeared in the literature from time to time, but some of them have concentrated on only one aspect of the subject, whilst the more comprehensive ones have failed in some respects to deal adequately with the subject.

The first part of this thesis is devoted to a comprehensive review of this subject and includes a description of a method of procuring marrow by sternal puncture and the technique of staining smears.

In the second part of the thesis is a detailed account of the value of marrow examination in diagnosis, prognosis and treatment of the diseases of the blood forming organs, based upon my own personal experience of approximately eighty cases, which necessitated the performance of over a hundred sternal punctures.

It had been my original intention to illustrate this thesis by coloured photographs, but I was unable to procure the necessary materials. In the place of these photographs I have substituted coloured /

coloured drawings which were painted for me by Mr. J. Varney.

My thanks are due to Dr. A. F. Hewat, who encouraged me to carry out this work and has given me much valuable help. The major part of the clinical work for this thesis was done in Dr. Hewat's wards whilst I was in the receipt of a senior government re-settlement grant for demobilised medical officers.

I also extend my thanks to Dr. T.R.R. Todd who allowed me to study all his haematological cases during the past year. Many of these cases are included in this thesis.

I also wish to thank the Librarian of the Royal Society of Medicine for his help in tracing the more obscure articles referred to in this work.

P A R T I.

I N T R O D U C T I O N.

The first reference to nucleated cells in the human bone marrow was credited to Robin (1849) who discovered that there were nucleated cells in the bone marrow, a tissue which had hitherto been regarded as functionless and a mere packing of the bones. He was unable to attribute any functions to these cells, and it was not until nineteen years later that Neumann (1868 and 1869) working with dead rabbits, demonstrated that these nucleated cells were responsible for the production of both the red and white cells of the blood.

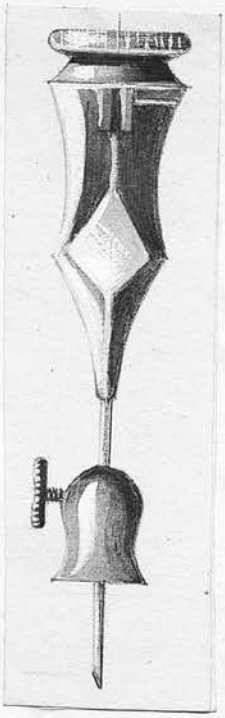
Cohnheim 1876 (cit. Merwe 1936) reported that the post mortem appearance of the marrow taken from a patient suffering from pernicious anaemia had an intense red appearance. Pianese - an Italian (cit. Coronia 1903) punctured the upper part of the epiphysis of the femur in living subjects with a trocar and aspirated the marrow by means of a Potain's apparatus. He did not leave any records of the appearance of the bone marrow in either health or disease, and it was not until Ghedini 1908 trephined the upper end of the tibia and removed some marrow with a curette that serious study of the cytological changes of the bone marrow was undertaken. Later in 1910 and 1911 he tried to co-relate the bone marrow appearances in various diseases with the blood picture made /

made at the same time. If the patient died, further post mortem samples of the marrow were obtained and compared with the earlier specimens of the marrow and blood. He claimed that he obtained diagnostic help by these methods in lymphosarcoma, tuberculous lymphoma, aleukaemic states, hypofunction of the bone marrow, dermatitis herpetiformis and certain diseases of infants. He left no details of this work so it is not of much value. Perhaps because of this lack of detail, these papers did not stimulate interest in this new study and it was not until Seyfarth (1922), impressed by the danger of haemorrhage following the diagnostic puncture of the spleen in suspected malaria cases, decided to search for the parasites in the cells of the bone marrow, that further interest in this tissue was shown. He trephined the sternum under a local anaesthetic and scraped out a piece of the marrow with a sharp spoon and from this sample made smears, thick drop preparations and paraffin sections. This method, involving as it did a minor surgical operation for its performance, did not gain wide favour amongst Haematologists. Arinkin (1929) overcame this objection by introducing a simple method of sternal puncture. This method consisted of puncturing the sternum with a stout needle and aspirating some of the bone marrow with a syringe. Smears were made from the aspirated material. The advantages and disadvantages of this method over the earlier method of sternal trephine were discussed by such authors as Vogel, Erf, and Rosenthal (1937), /

only certain way of assessing the degree of hypo or hyperplasia is by post mortem examination of the marrow in all the bones, so this disadvantage is common to both methods. This danger of placing too much importance in the degree of cellularity of the marrow is illustrated in two of my cases. Careful study of many smears, including a few "squashed" preparations, revealed a severe hypoplasia in the first case, but after two weeks iron therapy a marked normoblastic reaction in the marrow resulted. The smears of the other patient suffering from pernicious anaemia showed very few cells, but after four liver injections subsequent sternal marrow preparations revealed an intense normoblastic reaction.

Mandell and Meranze (1942) stated that the procedure of trephining the sternum should be reserved for those cases in which a 'dry' tap had resulted from sternal puncture. In their series two dry taps were experienced, one in a patient with Albers-Schoenberg's disease and the second in a case displaying dense sternal sclerosis with aplasia of the marrow.

D I A G R A M I.



S A L A H T Y P E N E E D L E.

TECHNIQUE OF STERNAL PUNCTURE.

I obtained the marrow by the following technique. The patient lay in bed or on a couch with the head supported by one pillow. The bone punctured was the sternum, because it contains the most marrow, and the site chosen for the introduction of the needle was half an inch below the sterno-manubrial junction. In obese subjects the sternum should be carefully palpated to make sure of its position. The area of skin over the site of the puncture was shaved, if necessary, and cleaned with ether, and iodine applied. The skin over the chosen site was anaesthetised by a small intradermal wheal of novocain, or similar local anaesthetic, using a thin sharp pointed needle, the subcutaneous tissues and periosteum were infiltrated, using in all about 2 c.c. of the anaesthetic. Three minutes were allowed to elapse before a stout needle, originally designed by Salah (see diagram I) with the stylet in place and the guard suitably adjusted, was driven through the anaesthetised area at right angles to the sternum, until the bone was reached. This was penetrated with a rotary movement of the needle. Moderate force was used. The stylet of the needle was withdrawn and a 5 or 10 c.c. syringe fitted into the needle and a half c.c. of marrow fluid, which had the appearance of blood, withdrawn. If a larger quantity was withdrawn it became grossly diluted with blood. The needle was removed and the puncture wound in the skin /

skin sealed with collodion. A dry dressing was applied. A small drop of the marrow fluid out of the needle was quickly placed on each of six clean slides, and smears made in the usual way. Six smears were necessary to make sure of obtaining a satisfactory specimen, and to insure against accidents in the subsequent staining procedures. The remainder of the fluid was transferred to a watch glass containing a few drops of citrate to prevent clotting. After thoroughly mixing the marrow juice with the citrate the excess fluid was carefully poured away and the marrow particles remained behind on the surface of the watch glass and were recognised as small bits of tissue of varying colour, according to the condition present. A few bits of the marrow were picked off the watch glass with fine forceps and transferred to a slide, and then smeared across the slide with the face of a second slide, using sufficient pressure to ensure an even distribution of the cells. These smears must be made within ten minutes after the marrow was obtained, as experience with the present method has shown that if a longer time interval is allowed to elapse, the details of the cell morphology were lost. The smears were dried in the air and stained by various methods which are described later. Throughout the procedure strict aseptic precautions are observed and the needle sterilised by boiling.

Only one failure in over 100 punctures resulted with this method and this was probably due to lack of experience /

experience as it occurred early in the series. This compares favourably with the experience of Young and Osgood (1935), who had five failures in 65 attempts. Sometimes fluid was slow to appear in the syringe and this was due I think to the point of the needle resting against the posterior lamina of the sternum and was rectified by a slight withdrawal of the needle. When great care was taken over the technical details, the operation was practically painless. Some patients experienced momentary discomfort or even pain when the suction was applied, due most probably to a sudden increase in the negative pressure within the sternal cavity. One of my patients complained of a severe pain in the left side of the chest radiating down the inner border of the left arm, which necessitated an injection of morphia for its alleviation. The pain did not return nor was it accompanied by any other symptom. I have had no complications resulting from this method of puncturing the sternum, and this is in agreement with the experience of other writers.

IMPORTANT /

IMPORTANT VARIATIONS OF THIS METHOD AS PRACTISED BY OTHER WORKERS.

Arinkin (1929) punctured the anterior lamina of the manubrium, but this is less satisfactory than puncturing the sternum as Zanaty (1937) has shown, that in the upper part of the manubrium there is only a spongy layer of marrow confined to a small narrow strip in the mid line, and even less in the lower part. Holmes and Brown (1933) punctured the junction between the two bones and they claimed that an easier entry into the sternum was gained by this method.

Davidson (1941) entered the sternum just to the right of the mid line, and held the needle at a slight angle to the bone.

Young and Osgood (1935) held the needle at an angle of 60° to the sternum. Tuschinski and Katlavando (1932) preferred an angle of 45°. Propp and Schwind (1944) entered the bone opposite the third rib.

Turkel and Bethell (1943) designed a large bore needle containing an inner trephining needle with a stylet, which they claimed gathered marrow as it thrust through the cavity containing the marrow.

Weller (1932) used a No. 18 guage spinal needle.

Hynes (1939) sterilised his needle in hot oil. The treatment of the aspirated fluid varies in the hands of different authors. Young and Osgood (1935) and Hynes (1939) aspirated 10 c.c. of the marrow juice /

juice and transferred it to a test tube containing 2 mgm. of potassium oxalate. After the tube had been corked and shaken thoroughly, smears were made from the resulting mixture. They claimed that an even distribution of the cells resulted.

Meutens (1932), Weller (1932), Reich (1935), Vogel and Bassen (1939) and Davidson (1941) allowed the fluid to clot and then made tissue sections.

Reich (1934), Schleicher and Sharp (1937) centrifuged the fluid and made smears and tissue sections from the buffy layer. Joppich and Leissens (1937) placed the aspirated marrow juice on a watch glass and inserted a filter paper in the juice, claiming that the paper absorbed the blood but left the marrow cells behind. There is no evidence to support this view. Weller (1937) managed to get enough marrow into his needle by merely inserting it into the marrow cavity, and he made smears by moving the needle backwards and forwards across a slide, over an area of $\frac{3}{4}$ of an inch. He claimed that he obtained a film free from blood by this method.

EXAMINATION OF THE ASPIRATED FLUID. /

EXAMINATION OF THE ASPIRATED FLUID.

The aspirated fluid consisted of a suspension of marrow cells mixed with a variable amount of blood. Enumeration of the total of the nucleated cells per c.mm. was practised by Young and Osgood (1935), Reid (1934), Holmes and Broun (1933), etc., but these counts were proved to be unsatisfactory by Falconer and Leonard (1941), Krumbhaar and Custer (1935), as they were so easily upset by the uncontrollable fluctuations of the amounts of blood present in the aspirated material. The greater the volume of fluid aspirated the greater the proportion of admixed blood (Scott 1939). In this work I have not counted the nucleated cells in the aspirated fluid and cannot recall one case in which the diagnosis would have been helped by this procedure. Most workers made smears from the aspirated fluid, using the technique which is commonly employed for making blood smears. Krumbhaar and Custer (1935) raised the objection that the cell relationship was lost by this method and they examined a piece of marrow smeared across the slide. Davidson (1941) utilized a similar method. He transferred the marrow juice to a watch glass, and with a pair of fine forceps placed a bit of marrow on a slide and smeared this across the slide, with the face of a second slide, the degree of pressure being regulated so that the marrow was spread with the minimum of destruction to the /

the marrow cells. I adopted both methods, and more often than not the required information was obtained as easily by one method as the other. The thin blood film type of smear gave excellent results when the bone marrow cells were numerous. On the other hand when the cells were scanty, "squashed" preparations gave better results as areas of the marrow, and hence a greater number of cells per microscopic field, could be examined relatively undisturbed. Examination of squashed films in suspected myelogenous leukaemia, because of the relatively undisturbed histology, sometimes facilitated the detection of invasion of the marrow tissue by the myelogenous cells. Davidson (1943) admitted that in the spreading of individual bits of marrow cellular damage was caused and aggregation of unrecognisable cells occurred in the films, but claimed that it was always possible to find other fields which were rich in undamaged cells, and maintained that a more rapid and accurate assessment of the prevailing cell types was obtained by this technique. Despite carefully following Davidson's technique I occasionally obtained smears containing so many damaged cells as to render them useless. On these occasions I found that the examination of thin films with their high proportion of undamaged cells indispensable. The smears were stained by one of the Romanowski techniques.

OBSERVATIONS ON STAINING /

OBSERVATIONS ON STAINING.

Romanowski, (1891) discovered that an excellent purplish red stain for the nuclei of malaria parasites could be made by mixing old mould covered solution of methylene blue with a solution of eosin. Freshly dissolved methylene blue lacked this quality. This mixture also stained the nuclei of tissue cells better than any previous dyes, and also demonstrated the various cytoplasmic constituents in different colours.

Romanowski's mould covered methylene blue solution owed these properties to a polychroming process, which decreased the degree of methylation of the methylene blue molecule by a form of oxidation. Unna (1891) found that an alkaline solution of methylene blue had the same composition as the mould solution and because of these meta-chromatic effects he called the solution polychromed methylene blue.

Simultaneously, but independently, Malachowski (1891) discovered these properties of methylene blue, and added the information that the best methylene blue solution was a very dilute aqueous solution of borax methylene blue, and the solution became known as Unna's polychromed methylene blue. Nocht (1900) combined Unna's polychromed methylene blue solution with eosin, and obtained consistent results similar to those obtained by Romanowski. These two solutions had to be freshly made every time they were required.

This /

This inconvenience stimulated Jenner (1899) to search for a better combination and he found that by precipitating methylene blue and eosin, and dissolving the precipitates in methyl alcohol, a single solution resulted which acted both as a fixative and a stain, but as he had not used polychromed methylene blue this stain lacked the metachromatic Romanowski nuclear effects. The final solution to this problem was achieved simultaneously by Reuter (1901(a) and Leishman (1901), who discovered that if the precipitates of polychromed methylene blue and eosin dissolved in methyl alcohol were first allowed to fix the tissues on the slide, and then were diluted with distilled water, the polychroming effect was produced and the nucleus, cytoplasm and cytoplasmic constituents were satisfactorily stained and differentiated. The same principle was used by Wright (1902) and Giemsa (1910).

This staining method and individual modifications of the technique were named after the first users of the stains, Jenner, Wright, Leishman, May-Grunwald, Giemsa and Pappenheim. Collectively these slightly different staining methods are known as the Romanowski technique. Romanowski stains gave inconsistent results if certain factors were not controlled. After the cells have been fixed by the methyl alcohol, the depth and quality of the staining of the cells depend on the concentration of the methylene blue and eosin used as well as on accurate dilution /

dilution with distilled water. As the depth of colour given by a known concentration of the dye in a fixed time interval, cannot be reproduced by allowing different concentrations of the dye to stain for proportionately longer or shorter time intervals, the same concentrations of the dye must always be used, and the time interval for staining kept constant. Accurately measured quantities of the dyes are ensured by careful dispensing of the stock solutions, and so the actual concentrations of the dyes in the solutions used, depend on the amount of the solvent which has not evaporated since the mixture was dispensed. This danger was minimized by keeping the solutions in well corked bottles and discarding them after prolonged use. The quantity of both the staining solution and the distilled water is often measured in drops from the same pipette. This practice results in variable dilutions of the staining solution, as the drops are not always the same size, and the drops of the dye solution are always smaller than the drops of distilled water. An accurate dilution was achieved by drawing the staining solution up to a mark on the pipette and transferring it to the slide, and then drawing up the distilled water to another mark on the same pipette, and diluting the staining solution with this quantity. When these details were carefully followed I found Leishman's modification of the Romanowski technique gave excellent results. I adopted this method for staining /

staining the smears. A carefully measured quantity of Leishman's stock solution was put onto the slide from a pipette and allowed to act for one minute. Distilled water was then added in the proportion of eight parts of the water to five parts of stain and allowed to act for eight minutes. The slide was washed in distilled water, dried and mounted in the usual way. Some solution of Leishman's stain tended to precipitate before the time required for perfect results had expired. The precipitate was redissolved with some undiluted stain and the slide again washed with distilled water and dried. Once a solution has shown a tendency to precipitate it must be filtered before being used again.

Davidson (1941) found Leishman's stain very useful, but used May-Grunwald-Giemsa's method as a routine. He clained that this method gave more consistent results and stained the cytoplasm of haemocytoblasts a darker blue. I agree that the cytoplasm of the haemocytoblasts were beautifully stained by this method, but no more so than by the modification of Leishman's technique which I used. I found that May-Grunwäld-Giemsa's technique stained the granules of the myelogenous cells better than Leishman's stain. I usually stained the smears by both these methods. Details of May-Grunwäld-Giemsa's technique which I used were :-

May- /

May-Grunwald's stain was poured in measured quantity over the slide and allowed to act for 3 minutes. Exactly the same quantity of distilled water was then added and the diluted stain left for 3 minutes. The slide was washed with distilled water, and a one-in-ten dilute Giemsa's stain poured onto the slide and allowed to act for 15 minutes. The slide was washed with distilled water, dried with a filter paper and mounted in the usual way. I also used Wright's and Jenner-Giemsa's methods. The former tended to precipitate before staining was satisfactorily completed, whilst the latter stained the cells a paler colour, with consequent poorer differentiation of the colours. I obtained such good results from the first two methods described that I did not continue with these less satisfactory techniques.

The smears were examined microscopically.

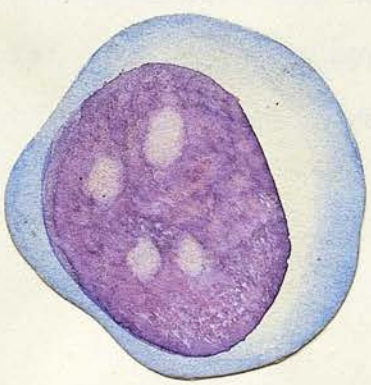
N O M E N C L A T U R E.

A clear definition of the nomenclature of the cells is essential as a preliminary to a thesis on this subject. All the cells which I consider to take part in the maturation of normal and abnormal haemopoiesis are named and described. The reasons for my choice of terminology are discussed later. The descriptions are based on cells stained by the modification of Leishman's technique /

technique which I have already described. The magnification was that obtained through the oil immersion lens of a good modern microscope.

HAEMOCYTOBLAST.

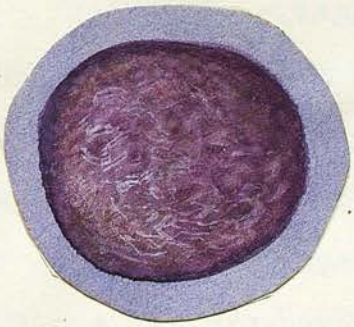
This is a large cell (20-30 u) and it is the earliest recognisable red cell precursor. The nucleus fills almost the entire cell and the nuclear membrane is sharply demarcated from the cytoplasm. The nuclear chromatin is arranged in finely woven threads and stains violet. There are two or three nucleoli. The cytoplasm stains a deep royal blue which sometimes shows an area of non-staining near the nucleus. I have been impressed by the royal blue staining of the cytoplasm which makes this cell distinctive, a fact not stressed in the literature. Other names for this cell are lymphoidocyte (Pappenheim 1911), primitive erythroblast (Cunningham, Sabin and Doan 1925), stem cell promegaloblast (Downey 1927), Megaloblast (Fowler 1945, Whitby and Britton 1946, Rhodes 1944, Krache 1941, Vogel and Bassen 1939, Dameshek 1935), Karyoblast (Osgood and Ashworth 1937), Erythroblast I (Davidson, Davis and Innes 1943), stem cell promegaloblast (Downey 1927).



The cell begins to ripen and by a series of almost imperceptible changes the nucleus becomes smaller, the chromatic network gradually condenses, and haemoglobinization of the cytoplasm procedes until finally a non-nucleated erythrocyte is formed, and gains access to the blood stream. At certain arbitrary stages in this procedure the cells are named.

PRO-ERYTHROBLAST.

The nucleus is somewhat smaller than the preceding cell, but still has a well defined network. Nucleoli are present and the chromatin stains a deep violet. The cytoplasm is basophil but not so deep a blue as in the haemocytoblast.



NORMOBLAST A. (15-24 u)

The nucleus takes in about two-thirds of the space of the cell and its chromatin structure is now condensing and may be arranged in small clumps and stains dark violet. There are still vestiges of nucleoli. Haemoglobinization has commenced in the cytoplasm which has a muddy grey appearance. Other names given to this cell are :- Prokaryocyte (Osgood /

(Osgood and Ashworth 1937), orthochromic normoblast (Downey 1927), macroblast (Piney 1930), Erthroblast II (Davidson, Davis and Innes 1943), early erythroblast (Whitby and Brittom 1946, Krache 1941 and Fowler 1945) early basophil normoblast (Israels 1943).

NORMOBLAST B. (10-15 u)

The nucleus has further condensed and is arranged in coarse clumps frequently arranged, like the spokes in a cart wheel, this was particularly noticeable in my preparations. The staining of the chromatic is almost black. The cytoplasm which is about one-third of the cell is polychromatic. Other names for this cell are :- Pro-normoblast (Downey 1927), macro-normoblast (Piney 1930), erythroblast III (Davidson, Davis and Innes 1943). Late erythroblast (Whitby and Britten 1946), karyocyte (Osgood and Ashworth 1937).

NORMOBLAST C. (8-10 u)

The nucleus is now pyknotic and stains black and is frequently eccentrically placed. The cytoplasm is fully haemoglobinized and therefore red. This cell is called by most authors a normoblast. Other names are erythroblast IV (Davidson, Davis and Innes 1943) and metakaryocyte (Osgood and Ashworth 1937).

When there is any serious disturbance in the supply of the haemopoietic factor to the maturing nucleated red cells an entirely different set of cells appear and are called megaloblast A, B and C., according to their maturity.

MEGALOBLAST A. (15-24 u)

The cell is fairly large and has a nucleus which fills more than two-thirds of the cell. The chromatin network of this nucleus is arranged in an open network but contains no nucleoli. The nucleus stains about the same colour as a normoblast A, which is a dark violet. The cytoplasm is partly haemoglobinized and so stains a muddy grey. The other name given to this cell is a haemoglobinized megaloblast (Whitby and Britton 1943).

Upon page

MEGALOBLAST B. (15-17 u)

This is a slightly smaller cell and the nucleus has not ripened much more, so it still has a relatively open network appearance, and still stains a dark violet. The cytoplasm is fully haemoglobinised and so is eosinophilic.

MEGALOBLAST C. (10-15 u)

The nucleus suddenly ripens and becomes pyknotic and the cytoplasm is eosinophilic. Some workers find this cell difficult to distinguish from a normoblast C. but it has not been my experience. It is always much larger than a normoblast C.

CHOICE /

CHOICE OF TERMINOLOGY.

THE MEGALOBLAST. There is much confusion over the name megaloblast and this arises because authors have given this name to entirely different cells found in widely varying circumstances. Ehrlich (1907) first applied the name megaloblast to a large haemoglobinised cell with a finely reticulated nucleus, found in the bone marrow and peripheral films of patients suffering from pernicious anaemia in relapse. Sabin (1922) called the primitive non-haemoglobinized cell of the developing cheek a megaloblast, and Doan, Cunningham and Sabin (1925) called the generations of developing red blood cells in the human embryo megaloblasts. Peabody (1927) called the large cells with scanty basophilic cytoplasm which he found in the bone marrow of patients with pernicious anaemia, megaloblasts, and Witts (1932) considered the megaloblast represented an early stage of red cell development requiring the haemopoietic principle for its further development. Israels (1939) pointed out that the appearance of a large haemoglobinized cell, with an open reticular nucleus, was strictly limited to conditions in which there was interference with the proper activity of the haemopoietic principle, and emphasized that this cell is always a pathological cell. I have only found this cell under these conditions, and in common with other authors (Israels 1939 /

TABLE M.

NOMENCLATURE USED BY DIFFERENT AUTHORS.

Davidson et alia		Whitby and Britton		Israels		Osgood and Ashworth	
Normal	H.F. def.	Normal	H.F. def.	Normal	H.F. def.	Normal	H.F. d
		Pro-erythroblast		Haemocytoblast		Karyoblast	
Erythroblast I	Haemoglobinised cell included in erythroblast II.	Megaloblast	Haemoglobinised megaloblast	Pro-erythroblast		Prokaryocyte	Haemog prokar
Erythroblast II		Early erythroblast		Normoblast A.	Megaloblast A.	Karyocyte	
Erythroblast III		Late erythroblast		Normoblast B.	Megaloblast B.	---	
Erythroblast IV		Normoblast		Normoblast C.	Megaloblast C.	Metakaryocyte	

1939, Davidson 1942, Peabody 1927) have observed their rapid disappearance from the bone marrow of patients with pernicious anaemia after an injection of liver. I am content, therefore, to agree with Israels (1939) that the term megaloblast should be reserved for the pathological cells found only in conditions where there is a disturbance in the activity of the haemopoetic principle. Again agreeing with Israels I think it is wise to indicate the maturity of the cell by the recognition of three types of megaloblast, A, B and C.

THE NORMAL RED CELL SERIES. The names given to the various cells concerned in the maturation of the normal red cells by the leading authors are given in table M. Davidson (1943) in a praiseworthy attempt to achieve simplicity did not recognise the primitive haemoglobinized cell as a distinct entity, but regarded it as a variant of his Type I erythroblast and writes thus - "Megaloblastic erythopoiesis is based upon the presence of a greatly increased frequency of the primitive basophil erythroblast I. Type II are also numerous, these may show various degrees of cytoplasmic haemoglobination and are characterised by the loosely reticulated openwork structure of their nuclei, which presents an appearance quite distinct from the lumpy condensed nuclei of the erythoblasts of equivalent degrees of haemoglobination seen in normoblastic marrow.". He did not mention megaloblast /

megaloblast in his terminology of the cells but did apply the term megaloblast to the type of erythropoiesis he wished to describe. The same kind of criticism can be directed against the term normoblastic erythropoiesis which he used when "the vast majority of the red cell precursors have compact condensed nuclear chromatin. An occasional primitive basophil erythoblast may be seen but the remaining erythoblasts which may include a proportion of the cells with basophilic cytoplasm varying with the degree or type of anaemia present are characterised by displaying lumpy chromatin patterns, the degree of condensation of which increases with the haemoglobinization of the cytoplasm". Again confusion arises if it is not realised that the erythoblast III and IV correspond to the normoblast. It is for these reasons that I think the objections to this terminology outweigh the advantages gained by its simplicity. I therefore rejected this system.

The terminology used by Whitby and Britton (1946) is unsatisfactory as they used the term megaloblast for one of the early primitive nucleated red cells which contained no haemoglobin. In the absence of proper quantities of the haemopoietic principle they said - "megaloblasts and early erythoblasts unable to mature in a normal manner become haemoglobinized and resemble in size and appearance and nuclear structure the haemoglobinized embryonic megaloblast of Ehrlich". They /

They have no distinct name for the primitive haemoglobinized cell. Another unsatisfactory feature of this system is the introduction of three different names for only five cells.

Osgood and Ashworth's terminology (1937) is very simple, as only one stem name is used, but they call the haemoglobinated nuclear forms produced by haembpoietic factor deficiency prokaryocytes, thereby making no distinction between these cells and those early ones found in normal maturation. This causes confusion and lessens the value of this system.

Israels (1939) in his classification made a clear distinction between the normal erythropoiesis and the erythropoiesis found in haemopoietic factor deficiency. He used the term megaloblast to describe the cells which were found in bone marrow smears consequent upon a deficiency in the haemopoietic factor. As I think this terminology prevents confusion I have adopted it, and it can be summarised thus :-

HAEMOCYTOBLAST
PRO-ERYTHROBLAST

NORMOBLAST A	MEGALOBLAST A
NORMOBLAST B	MEGALOBLAST B
NORMOBLAST C	MEGALOBLAST C
ERYTHROCYTE	MACROCYTE

Israels' classification has not escaped criticism. Davidson et alia (1942) stated in their experience with /

with differential marrow films the classification is an impracticable working proposition for in numerous instances they found it impossible to decide with certainty whether a given cell should be classified in the early normoblast or late megaloblastic series. Thomson (1939) on the other hand praised Israels' classification and wrote that many haematologists do not recognise the megaloblast B but restrict the term to pro-erythroblast and megaloblast A, and many mistakes arise in interpretation of myelograms by including normoblast A as megaloblasts.

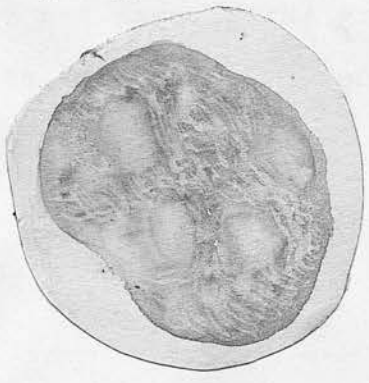
After prolonged practice, I have experienced no difficulty in the differentiation of these cells, with the possible exception of distinguishing a normoblast C from a megaloblast C. Even here a mistake can be avoided by careful attention to the size of the cell. The megaloblast C is always a much larger cell than the normoblast C.

THE WHITE CELL SERIES. There are three distinct groups of cells in this series. They are the myeloid, lymphoid and monocytic groups.

THE MYELOID GROUP.

The most primitive cell is the myeloblast and it is difficult to distinguish it from the haemocytoblast especially in normal smears, but I was able to study the /

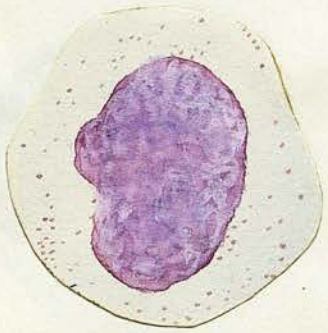
the structure of this cell from the films I prepared from a case of acute myelogenous leukaemia. It is a medium sized cell (11-15 u), with a centrally placed nucleus, which has an open reticular network and stains a rather pale violet and contains 3 - 5 nucleoli. My impression is, that the nucleus stains a lighter shade of violet than the haemocytoblast. The pale blue cytoplasm forms a rim round the nucleus. I have noticed, in some of these cells that the cytoplasm has a somewhat reticulated appearance as if there was already a suspicion of granulation. I am in agreement with Davidson (1943) who stated that this cell is usually smaller than the haemocytoblast (erythroblast I). This cell was called a myeloblast by Davidson (1943), Israels (1939), Vogel and Bassen (1939), Dameshek (1935), Hynes (1939) Mendell and Meranze (1942) and Downey and Kato (1927), a haemocytoblast by Ferrata (1918), a lymphoidocyte by Pappenheim (1912) and a granuloblast by Osgood and Ashworth (1937).



THE PROMYELOCYTE.

This cell is a little smaller than its predecessor. The nuclear chromatin is beginning to condense and contains vestiges of nucleoli. The nucleus /

nucleus is still large and slightly indented and stains a dark violet. Scattered throughout the light blue cytoplasm are a few asurophilic granules which I have noticed always stain the exact colour of the nucleus. This cell is called a megaloblast by Davidson (1943), Dameshek (1937), Mandell and Meranze (1942), Vogel and Bassen (1939), a premyelocyte by Hynes (1939) and an early myelocyte by Israels (1939).



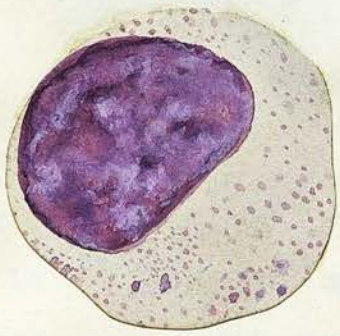
THE MYELOCYTE.

The nucleus is now kidney shaped and the chromatin is condensed so that it appears lumpy. The granules are basophilic, numerous and coarse. This cell is called a myelocyte by most authorities except Osgood and Ashworth (1937) who name it the granulocyte. After this stage the granules differentiate into their different colours - neutral, basophil and eosinophil.

THE /

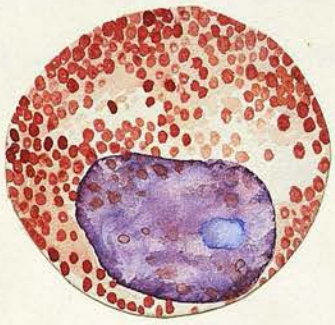
THE NEUTROPHIL METAMYELOCYTE.

This cell is approaching the size of the mature forms. The nucleus is bean or kidney shaped with a coarse chromatin structure. The lumpy parts I have noticed stain black, whilst the remainder stains a deep violet. The neutrophil granules are always fine and remain distinct and are scattered throughout the cytoplasm which is still a light blue colour.



THE EOSINOPHIL METAMYELOCYTE.

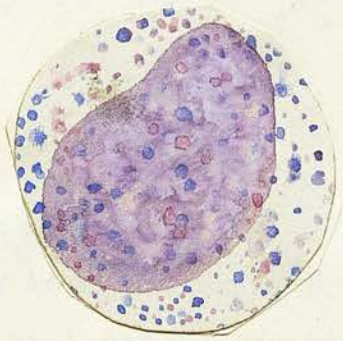
The nucleus is bean or kidney shaped with coarse lumpy chromatin, the stain varying in colour from black to deep violet. The cytoplasm is filled with coarse red granules, which in a few instances I have noticed are superimposed upon the nucleus.



THE BASOPHIL METAMYELOCYTE.

I have only seen this cell on two or three occasions. It is about the same size as the other metamyelocytes /

metamyelocytes with a similar nucleus, but the cytoplasm is filled with irregular coarse granules, ranging in colour from a deep blue to black, many are superimposed upon the nucleus, and may obscure its structure.



The term metamyelocyte is used by the majority of haematologists, is called a late myelocyte by Israels (1939), a myelocyte C by Cunningham, Sabin and Doan (1925), a non-segmented myelocyte by Vogel (1939), a metagranulocyte by Osgood and Ashworth (1937).

THE BAND CELL.

These again have either neutrophil, eosinophil or basophil granules. These cells are young polymorphonuclear cells and are not illustrated. The polymorphonuclear cells are illustrated later.

THE NEUTROPHIL BAND CELL.

The nucleus is curved, rod shaped or curved upon itself. The chromatin is dark and condenses, and the cytoplasm /

cytoplasm which is plentiful is full of fine neutrophilic granules.

THE EOSINOPHILIC BAND CELL.

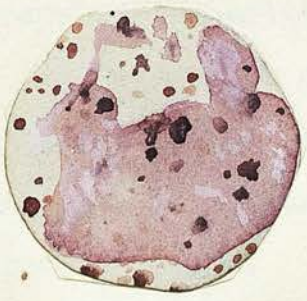
The nucleus is like a curved or coiled rod, which may be constricted in the middle. Coarse red granules, may be superimposed upon the nucleus.

THE BASOPHILIC BAND CELL.

The nucleus is curved or rod shaped and fills a large proportion of the cell. The cytoplasm is filled with coarse black or blue granules, many of which are superimposed upon the nucleus.

THE BASOPHIL LEUCOCYTE.

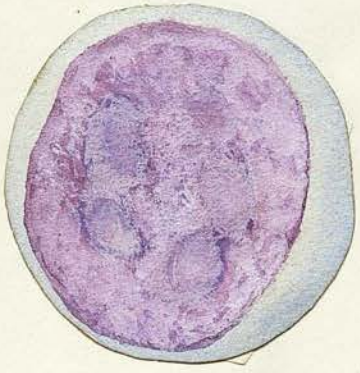
The nucleus, which in my sections was never properly lobed, is condensed and stains almost black, and fills about the whole of the cell. The cytoplasm is filled with blue or black coarse granules, many of which are superimposed upon the nucleus.



THE LYMPHOID SERIES.

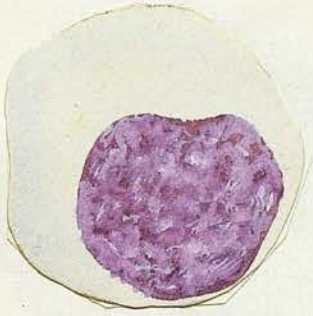
THE LYMPHOBLAST.

A medium sized cell 15-20 u. The nucleus fills almost the entire cell and is finely reticulated and contains 2 - 3 nucleoli. The nuclear chromatin stains violet. A small rim of light blue cytoplasm surrounds the nucleus. I have sometimes observed a few asurophil granules in the cytoplasm. The majority of authors agree with this name but Ferrata (1918) calls it a haemocytoblast, Downey and Kato (1917) a myeloblast, and Pappenheim (1912) a lymphoidocyte.



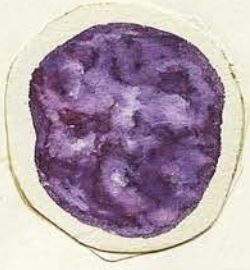
LARGE LYMPHOCYTE (12-15 u)

A large eccentrically placed nucleus fills almost two-thirds of the cell. The chromatin network is coarse and even lumpy in places and stains unevenly. The cytoplasm stains a clear light blue and scattered here and there throughout it may be fine asurophil granules. These granules are present in many of the cells I observed. Again there is common agreement to the name, except Downey and Kato (1927) call it an atypical leucocytoid lymphocyte, and Osgood and Ashworth (1937) a prolymphocyte.



SMALL LYMPHOCYTE (7-8 u)

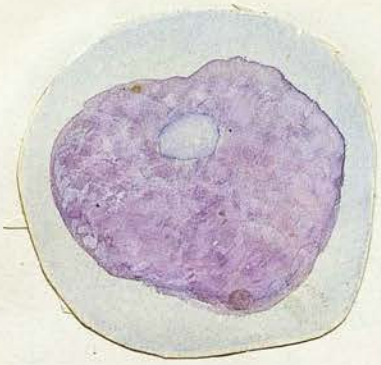
A round, intensely blue nucleus fills almost the entire cell and is surrounded by an agranular rim of light blue cytoplasm. This rim of cytoplasm, in my smears from cases of chronic lymphocytic leukaemia, seems to be easily detached from the nucleus.



MONOCYTIC SERIES.

MONOBLAST (20-30 u)

This is a very large cell with a round or oval nucleus which fills most of the cell. The chromatin is arranged in a very fine skein and stains a light violet and always contains one or two clearly demarcated nucleoli. The cytoplasm is a light greyish blue which gives it a typical ground glass appearance. I have never been able to make out any granules. Many haematologists cannot differentiate this cell from a myeloblast or a lymphoblast.

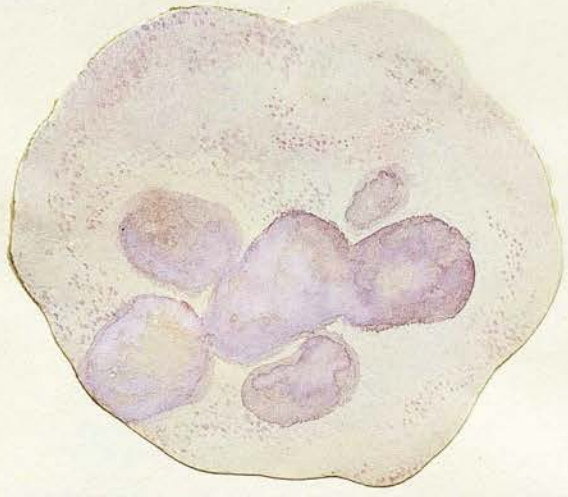


MONOCYTE (15-20 u)

The nucleus may be kidney shaped, bean shaped, oval lobulated or even convoluted. The chromatin network is coarse but still distinct and stains a pale violet. There are no nucleoli. The cytoplasm is a light greyish blue which gives it a typical ground glass appearance. I have never seen granules in the cytoplasm but Osgood and Ashworth (1937) state "the finely scattered asurophil granulation is easily overlooked when poorly stained or when viewed through an indifferent microscope.

MEGAKARYOCYTE (40 u)

A large cell with an irregular outline. It is formed by the fusion of several endothelial cells. It contains many nuclei which are arranged in an irregular ring and may contain a few coarse granules. The cytoplasm stains purple. Individual granules cannot be seen but the cytoplasm does not stain evenly.



UNCLASSIFIED CELLS.

Two other cells, which I am not prepared to classify appeared in my smears with regularity. These are the plasma cell and the haematogone.

THE PLASMA CELL (12-15 u)

The nucleus is always eccentrically placed. The chromatin is lumpy and these are arranged like the spokes in a cart wheel. The chromatin stains unevenly and is mainly dark violet. The cytoplasm stains a deep blue and usually contains vacuoles but never granules.

THE HAEMATOGONE.

I have observed quite a number of these cells especially in my normal smears. The cell is remarkably like a small lymphocyte but it is smaller than a lymphocyte. The nucleus is composed of a dense chromatin network and is surrounded by a rim of pale blue cytoplasm. Maximoff and Bloom (1930) considered it was an undifferentiated primordial cell or haemocytoblast, Falconer and Leonard (1941) called it a primitive cell but did not attempt to place it in any category, whilst Vogel, Erf and Rosenthal (1937) called it the mystery cell /

TABLE N.
NORMAL MYELOGRAMS BY VARIOUS AUTHORS.

Cells	Arinkin (1908)	Tampka and Braun (1932)	Holmes and Brown (1933)	Segerdahl (1935)	Nordenson (1935)	Reich (1935)	Young and Osgood (1935)	Rohr (1935)	Markoff (1936)	Vogel et alia (1937)
Haemocytoblast	12.3	12.2	12.1	12.3	18.2	14.8	14.8	30.1	13.06	29.48
Pro-erythroblast	1.4			-	0.4			4.4	0.01	-
Normoblast A				-	1.3	12.7			2.5	7. 1
Normoblast B	16.5	4.9	6.9	-	-			9.3	-	-
Normoblast C		7.3	5.2	-	16.5		12.7	16.4	-	-
Myeloblast	1.7	2.4	2.4	1.3	2.7	2.0	0.6-1.3		10.55	22.6
Promyelocyte	1.9	7.0	7.0	1.5	10.3		3.9-9.5		2.5	1.6
Myelocyte	7.7	5.4	-	14.8	3.0	2.0	1.3-6.6		1.5	0.1
Metamyelocyte		-			22.2		1.3-8.8		17.5	21.5
Band cell	49.	13.4	20.7	8.3	4.4	15.0	24.4-41		12.0	-
Leucocyte		16.4	17.4	22.3	16.6	25.0			32.5	30.2
Plasma cell	-	0.9	-	0.5	1.		13.3-17 0.5 -		-	3.4
Megakaryocyte	3.9	2.2	-	-	1.	-	- -		0.5	-
Lymphocyte	11.9	2.6	24.9	19.5	10.	10.4	10.4-11		15.0	-
Monocyte	5,0	1.2	9.0	0.1	1.0	2.1	- -		1.0	-

TABLE N.

Vogel et alia (1937)	Klima (1938)	Scott (1939)	Hynes (1939)	Mandell and Meranze (1942)
29.48	26.5	0.43	0.4	1.0
-	5.0	-	2 - 7	5.3
7. 1	7.0	1.95	-	-
-	-	-	-	-
-	-	-	7.0 -19.0	9.1
22.6	18.0	-	-	-
1.6	1.0	1.7	2.5	-
0.1	3.0	4.5	5.5	1.0
21.5	14.0	13.05	2.5- 12.0	0.5
-	14.0	15.70	2.0- 4.0	10.0
30.2	11.6	16.0	9.0- 30.0	12.4
3.4	18.0	14.7	-	3.5
-	-	-	-	0.1
-	-	10.85	5.0- 20.0	13.5
-	-	0.83	0.5	0.7

cell of the marrow, and I am quite content to agree with them.

It was always impossible to classify all the cells seen in bone marrow smears and many nuclei without cytoplasm were always present.

IMPORTANCE OF MYELOGRAMS.

Marked variations in the percentages of each important cell is illustrated in Table N. As there are such wide variations in the normal figures only grosser changes in the cell percentages are significant Experience gained from the examination of films is the only real guide to diagnosis in doubtful cases. Any attempt to arrive at a diagnosis by placing reliance in minor variations in the percentages of the cells away from normal, undoubtedly leads to serious errors. The marrow picture was diagnostic after the examination of a few fields, in the majority of my cases, but a myelogram was always constructed as it was interesting to compare the percentages of the cells in the same diseases affecting different patients. A myelogram gave the greatest help in the diagnosis /

diagnosis of a case of chronic lymphocytic leukaemia, where a significant rise in the total lymphocyte count established the real cause of the patient's illness. In two other cases a diagnosis of chronic myelogenous leukaemia was suspected because of a raised leucocyte count and many myelocytes in the blood film. Myelograms in both these patients were normal so the diagnosis of leukaemia was abandoned. The importance of myelograms will be fully illustrated in other sections.

P A R T II.

THE VALUE OF THE EXAMINATION OF THE MARROW IN MYELOGENOUS LEUKAEMIA.

There are two commonly accepted variations of the nomenclature used in the classification of the leukaemias involving the myeloid series. They are:

Acute myelogenous leukaemia.
Subacute myelogenous leukaemia.
Chronic myelogenous leukaemia.

and

Acute leukaemic myelosis.
Subacute leukaemic myelosis.
Chronic leukaemic myelosis.

The real difficulty arises over the nomenclature employed to describe the group of cases with a low peripheral white cell count. Forkner (1938) in his book listed the following names applied to this group. Pseudoleukaemia, anaemic pseudoleukaemia, pseudo-leukanaemia, splenic pseudoleukaemia, aleukaemic leukosis, aleukaemic myelogenous leukaemia, aleukaemic reticulosis, aleukaemic reticulo-endotheliosis, aleukaemia, aleukia, aleukaemic erythroblastosis, aleukocythaemic leukosis, medullary leukaemia, aplastic leukaemia, leukanaemia, subleukaemic leukaemia and leukopenic leukaemia.

Perhaps the most popular term in Britain is aleukaemic myelogenous leukaemia, which is a contradiction /

contradiction of terms, but is in harmony with the myelogenous nomenclature. Aleukaemic myelosis is the best suited to fit in with the leukaemic myelosis variation which I have chosen, and so is adopted in this thesis.

It is accepted without any dissension that the marrow is invaded in acute leukaemic myelosis with myeloblasts and promyelocytes, and in chronic leukaemic myelosis by more mature cells such as the myelocytes and metamyelocytes. This invasion takes place whether or not there is an increase in the white cells of the blood. The white cells are slightly more mature in the blood than those found in the marrow. Examination of the bone marrow is unnecessary when the diagnosis is obvious from the clinical features and blood examination, and it is in the aleukaemic myelosis group that the knowledge of the marrow cytology gives the greatest help in diagnosis. The examination of the marrow is also of importance, where chronic leukaemia myelosis is confused with other conditions and often decides the diagnosis. The value of marrow examination in this respect is not stressed by other writers.

Cases from my series will stress this importance.

For the purpose of illustrating these statements I examined the marrow in three groups of patients suffering from leukaemic myelosis.

(1) /

(1) Those in which a confident diagnosis had been made from the peripheral film and clinical features.

(2) Those suspected to have leukaemic myelosis with an aleukaemic blood picture.

(3) Those in which the diagnosis of chronic leukaemic myelosis was suspected for one reason or another.

(a) Differentiation from lymphadenoma.

(b) Differentiation from leukaemoid states.

In the first group I always found the accepted marrow picture, i.e., great infiltration with myelocytes and metamyelocytes, and depending on the acuteness of the process, promyelocytes and myeloblasts.

Details of one case are given as representative of this group.

Case Z. L.F. Aged 41.

History. Six weeks prior to admission he was working as a roadman and began to suffer from tiredness, loss of energy, and all the common symptoms of a severe anaemia.

Examination.

General. There was a swinging intermittent temperature, rapid pulse and moist skin. A mild generalized enlargement of all his lymph glands with moderate increase in the size of the liver and spleen were discovered.

Haemopoietic /

Haemopoietic system. R.B.C. 2,500,000. Hb. 35%. C.I. 0.7. W.B.C. 41,000. Film. The red cells showed mild haemoglobin deficiency and the differential white cell count was

Myeloblast	6	per cent
Promyelocyte	8	" "
Myelocyte	22	" "
Metamyelocyte	18	" "
Polymorph	42	" "
Lymphocyte	4	" "

Marrow examination. The film was invaded by promyelocytes and myelocytes. A few myeloblasts were present. Details of the myelogram were

Myeloblast	10	per cent
Promyelocyte	55	" "
Myelocyte	20	" "
Metamyelocyte	5	" "
Polymorph	10	" "

There were no red cells precursors present. The marrow appearances in this and many other similar patients confirmed that the blood reflects the marrow cytology and that the percentage of the more immature cells is slightly higher in the marrow than found in the blood.

The aleukaemic group.

Case A 1. B. McK. Aged 67.

History. For nine months had suffered from signs and symptoms of a severe anaemia, such as, intense pallor /

pallor, breathlessness, tiredness and irritability.

Examination.

Haemopoietic system. R.B.C. 1,100,000. Hb. 26%. C.I. 1.2 (approx.). W.B.C. 2,400. Film. There was marked variation in size and shape of the red cells with an occasional nucleated form of the type normoblast C. The differential white cell count was

Polymorph	68	per cent
Lymphocyte	24	" "
Monocyte	6	" "
Eosinophil	2	" "

The B.T. and C.T. were normal.

Other systems. There was no enlargement of the lymph glands, liver or spleen. Nil else found.

Marrow examination. The marrow was invaded by myelocytes and promyelocytes, an occasional cell of the nucleated red cell series was seen.

Prior to marrow examination, the diagnosis rested between pernicious anaemia, aplastic anaemia, and a macrocytic anaemia produced by other conditions of the bone marrow. Without this examination it was impossible to differentiate these three conditions.

Case A 2. A.R. Aged 73.

History. For four weeks he had been feeling very ill with pains in his stomach. He collapsed and was admitted to hospital for further investigation.

Lymphatic /

Lymphatic system. A few scattered enlarged lymph glands were discovered but there was no clinical evidence of an increase in the size of the liver or spleen.

His blood pressure was 180/104. Little else was discovered. Before death he became uraemic.

Haemopoietic system. R.B.C. 1,700,000. Hb. 30%. C.I. 0.9. W.B.C. 1,400. Film. There were a few normoblasts B. and C. present. The red cells were well filled with haemoglobin and varied in size and shape. The differential white cell count was

Myelocyte	4	per cent
Polymorph	62	" "
Lymphocyte	26	" "
Eosinophil	2	" "
Monocyte	6	" "

A sternal puncture revealed a marrow infiltrated with promyelocytes and myeloblasts. Details of the myelogram were

Myeloblast	6	per cent
Promyelocyte	4	" "
Myelocyte	62	" "
Metamyelocyte	20	" "
Polymorph	3	" "
Normoblast B.	1	" "
Normoblast C.	4	" "

Before sternal puncture the diagnosis amongst many other conditions might have been pernicious anaemia complicated by a certain amount of white cell irritation /

irritation in the marrow, aplastic anaemia, and aleukaemic myelosis. The marrow findings made the last diagnosis obvious.

Differentiation from lymphadenoma.

Case A 3. L.G. Aged 26.

History. For one year had been suffering from loss of energy, tiredness, anorexia, breathlessness on exertion, and he had also noticed the gradual appearance of lumps in his neck and a swelling in the left side of his abdomen.

Examination.

Lymphatic system. The enlarged glands involved the posterior cervical chain, more on the right than the left. Each gland was about the size of a pigeon's egg, and was hard, discrete, painless and not in any way attached to the surrounding tissues. The spleen was moderately enlarged and not tender. The liver appeared normal.

Haemopoietic system. R.B.C. 4,300,000. Hb. 83%. C.I. 1. W.B.C. 12,200. Differential white cell count

Polymorph	70	per cent
Lymphocyte	25	" "
Monocyte	5	" "

A careful search of the film failed to reveal any early cells of the myeloid series. The diagnosis at this stage rested between lymphadenoma and chronic leukaemic myelosis.

Marrow /

Marrow examination. Normal.

Chronic leukaemic myelosis was ruled out of the diagnostic picture by these findings and lymphadenoma was strongly suspected, and this diagnosis was subsequently confirmed by pathological examination of one of the glands.

Case A 4. A. C. Aged 21.

History. For one year had suffered from loss of energy, fatigue, lethargy and gradually increasing pallor.

Examination.

Lymphatic system. There was a generalised adenopathy. Each gland was about the size of a marble, painless, discrete and moderately hard. The spleen was slightly enlarged and the liver was normal.

Haemopoietic system. R.B.C. 3,800,000. Hb. 52% C.I. 0.7 (approx.) W.B.C. 8,200. Film.

Polymorph	74	per cent
Myelocyte	2	" "
Lymphocyte	20	" "
Monocyte	4	" "

These clinical findings suggested a mild lymphadenoma.

Marrow examination.

Myeloblast	1	per cent
Promyelocyte	22	" "
Myelocyte	18	" "
Metamyelocyte	16	" "
Polymorph	30	" "
Normoblast B	2	" "
Normoblast C	19	" "

The clinical findings and peripheral blood examination pointed to a diagnosis of lymphadenoma, but the sternal marrow ruled out this and the diagnosis of an early chronic leukaemic myelosis was made.

Differentiation from leukaemoid states.

Peripheral blood pictures indistinguishable from those familiar in chronic leukaemia myelosis sometimes occur in

(a) Acute infections, i.e., meningococcal meningitis, diphtheria and tuberculosis.

(b) Intoxication, e.g., eclampsia, severe burns and mercury poison.

(c) Malignancy.

(d) Occasionally following severe haemorrhages or sudden haemolysis of blood.

Marrow examination is only of help in differentiating on the leukaemoid picture consequent upon an acute infection, from chronic leukaemic myelosis. This is illustrated by the following case.

Case A 5. E.J. Aged 18.

History. For many months he had suffered from increasing fatigue and general malaise.

Examination.

General. There was no enlargement of the spleen or liver but there was a mild general adenopathy. No evidence of sepsis was found at first.

Haemopoietic /

Haemopoietic system. R.B.C. 3,400,000. Hb. 50% C.I. 0.76. W.B.C. 16,400. Film: The differential white cell count was

Polymorph	82	per cent
Myelocyte	8	" "
Lymphocyte	8	" "
Monocyte	2	" "

The possibility of an early leukaemia was suspected. Marrow examination revealed a mild polymorphonuclear increase. The myelogram was

Myeloblast	1	per cent
Promyelocyte	1	" "
Myelocyte	6	" "
Metamyelocyte	10	" "
Band cell	15	" "
Polymorph	45	" "
Normoblast A	1	" "
Normoblast B	1	" "
Normoblast C	15	" "
Lymphocyte	5	" "

This result enabled the serious diagnosis of leukaemic myelosis to be abandoned. About a week after this examination the signs and symptoms of an acute infection of his left antrum arose and this diagnosis was confirmed by an E.N.T. consultant.

It is generally accepted that the later cells of the myeloid series are increased in such an acute infection, but there is lack of uniformity of opinion /

opinion whether the marrow can be myeloblastic or not in leukaemoid states due to acute infections. Scott (1939) observed that a myeloblastic marrow usually necessitated a diagnosis of myelosis, but admitted the possibility of a myeloblastosis in occasional cases of leukaemoid reaction remained. Leibowitz (1938) reported a proved case of tuberculosis with a blood picture identical with that of myelosis with many myeloblasts and myelocytes in the marrow, but Klima (1938) denied that there was ever any evidence of a myeloblastic reaction in the marrow in acute infection.

Examination of the marrow was of minor importance in the confirmation of a diagnosis of chronic leukaemic myelosis complicating a case of pernicious anaemia. There are only two references to pernicious anaemia ending in chronic leukaemic myelosis in the literature. (Sterne et alia, 1941, and Wolley, 1944). When such a rare occurrence is encountered it is wise to confirm such a diagnosis by every means possible. The examination of the marrow confirmed the diagnosis of chronic leukaemic myelosis complicating a proved case of pernicious anaemia.

Case A 6. I.P. Aged 64.

History. Since 1940 had been suffering from pernicious anaemia which had responded satisfactorily to liver injections until early in 1946 when she began to suffer from increasing pallor, tiredness, breathlessness, etc.

Examination. /

Examination.

There was a generalised adenopathy. Liver and spleen were also enlarged.

Haemopoietic system. R.B.C. 3,760,000. Hb. 58% C.I. 0.78. W.B.C. 36,000. Film. A differential white cell count showed

Polymorph	33	per cent
Lymphocyte	5	" "
Monocyte	4	" "
Eosinophil	4	" "
Myeloblast	3	" "
Myelocyte	48	" "
Eosinophil myelocyte	3	" "

Sternal marrow examination. The film was invaded throughout by myelocytes and metamyelocytes.

The diagnosis of chronic leukaemic myelosis was therefore confirmed.

THE VALUE OF MARROW EXAMINATION IN MONOCYTIC LEUKAEMIA

There are no references in the literature to an aleukaemic form of monocytic leukaemia. The only case of monocytic leukaemia studied in this series was an example of this type and marrow examination proved very useful in helping to arrive at the correct stem cell diagnosis.

Case A 7. L.W. Aged 62.

History. For two years had suffered from periodic attacks of weakness, breathlessness and fits of depression. A day before admission he was seized with a severe precordial pain, and admitted with a provisional diagnosis of coronary thrombosis.

Cardio-vascular system. The heart was dilated and there was a soft systolic murmur in the mitral area which disappeared when he sat up. The B.P. 110/80

Lymphatic system. One slightly enlarged gland was found in the right side of the neck.

Haemopoietic system. R.B.C. 1,500,000. Hb. 40%. C.I. 1.3. W.B.C. 1,000. Film. The red cells showed a marked variation in size and shape and were well filled with haemoglobin. No nucleated red cells were seen. The differential white cell count was

? Early white cells	90 per cent
Myelocyte	5 " "
Lymphocyte	2 " "
Polymorph	3 " "

It /

It was obvious from these findings that the cause of his illness was an aleukaemic leukaemia, but the stem cell responsible for this process remained in doubt. Close examination of the undifferentiated white cells gave the impression that they might have contained granules and the nuclei of some of the cells were indented.

Sternal marrow examination. The marrow was invaded by large immature white cell precursors. The nuclei of these cells were irregular in shape and were composed of a rather fine reticular chromatin. No nucleoli were present. The cytoplasm was greyish-blue and had the definite appearance that granules were present although individual granules could not be distinguished. There was an increase in the myelocytes.

Details of the myelogram

Monoblast	75 per cent
Myelocyte	12 " "
Polymorph	10 " "
Normoblast C.	3 " "

This marrow appearance was that of an acute monocytic leukaemia sometimes referred to as the Naegeli type, because of the myelocytes which were found increased in both the film from the marrow and blood.

THE IMPORTANCE OF EXAMINATION OF THE MARROW IN DISEASES AFFECTING THE LYMPHOID TISSUE.

The diagnostic importance of sternal puncture in diseases resulting from disordered proliferation of the lymphoid tissue cannot be evaluated without a clear understanding of their classification, over which there is much disagreement and confusion. The best classification of this group of diseases was given by Wiseman (1935 and 1943) and also used by Bethell (1943) Although no satisfactory concept of the identity, life history and specific functions of the lymphocyte has yet been discovered, normal lymphopoiesis is thought to start by the multiplication of the fixed reticulum cell with the production of a primitive round cell. These two cells are theoretically totipotential, but because of environmental conditions the conditioning stimuli received, or both, these precursors mature only in the direction of the next cell which is the lymphoblast. This cell and all others produced from it are fully differentiated and are incapable of producing any other cells other than lymphocytes. Normal maturation proceeds through the large lymphocytes to the small lymphocytes. Lymphopoiesis may also be affected to an important degree by a rapid amitotic division of the large and small lymphocytes. Abnormal lymphopoiesis is theoretically possible by multiplication without maturation of the cells at any level since all the cells /

cells have the power to reproduce themselves, furthermore neoplastic changes may occur at any of the stages in the development. In other words, there are three mechanisms by which lymphoid tissue can become hyperplastic :-

(1) When there is excessive multiplication and maturation of the cells occurring simultaneously, e.g., infectious mononucleosis.

(2) When there is excessive multiplication with little or no concurrent maturation, e.g., lymphocytic leukaemia.

(3) When there is multiplication of the cells with neoplastic alteration, e.g., lymphosarcoma.

The first mechanism is not discussed further here as it is not a true leukaemia. In the second mechanism two cells can be involved in the multiplication - the lymphoblast and lymphocyte. When the former is implicated the disease is known as acute lymphoblastic leukaemia, and when the latter is implicated it is known as chronic lymphocytic leukaemia. These two diseases are quite distinct entities. The lymphoblast although not showing maturative phenomena does possess all the attributes of a neoplastic cell, whilst the lymphocytes do not possess any of these features of neoplasia.

The classification can be tabulated thus :-

A. Acute lymphoblastic leukaemia.

B. Acute lymphosarcoma cell leukaemia.

C. Chronic lymphocytic leukaemia.

This classification is by no means accepted by the majority /

majority of Haematologists and Pathologists. The chief objection to the classification is the inclusion of lymphosarcoma. The usual classification of this group of diseases is based on whether the disease is acute or chronic, leukaemic, subleukaemic or aleukaemic. These terms are sometimes combined with the dominant cell.

Acute (lymphocytic) leukaemia.

Subacute (lymphocytic) leukaemia.

Chronic (lymphocytic) leukaemia.

Aleukaemic (lymphocytic) leukaemia.

Others indicate the origin of the cell.

Acute lymphogenous or lymphoid leukaemia.

Subacute lymphogenous or lymphoid leukaemia.

Chronic lymphogenous or lymphoid leukaemia.

Aleukaemic lymphogenous or lymphoid leukaemia.

Yet another group of workers prefer to use nomenclature with a neoplastic significance, such as,

Acute, Subacute, Chronic, Aleukaemic leukaemic lymphoblastoma.

Many combinations of the above terms are also used. Finally, there has been some tendency to substitute the term lymphadenosis for leukaemia on the grounds that this term indicates a more fundamental haemopoietic change. The literal translation of the term leukaemia is "white blood".

Whether lymphosarcoma should be classified along with the leukaemias of lymphogenous origin presents a difficult problem. Textbooks of Pathology do not as a rule /

rule classify these diseases together, but while some, Beattie and Dickson (1943), Boyd (1945), Moore (1945), admit a close relationship, none deny it altogether. McCallum (1940) includes Sternberg's (1904) classification in his book, which includes both leukaemia and lymphosarcoma in the hyperplasia of the lymphoid tissue. Mallory (1914) placed lymphosarcoma, lymphadenoma and lymphogenous leukaemia in one category, lymphoblastoma. It is my opinion, which is based on the evidence already submitted, that lymphosarcoma should be classified with the lymphocytic leukaemias, but admit that absolute proof of the etiology is lacking. The classification I have adopted, therefore, is

A. Acute lymphoblastic leukaemia.

B. Acute lymphosarcoma cell leukaemia.

   (1) Without leukaemia.

   (2) With leukaemia.

C. Chronic lymphocytic leukaemia.

   (1) With leukaemia.

   (2) Without leukaemia.

The next question to be considered is where does the initial lesion of these three diseases start. Does acute lymphoblastic leukaemia start simultaneously in lymph nodes and the bone marrow? Does acute lymphosarcoma cell leukaemia start regionally in the lymph nodes and only in the terminal stages invade the marrow? Does chronic lymphocytic leukaemia arise spontaneously throughout the lymph tissue of the body? Obviously the diagnostic value of sternal puncture in the /

the early stages of these varieties of leukaemia is intimately linked with the accurate interpretation of these questions. The evidence on which satisfactory answers can be given to these and similar questions is confusing and will be reviewed before any further evidence based on the study of my own cases is advanced.

It is difficult to find many references in the literature which throw light on the starting point of lymphosarcoma. It is generally stated that the bone marrow is unaffected in lymphosarcoma. Kundat (1893) recognised that lymphosarcoma was a widespread growth arising from a group of lymph glands (more rarely from a single one) or from a tract of lymphoid tissue such as occurs in the intestinal wall, pharynx, etc. It fails to respect the capsule of the lymph glands and grows rapidly and invades adjacent tissue. Isolated metastases in distant organs are rare but when they do occur they remain as sharply localised infiltration of cells. He did not mention if the metastases were found in the bone marrow. McCallum (1940) studied eight cases. Three showed thoracic masses apparently derived from mediastinal lymph glands and limited in their extension to the thorax, whilst five were equally limited to the abdominal cavity. In two of these cases there were many cells in the bone marrow which resembled precisely those of a tumour growth. Scott (1939), Friedmann (1945), both found that the bone marrow was unaffected by lymphosarcoma, but this was /

was not the experience of Wiseman (1943) and Bethell (1943) who both found early invasion of the marrow in this disease.

Neumann (1870) showed that the marrow was infiltrated with lymphocytes in post mortem studies of chronic lymphocytic disease. Leube and Fleisher (1881) found normal cellular marrow in the femur of a gangrenous leg amputated from a patient said to have lymphocytic leukaemia. Banti (1904) stated that in the early stages of lymphocytic leukaemia the marrow is hyperaemic and that this is followed by hyperplasia of all the elements in the marrow. As the disease progresses focal collections of lymphocytes appear amongst this hyperplastic tissue. These foci enlarge and coalesce until the marrow is almost entirely replaced by lymphocytes. He was therefore quite certain that the early lymphocytic lesion is outside the bone marrow. No further references to this subject appear in the literature until Gulland (1925) basing his statments entirely on the clinical features of his patients and without examining the marrow wrote that in chronic lymphocytic leukaemia the process certainly starts in the lymphatic glands and gradually involves one group after another, the final anaemia does not set in until the marrow becomes completely involved. He went on to comment that there is no reason why the process should not start in any bit of the lymphatic tissue in the body. In cases where the disease starts outside the marrow the chronicity or the acuteness of the individual case seems /

seems to depend entirely on the period when the marrow becomes involved or on the rate at which the marrow space is filled with lymphocytes. The assessment of the value of this evidence is difficult without the knowledge of the marrow histology. It is tempting to accept the evidence of this experienced clinician without further questions but this cannot be contemplated as the argument could always be advanced that if sternal biopsy had been performed infiltration of the marrow, even if only in a minor degree, would have been found every time. Warren (1929) pointed out that study of post mortem material of lymphocytic leukaemia gives little help in determining the origin of the disease, as by this time there is always gross involvment of the spleen, lymph nodes and bone marrow. Italo (1930), Rossle (1930), Livingstone (1932), and Zanaty (1934), reported cases in which there was marked infiltration of the marrow without any infiltration of the lymph glands.

Quite the opposite view was expressed by Krachi and Garver (1935) who thought that there was a reversion to embryonic conditions in lymphogenous leukaemia, in which extra-medullary haemopiesis occurs, so the origin of the disease is always in the lymph nodes, and there is only a terminal proliferation of the lymphoid tissue in the marrow. On what evidence they based this categorical statement was not given. Scott (1939) stated that there is a general agreement that /

that with a marked peripheral lymphocytosis (over 50,000 lymphocytes per c.mm.) the sternal marrow always shows about 98% lymphocytosis. By the time such a marked peripheral lymphocytosis is present the disease is well advanced so his experience does not help to pinpoint the origin of the disease. Klima (1938) and Weiner and Kaznelson (1925) expressed the view that marrow lymphocytosis may be absent or only slight in chronic lymphocytic leukaemia but this is denied by Henning, who thought that absence of lymphocytosis of the marrow almost excludes leukaemia.

Caird (1940) reported the results of his studies of a few patients suffering from chronic lymphocytic leukaemia. Two of his patients had no glandular enlargement, early disappearance of the thrombocytes and granular cells from the circulation, together with relatively early irritative reaction of the red cells. From these facts he drew the conclusion that the process must have started in the marrow. I admit that these features do point to marrow involvement with the leukaemic cells, but without corroborative evidence from marrow smears too much attention cannot be paid to them. In the third patient he argued that because the blood picture did not become frankly leukaemia for five years after the first enlargement of the glands that this was proof that the marrow was invaded from without. Lymphocytic reinforcements of the /

the blood come in the main from the lymphoid tissue outside the marrow and this area when compared with that available in the marrow is so large that it makes any contribution to the lymphocytosis of the blood from the marrow relatively small. McCallum (1940) in his textbook of Pathology wrote that it is impossible to determine where the disease starts because at a post mortem all the lymphoid tissue, as well as the marrow, is packed with lymphocytes. He reported one case of acute lymphoblastic leukaemia in which all the lymphoid tissue outside the marrow was unaffected but was itself packed with lymphocytes. Naegeli (1913) dogmatically stated that acute and chronic lymphocytic leukaemia never begin in the marrow alone but are essentially systemic diseases affecting all the lymphoid tissue.

Dibble and Davie (1943) thought that the marjority of the lymphocytes in the marrow are there as a result of passive infiltration, and may represent foci of active lymphopoiesis. In Boyd's (1945) opinion the marrow in chronic lymphocytic leukaemia is invaded by lymphoid cells. At first these form only isolated islands which eventually coalesce and finally replace the marrow cells. He did not express an opinion where the disease starts but agreed with Banti (1904) as to the nature of the invasion of the marrow.

Beattie and Dickson (1943) also stressed this patchy /

patchy distribution of the lymphocytic cells and had this to say of their origin - "as to the nature of these lymphocytic cells opinion varies greatly, some regard them as infiltration of the tissue by cells produced elsewhere, but opponents of this theory point out that the marrow is extensively affected in cases where these nodes show little or no change. Lately the view that these cells are in reality early members of the myelocytic series has gained ground and it is believed that lymphoblast like cells, especially the large varieties, are simply non-granular promyelocytes or myelocytes in which the granules have not developed, and the two series of cells have a common ancestral cell".

The difficulties which arise when attempts are made to answer the questions referred to earlier in this section are made abundantly clear by this review of the relevant facts. The conclusions to be drawn from this mass of conflicting evidence are (1) simultaneous involvement of the marrow and lymphatic tissue elsewhere has not been proved so a normal marrow count in suspected chronic lymphocytic leukaemia does not exclude the disease. (2) The marrow is often involved early in the disease and if a marrow lymphocytosis of over 40% is found then the diagnosis of chronic lymphocytic leukaemia is certain. Lower lymphocytic counts suggest chronic lymphocytic leukaemia but further marrow biopsy must be performed to confirm or disprove the diagnosis.

(3) /

(3) In advanced chronic lymphocytic leukaemia the marrow is always heavily invaded with lymphocytes whether or not there is a lymphocytosis in the blood. A sternal biopsy gives valuable confirmatory evidence in the aleukaemic forms. (4) The confident opinion expressed by some British writers that lymphosarcoma cell leukaemia does not invade the bone marrow is not supported by the writings of some of the American authorities.

Cases from my series which are useful in helping to solve these problems are now described.

Case O. R.C. Aged 16½

History. For six weeks he had been suffering from increasing breathlessness, loss of weight and pain in the chest.

Examination.

The lymph glands, liver and spleen were normal. Lungs. There was a little moisture at both bases. X-ray of the chest showed a large round mass involving the mediastinal glands.

Haemopoietic system. R.B.C. 5,000,000. Hb. 96%. C.I. 0.96. W.B.C. 12,000. Film. A differential white cell count revealed polymorph 56%

small lymphocyte 28%

large lymphocyte 11%

monocyte 5%

A film made from the centrifuged specimen of blood showed a few large immature lymphocytes. There was thickening of the /

the nuclear membrane, a diagnostic feature of the lymphosarcoma cell (Bethell 1943).

Prior to sternal puncture the conditions which had to be considered in the differential diagnosis were lymphadenoma, acute or subacute lymphoblastic leukaemia, chronic lymphocytic leukaemia and lymphosarcoma cell leukaemia. Marrow examination revealed a gross infiltration of this tissue with immature lymphocytic cells which had centrally placed round or slightly indented nuclei with a rim of cytoplasm round this nucleus. Close examination showed that there was a distinct thickening of the nuclear membrane, which was easier to see in the marrow films than in the blood smears. On the basis of these findings taken in conjunction with the other clinical features (site of origin, more or less normal peripheral blood findings, etc.) a diagnosis of lymphosarcoma cell leukaemia was made. The diagnosis was proved by a post mortem examination.

This case supported the experience of McCallum (1940) Wiseman (1943) and Bethell (1943) but was not in agreement with the opinions expressed by Scott (1939) and Friedmann (1945).

Case P. J.A.M.C. Aged 70.

History. He suffered from severe pains in both hands and feet for one month and his own doctor discovered an enlarged spleen on routine examination. He had been a lifelong worker with lead paint.

Examination. /

Examination.

C.N.S. There was bilateral weakness and wasting of the small muscles of both hands and feet with acute tenderness on deep pressure, but with no actual sensory loss. The arm jerks were present but sluggish and the right ankle jerk was absent.

Abdomen. There was moderate enlargement of liver and spleen.

Haemopoietic system. R.B.C. 5,200,000. Hb. 99%. C.I. 1.0 (approx.). W.B.C. 12,000. Film. A differential white cell count was

large lymphocyte	45 per cent
small lymphocyte	25 " "
polymorph	26 " "
monocyte	4 " "

Other examinations.

The lead content of a 24-hour specimen of urine was below normal and the W.R. was negative in the blood and C.N.S.

The differential diagnosis rested between a chronic syphilitic infection, infectious mononucleosis, Banti's disease and chronic lymphocytic leukaemia. The first two conditions were ruled out by a negative W.R. and Paul-Bunnell agglutination test. A low total white cell count affecting mainly the granulocytes, giving a relative increase in the lymphocytes, is common in Banti's disease, whilst such a low count is rare in chronic lymphocytic leukaemia. A sternal puncture revealed /

revealed

large lymphocyte	32	percent
small lymphocyte	21	" "
normoblasts	16	" "
promyelocyte	2	" "
myelocyte	10	" "
polymorph	19	" "

There was therefore a total percentage of lymphocytes of 53. A close examination of the lymphocytes revealed that many of them were more primitive than normal. The nuclear chromatin was arranged in loose reticular network, whilst a few of the nuclei had vestiges of nucleoli. Many of the small lymphocytes had a very narrow rim of cytoplasm round their nuclei. A diagnosis of chronic lymphocytic leukaemia was proved. In this patient there was an early generalised infiltration of the lymphatic tissue both outside and within the marrow, and so study of this marrow gave a certain amount of support to the view that the lymphatic tissue throughout the body is spontaneously involved in lymphocytic leukaemia. Further, marrow examination by revealing the true nature of the disease enabled X-ray therapy to be confidently advised.

Case Q. K.K. Aged 31.

History. For six months she had been gradually becoming weaker with loss of energy, irritability, etc. Her own doctor was confident that she was suffering /

suffering from chronic lymphocytic leukaemia.

Examination.

Haemopoietic system. R.B.C. 3,400,000. Hb. 54% C.I. 0.77. W.B.C. 6,600. Film. The red cells showed a mild degree of hypochromia. The differential white cell count was

large lymphocyte	30	per cent
small lymphocyte	29	" "
polymorph	35	" "
monocyte	6	" "

The lymphocytes were normal in appearance. The remaining investigations were all negative.

A sternal marrow examination showed that 29% of the total number of cells present were lymphocytes. The diagnosis therefore rested on whether or not this percentage was significant of lymphocytic leukaemia. The total lymphocytic cell count of the peripheral blood was low, therefore few of the cells making up this percentage could have come from the blood enmeshed in the marrow spaces; there was therefore a real increase of the lymphatic elements in the marrow. However, this figure was 11% below the lowest figure accepted by Scott (1939) and 21% below that accepted by Israels (1936) as significant of lymphocytic leukaemia. On the other hand it was higher than the highest figure claimed as normal by Holmes and Broun (1933) which was 24.9%. The lymphocytosis of 29% taken in conjunction with the other clinical features which /

which were not in the least suggestive of chronic lymphocytic leukaemia, did not allow of a final diagnosis of chronic lymphocytic leukaemia being made. A second sternal puncture was performed fourteen days after the first one, following a slight improvement in the peripheral blood picture, and on this occasion was within normal limits, and so the diagnosis of chronic lymphocytic leukaemia was abandoned. Marrow examination was therefore of the utmost value in dismissing this fatal diagnosis.

Case R. W.L. Aged 39.

History. Six years before admission the glands began to enlarge in the right side of his neck and he began to complain of general malaise. A pathological report in 1944, on a gland removed from the neck, stated that the architecture of the gland was completely destroyed by lymphocytes. The appearance was not that of lymphosarcoma, but more that of a simple lymphocytic hyperplasia or a leukaemia. The radiologist thought the glands were those of a generalised lymphadenoma.

Examination.

Lymphatic system. The glands were about the size of a cherry. They were not tender but were moderately hard and freely mobile. The spleen was slightly enlarged but the liver was normal.

Chest. An X-ray showed slight enlargement of the mediastinal glands.

Other /

Other investigations. W.R. was negative.

Haemopoietic system. R.B.C. 3,600,000. Hb. 52% C.I. 0.72. W.B.C. 18,000. Film. The differential white cell count showed

small lymphocyte	61 per cent
large lymphocyte	20 " "
polymorph	15 " "
monocyte	4 " "

The diagnosis was almost certainly chronic lymphocytic leukaemia as only rarely has a lymphocytosis been recorded in lymphadenoma, but on account of the slight doubt thrown on the diagnosis by the earlier report on the pathology of the gland a sternal puncture was performed and a marrow found heavily invaded by small lymphocytes. They comprised 93% of the total number of cells. The diagnosis of chronic lymphocytic leukaemia was confirmed beyond all doubt.

VALUE OF MARROW EXAMINATION IN ANAEMIA DUE TO HAEMOPOIETIC DEFICIENCY.

Classification of anaemia due to deficiency of the haemopoietic factor.

A. Dietetic, due to extrinsic factor deficiency.

(1) Tropical macrocytic anaemia.

(2) Pernicious anaemia of pregnancy.

B. Deficiency secondary to gastro-intestinal disturbances.

(1) Pernicious anaemia.

(2) Carcinoma of the stomach.

(3) Gastric syphilis.

(4) Gastric polyposis.

(5) Corrosion of the stomach with poisons.

(6) Strictures.

(7) Following gastrectomy.

(8) Following gastro-enterostomy.

(9) Following resection of the intestines and various anastomoses.

(10) Jejuno-ileal absorptive deficiency.

(11) Regional ileitis.

(12) Chronic pancreatic disease.

C. Following liver damage.

(1) Cirrhosis.

(2) Malignancy.

D. Infestation with tapeworms.

(1) Diphyllobothrium Latum.

(2) Taenia saginata.

(3) Ascaris lumbricoides.

Marrow /

Marrow examination is of great value in pernicious anaemia in relapse, tropical macrocytic anaemia and pernicious anaemia of pregnancy, as a megaloblastic picture is always present. A megaloblastic picture is usually present in sprue, but this disease is easily diagnosed from the other clinical features so marrow examination is rarely necessary. In the other diseases listed in the classification, a megaloblastic reaction is not constantly present, so examination of the marrow is of little help in diagnosis.

I have not had any opportunity to study macrocytic anaemia of pregnancy, but Callender (1945) summed up the diagnostic importance of marrow examination in pernicious anaemia of pregnancy thus "the only two features which may be relied upon for the certain diagnosis of pernicious anaemia of pregnancy are the appearance of megaloblasts in the peripheral blood and the finding of a megaloblastic change in the bone marrow".

IMPORTANCE OF MARROW EXAMINATION IN PERNICIOUS ANAEMIA.

but in remission

There is always a megaloblastic reaction in the marrow in pernicious anaemia and so the demonstration of this picture is strong confirmatory evidence of this disease. Davidson (1941) did not consider that a sternal puncture was required for the routine diagnosis of pernicious anaemia but expressed the opinion that it should be reserved for all cases of macrocytic anaemia which failed to respond adequately to potent liver preparations. If sternal puncture /

puncture is always withheld until the failure to respond to a potent liver preparation is proved, many patients whose initial macrocytic anaemia was due to such conditions as leukaemic myelosis, macrocytic haemolytic anaemia, or anaemia secondary to infiltration of the bone marrow with carcinoma or lymphadenoma, which never give a megaloblastic picture, will be subjected to an unnecessary delay in diagnosis. Such delays can be prevented by routine examination of the marrow in all cases of macrocytic anaemia as soon as possible after admission to hospital. Two cases in this series serve to emphasize this argument.

Case A. Mrs. McK. An old lady admitted with a peripheral blood typical of pernicious anaemia but a routine examination of the marrow revealed a myelogenous infiltration. This case is reported fully in section dealing with leukaemic myelosis.

Case B. A.G. The correct diagnosis in this patient was delayed for nine months because of the failure to perform a routine examination of the bone marrow.

History. Admitted to hospital in August 1945 with a long standing history of persistent tiredness and left-sided abdominal pain.

Haemopoietic system. R.B.C. 3,600,000. Hb. 80%. C.I. 1.1 (approx.). W.B.C. 7,400. Film. The red cells were well filled with haemoglobin and they varied markedly in size and shape, the majority being macrocytes.

Other systems. There was slight enlargement of the /

the spleen, histamine fast achlorhydria and a raised icteric index. On this evidence a diagnosis of pernicious anaemia was made and he was treated with liver extract for three weeks in hospital and then discharged home, symptomatically greatly improved, but his blood in almost the same condition as when he was admitted. However, he was regarded as a pernicious anaemia who was responding slowly to treatment. Nine months later he was readmitted with the same signs and symptoms as previously, having had no liver injections for three months prior to admission. Investigation proved that clinically he was in the same condition as the first time he was admitted. In addition a persistent reticulocytosis of 7% was noted. A sternal puncture revealed an intense normoblastic reaction of the bone marrow. The details of the myelogram were

Haemocytoblast	1	per cent
Pro-Erythroblast	2	" "
Normoblast A	9	" "
Normoblast B	15	" "
Normoblast C	11	" "
Promyelocyte	2	" "
Myelocyte	5	" "
Metamyelocyte	15	" "
Polymorph	34	" "
Plasma cell	2	" "
Lymphocyte	4	" "

The final diagnosis in view of the intense normoblastic reaction of the marrow, the persistently raised /

raised reticulocyte count, slightly enlarged spleen and relatively high total white count, was haemolytic disease of old age of unknown origin. Presumably if a marrow examination had been performed on his first admission this normoblastic marrow picture would have been discovered and a diagnosis of pernicious anaemia abandoned.

In the next three cases the diagnosis of pernicious anaemia was only considered as an outside possibility and a sternal marrow examination made with a rather optimistic hope that it might help in arriving at a diagnosis.

Case C. H.B. Aged 45. His presenting symptom was severe epistaxis. He had four attacks during the first two days in hospital, after which they ceased altogether.

Examination. Heart, blood vessels, chest and C.N.S. were all normal. B.P. 135/75.

Alimentary system. His tongue was not atrophied. The only abnormality detected on barium meal examination was a degree of spasm of the pyloric end of the stomach with no suggestion of malignancy.

Gastric analysis. Histamine fast achlorhydria.

Urine. Urobilinogen present.

I. I. 7.

Haemopoietic system. R.B.C. 2,800,000. Hb. 47%. C.I. 1 (approx.). W.B.C. 7,000. Reticulocytes 1%. B.T. and C.T. Normal. Film. Red cells were well filled with haemoglobin but there was little variation in size and shape of these cells. A differential white cell count was normal.

These clinical features of epistaxis, a relatively high white cell count and the peripheral film findings did not suggest the diagnosis of pernicious anaemia. There are no reports in the literature of pernicious anaemia giving as its first symptom epistaxis. But the evidence of haemolysis, the histamine fast achlorhydria and the normal C.I. after four attacks of severe epistaxis made the diagnosis of pernicious anaemia a possibility. Examination of the marrow revealed a megaloblastic hypoplastic picture strongly suggestive of pernicious anaemia. The possibility of a megaloblastic type of refractory anaemia could not be ruled out at this stage. After fourteen days of intensive liver therapy the blood count, Hb., and reticulocyte percentage remained unaltered, but a second sternal puncture performed at this time revealed a marrow which was intensely normoblastic. Four days after this puncture a brisk reticulocyte response resulted and the blood gradually returned to normal. The diagnosis based mainly on the marrow examinations was therefore pernicious anaemia. The hypocellular appearance of the first marrow smear serves as a warning that a hypercellular marrow is not always found in this disease.

Case D. G.R. Aged 52.

History. Two years before admission to hospital she had a severe vaginal haemorrhage but since then she has had no further haemorrhages apart from her normal periods. Since then she has, however, become increasingly /

increasingly tired, together with the usual symptoms of anaemia.

Examination.

Alimentary system. Her tongue was atrophied. There was no enlargement of the liver or spleen.

Other systems. Negative.

Gynaecological report. Negative.

I.I. 17.

Special examinations. Histamine fast achlorhydria and benzidine negative reaction in the stool.

Haemopoietic system. R.B.C. 3,260,000. Hb. 46%. C.I. 0.7. W.B.C. 5,800. Reticulocytes 2%. Film. There was macrocytosis, poikilocytosis and anisocytosis of the red cells which definitely showed haemoglobin deficiency. The differential white count was normal.

The clinical features, apart from the achlorhydria and evidence of haemolysis, did not encourage a diagnosis of pernicious anaemia, but sternal puncture revealed a megaloblastic hypercellular marrow. Many haemocytoblasts, pro-erythroblasts and megaloblasts A., B. and C. were present. The myelocytes and metamyelocytes were also increased in numbers and showed signs of immaturity of the cytoplasm. These findings were highly characteristic of pernicious anaemia in relapse. Treatment with liver preparations proved effective and so the diagnosis was proved.

Case E. A.D. Aged 58.

Three weeks prior to admission he suffered from attacks /

attacks of flatulence and vomiting. The vomitus was bile stained. These symptoms became alarming so he was admitted to hospital. The last attack of vomiting was accompanied by deep jaundice. On a few occasions severe epigastric pain complicated these symptoms. It usually disappeared in about ten minutes. He also suffered from the usual signs and symptoms of a severe anaemia.

Examination.

Alimentary system. There was some tenderness in the mid epigastrium on deep palpation, but no tumour was felt. There was also a histamine fast achlorhydria and the benzadine test was negative in the stools. No other abnormalities were found. He was too ill to have a barium meal.

Haemopoietic system. R.B.C. 1,200,000. Hb. 24%. C.I. 1. Film. Red cells were well filled with haemoglobin and showed marked changes in size and shape. No nucleated red cells were discovered. The white cells were normal in numbers and appearance.

The clinical features, apart from the blood findings were more suggestive of a gastric neoplasm than pernicious anaemia. Examination of the marrow, however, showed that it was hypercellular and filled with promegaloblasts, pro-erythroblasts and megaloblasts A., B. and C. The promyelocytes and myelocytes showed signs of immaturity.

On this evidence a diagnosis of pernicious anaemia was made although it was realised that a similar marrow picture can be present in carcinoma of the /

the stomach. Before carcinoma of the stomach can destroy the glands producing the intrinsic factor to such an extent that a profound megaloblastic bone marrow picture is produced the test meal, apart from the achlorhydria present, would have shown other features of neoplasm. The stools would also have shown occult blood. He was treated with liver and a satisfactory response resulted, and a subsequent barium meal excluded any possibility of neoplasm, so the original diagnosis of pernicious anaemia was upheld.

There is another group of cases in which examination of the marrow helps to exclude the diagnosis of pernicious anaemia when it has been suspected on clinical grounds. The following cases from my series are illustrative of this group.

Case F. M.W. Aged 64.

History. For three years had been suffering from increasing tiredness, loss of appetite, breathlessness, and palpitation. She had a sore tongue on occasions, and her appetite had failed. She was admitted to hospital for investigation with the provisional diagnosis of pernicious anaemia.

Examination.

Heart. There was moderate hypertrophy of the heart. B.P. 190/100.

Alimentary system. Her tongue was smooth but not ulcerated. Nil else.

C.N.S. Normal.

Haemopoietic system. R.B.C. 3,400,000. Hb. 80% C.I. /

C.I. 1.2. W.B.C. 4,000. Film. Red cells were well filled with haemoglobin and there was a slight degree of anisocytosis and poikilocytosis. The overall picture was one of macrocytosis. The differential white count was lymphocytes 54%, polymorphs 44%, monocytes 2%.

Special investigations. There was a histamine fast achlorhydria and there was no occult blood in the stools. The barium meal proved negative.

The mild hyperchromic macrocytic anaemia with the histamine fast achlorhydria and normal barium meal all suggested the possibility of a mild pernicious anaemia. Examination of the marrow revealed that it was normal and so the diagnosis of pernicious anaemia could not be entertained.

Case G. C.W. Aged 72.

History. For the past three months he had complained of dizziness, weakness, anorexia, nausea and vomiting. He had gained 1 stone in weight during the past year.

Examination. He was a well built man with a sallow complexion. The rest of the clinical examination was essentially negative except for findings in the C.N.S. and haemopoietic system.

C.N.S. There was some lack of co-ordination in both legs. Both ankle jerks were absent and Babinski's plantar response was bilaterally positive.

Haemopoietic system. R.B.C. 4,000,000. Hb.100% C.I. 1.2. W.B.C. 6,400. Film. The red cells were well filled with haemoglobin and showed slight variations /

variations in size and shape. An occasional normoblast C. was present.

Special investigations. There was a histamine fast achlorhydria and the I.I. was 5. There was no occult blood in the stools.

These signs and symptoms were suggestive of pernicious anaemia with early postero-lateral sclerosis and so a sternal puncture was performed in an attempt to confirm this diagnosis but the marrow was normal. The diagnosis of pernicious anaemia was abandoned and the final diagnosis proved to be myxoedema.

Case H. M.W. Aged 67.

History. For the past six months had been suffering from severe breathlessness on exertion, loss of power of concentration, anorexia, nausea and vomiting. She had one attack of epistaxis about a fortnight before admission.

Examination.

Cardio-vascular system. The heart was markedly enlarged with a systolic murmur in the mitral area, together with a systolic and diastolic in the aortic area. The pulse was regular and there was a marked difference between the systolic and diastolic blood pressures. The figures were 210/60.

Haemopoietic system. R.B.C. 1,400,000. Hb.28%. C.I. 1. W.B.C. 6,000. Film. Red Cells showed marked changes in size and shape with an occasional normoblast C. The differential white cell count was normal.

Special /

Special investigations. There was a histamine fast achlorhydria and there was no occult blood in the stools. The W.R. was negative.

Amongst many other conditions too numerous for discussion in this thesis, pernicious anaemia was suspected because of the blood findings and the achlorhydria, but a sternal puncture revealed a normal marrow and this diagnosis was abandoned.

It has been my experience that for some unexplained reason a number of proved cases of pernicious anaemia, although having regular monthly liver injections, relapse. When further liver injections are given in hospital they respond immediately. These patients cannot be classified as suffering from true refractory anaemia - a condition discussed elsewhere - as it can never be proved that they were refractory to liver injections as those they received outside the hospital may not have been potent. The marrow of such patients should be examined as soon as possible after admission to hospital as thereby a few will be shown to have other conditions affecting the marrow and so causing this lack of response to treatment. These conditions are aplasia of the bone marrow and early leukaemic infiltration of the marrow. The following case from my series serves to illustrate this point.

Case I. J.M. Aged 62.

History. Eighteen months prior to admission he had been proved to be suffering from pernicious anaemia. He made good progress until six weeks before admission /

admission to hospital when all his old symptoms of anaemia gradually returned.

Examination. This was essentially negative except for the abnormalities found in the haemopoietic system.

Haemopoietic system. R.B.C. 1,100,000. Hb.40%. C.I. 1.9. W.B.C. 3,100. Reticulocytes 0.5%. Film. The Red Cells were well filled with haemoglobin and showed great changes in size and shape. There were large numbers of macrocytes present but no nucleated red forms. The differential white cell count was normal.

A sternal puncture revealed an intense megaloblastic hyperplasia. A diagnosis of pernicious anaemia in relapse was made although the possibility of a true refractory anaemia was not excluded. However, he immediately responded to liver injections and the diagnosis of uncomplicated pernicious anaemia was upheld.

ABNORMAL LEUKOPOIESIS IN HAEMOPOIETIC FACTOR DEFICIENCY ANAEMIA.

Wintrobe (1946) and other writers have drawn attention to the abnormal leukopoiesis in haemopoietic factor deficiency anaemias, especially in pernicious anaemia. There is general agreement that an increase in the myelocytes and metamyelocytes takes place. These cells also show signs of cytoplasmic immaturity as well as changes in the nuclei. The nuclei are very large and have an irregular outline. The cytoplasm is blue and less mature than would be expected /

expected from the appearance of the nucleus, and contains a few neutrophil granules and occasionally vacuoles. The majority of my cases of pernicious anaemia had these changes but in two cases the white cell series showed no abnormality. It is difficult to decide whether the myelocytes and metamyelocytes are increased above the normal figure as the highest normal figures given are 14% (Klima 1938) and 15.7% (Scott 1939). Many other authors quote much lower figures than this. The highest figures I have found in normal smears are 8% and 6% respectively. Only four cases in my series of pernicious anaemia showed figures above those of Klima and Scott. This study convinces me that too much importance has been attached to the increase in the percentage of these cells in pernicious anaemia and further study is required to clear up this confusion.

VALUE OF MARROW EXAMINATION IN REFRACTORY ANAEMIA.

The first workers to classify refractory anaemias were Bomford and Rhoads (1941) who included in their classification all disorders of the blood which failed to yield to any treatment except transfusions of blood. No case of anaemia secondary to such diseases as cancer, tuberculosis, lymphogranuloma, nephritis, cirrhosis of the liver, sepsis, infective endocarditis and frank leukaemia were included. The classification of these anaemias was based on the histological appearance of the marrow. Their cases fell into four groups.

I. Those with partly mature cellular marrow, i.e., slight hold-up in maturation to the stage of normoblast and polymorph.

II. Those with hypocellular normoblastic marrow.

III. Those with immature cellular marrow of a normoblastic type.

IV. Those which were replaced by fibrosis, giant cell hyperplasia of the marrow and replacement of the marrow by other tissues.

These authors therefore included in their classification all conditions which at one time were grouped rather unsatisfactorily under the heading of aplastic anaemia. Thomson et alia (1934) and Rhoads and Miller (1938) studied the marrow appearances in aplastic anaemia and recognised the different phases of maturity in the cells /

cells but included all types of aplastic anaemia however caused in their classification. Davidson, Davis and Innes (1943) clarified the classification of refractory anaemia thus :

A. Refractory anaemia with hypocellular normoblastic marrow.
   (1) Secondary to the exposure of toxic substances.
   (2) Idiopathic.
      (a) Progressive hypoplastic anaemia with fatal termination.
      (b) Chronic hypoplastic anaemia surviving for about two years.
      (c) Relapsing hypoplastic anaemia.

B. Refractory anaemia with hypercellular megaloblastic marrow.
   (1) Occurring in pregnancy and the puerperium.
   (2) Idiopathic.

C. With cellular normoblastic marrow and arrested myelocytic maturation.

Marrow examination must be performed as soon as possible in order that treatment can be placed on a scientific basis and an accurate prognosis given. It is therefore in this group of diseases that sternal biopsy can be described as indispensable, as the following cases prove.

Case /

Case J. J.G. Aged 52.

History. In 1939 he was diagnosed as suffering from a typical pernicious anaemia and treated satisfactorily with liver injections and continued with a maintenance dose of 2 c.c.s of Anahaemin every month. In 1942 and 1945, despite these injections, he had relapses with the return of all the symptoms of anaemia, but on both occasions after a short stay in hospital with further intensive treatment with liver injections his blood responded and was restored to normal. Six weeks prior to admission all his symptoms returned so he was admitted to hospital for full investigation.

Haemopoietic system. R.B.C. 1,200,000. Hb. 32% C.I. 1.3 (approx.). W.B.C. 2,400. Film. Red cells were well filled with haemoglobin and showed marked variations in size and shape. No nucleated forms were seen. Many large macrocytes were present.

Myelogram.

Haemocytoblast	13	per cent
Pro-erythroblast	27	" "
Megaloblast A.	5	" "
Megaloblast B.	15	" "
Megaloblast C.	2	" "
Normoblast	4	" "
Promyelocyte	1	" "
Myelocyte	18	" "
Metamyelocyte	5	" "
Polymorph	10	" "

Other /

Other investigations revealed a histamine fast achlorhydria, an I.I. of 14, and urobilinogen in the urine, and so completed the picture of a pernicious anaemia in relapse. Intensive liver therapy from a batch of ampoules of proved potency failed to bring about a response and further marrow examination fourteen days after the commencement of this treatment revealed a picture identical with the first smear. These findings supplied the final proof that this was an example of a refractory anaemia with a hypercellular megaloblastic bone marrow. The prognostic importance of finding such a hypercellular marrow is emphasized by Davidson, Davis and Innes (1943), who found that in six cases of refractory anaemia with a hypercellular megaloblastic marrow, recovery resulted after a variable refractory phase if treatment was continued with liver and life maintained during the interim period with repeated blood transfusions. In Davidson's (1943) cases this time interval varied from four to ten weeks.

Davidson (1945) thought that the finding of a megaloblastic picture in refractory anaemia was an indication for the substitution of liver injections with proteolysed liver, this view being supported by experience gained from this patient. The explanation of the cause of this patient's refractory state is that he required an unknown factor, present only in small quantities in Anahaemin, to give him a satisfactory haemopoietic response. Intensive treatment with Anahaemin in 1939 provided /

provided enough of this unknown substance to give him a satisfactory response, but when he returned to monthly injections he was supplied with insufficient quantities so he gradually relapsed. In 1942 after further intensive Anahaemin injections he again responded, only to relapse in 1945 on reverting to monthly injections. Further intensive liver injections restored the missing factor. During these years he has become more and more resistant to this unknown factor that in 1946 he was incapable of responding any more to liver by injection. It appears that this missing factor might be destroyed to a large extent in the preparation of the highly purified form of Anahaemin and that perhaps this missing factor was present in proteolysed liver, which is merely a papain digest of whole liver. Proteolysed liver, 1 teaspoonful three times a day, was administered and after ten days a reticulocyte response of 12% occurred and a sternal puncture performed on the same day provided a normoblastic bone marrow giving final proof of the efficacy of this treatment. The patient has remained well on this treatment up to the present time.

My second case of refractory anaemia was somewhat different.

Case K. J.M. Aged 53.

History. For two years he has been a known case of pernicious anaemia and has been treated with the usual monthly injections of Anahaemin. A few weeks prior to admission there was a return of all his old symptoms of lassitude, weakness, sore tongue, etc.

Examination. /

Examination.

Haemopoietic system. R.B.C. 1,720,000. Hb. 45% C.I. 1.3. W.B.C. 2,800. Film. Red cells were well filled with haemoglobin and varied greatly in size and shape. Nucleated forms of the type Megaloblast B. were present. The differential white cell count was normal.

Other investigations revealed a histamine fast achlorhydria, an I.I. of 14, and urobilinogen in the urine. A sternal puncture was not performed on admission, a mistake emphasized earlier in the thesis, as she was considered to be a straightforward case of pernicious anaemia, despite the evidence of treatment by liver injections. Daily injections of Anahaemin were prescribed but no response was forthcoming after ten days. She developed urticaria so Neohepatex was substituted for the Anahaemin in doses of 2 c.c.s every other day. After a further period of ten days there was no response. A sternal puncture was performed and the result was a hypercellular marrow with an intense megaloblastic reaction. The details of the myelogram were

Haemocytoblast	6	per cent
Pro-erythroblast	17	" "
Megaloblast A.	3	" "
Megaloblast B.	23	" "
Megaloblast C.	16	" "
Normoblast A.	1	" "
Normoblast B.	3	" "
Normoblast C.	8	" "
Promyelocyte	1	" "
Myelocyte	2	" "

Metamyelocyte /

Metamyelocyte	4 per cent
Polymorph	14 " "
Lymphocyte	2 " "

Marrow examination therefore revealed that she was suffering from a refractory anaemia with an intensely hyperplastic megaloblastic picture. It was decided to carry on with the Neohepatex to try and find out if she would respond to parenteral liver as was the case with Davidson's (1941) patients. In three weeks' time a brisk reticulocyte response of 30% occurred and she made an uninterrupted recovery and has remained well ever since. It remains to be seen whether she will relapse as Case J. did. Without a marrow examination the true nature of this case would not have been revealed.

My third patient in this series illustrates a rather different value of sternal puncture. He suffered from pernicious anaemia and was apparently slipping into a refractory state.

Case L. M.C. Aged 74.

History. This old man was admitted to the ward with a history of pernicious anaemia for the past five years adequately treated with monthly injections of Anahaemin. The pertinent clinical details were :-

Haemopoietic system. R.B.C. 1,600,000. Hb. 50%. C.I. 1.6 (approx.). W.B.C. 3,500. Film. Red cells were well filled with haemoglobin with intense anisocytosis and poikilocytosis. No nucleated forms were seen in the red cell series. The differential white count was normal.

The other investigations supported a diagnosis of pernicious /

pernicious anaemia. A sternal puncture revealed a remarkable marrow. There was an intense early normoblastic reaction together with a megaloblastic picture.

Haemocytoblast	7	per cent
Pro-erythroblast	5	" "
Megaloblast A.	3	" "
Megaloblast B.	8	" "
Megaloblast C.	10	" "
Normoblast A.	21	" "
Normoblast B.	12	" "
Normoblast C.	4	" "
Promyelocyte	1	" "
Myelocyte	3	" "
Metamyelocyte	10	" "
Polymorph	14	" "
Lymphocyte	2	" "

I have never come across such a picture before and the only reference I could find to a similar case in the literature was by Davidson (1945). He reported his cases as examples of refractory anaemia which ultimately responded to liver therapy. This case therefore supports Davidson's (1945) supposition that in some instances there is a dimorphic appearance in the bone marrow in refractory anaemia. It might be argued that in this patient the haemopoietic system at the commencement of liver therapy was quite sensitive to the haematinic factors supplied by the Anahaemin, but as the years passed there /

there was a gradual decline in this sensitivity and at the time of performing the sternal puncture he was just passing into the refractory stage and his marrow was reflecting the reversal of the normal process. This patient was treated with 1 teaspoonful three times a day of proteolysed liver and within a week a satisfactory response resulted and further marrow examination gave a normal marrow. The value of proteolysed liver in refractory anaemia which ultimately responds to treatment was further demonstrated, as well as the value of marrow examination in assessing progress under treatment. The experience of this case, together with that of Davidson, emphasizes that the classification of refractory anaemia into groups with normoblastic or megaloblastic marrows is too rigid and a further group with a mixed normoblastic megaloblastic picture must be added.

The next example of refractory anaemia does not fall neatly into any of the sub-groups mentioned by Davidson (1943).

Case M. C.M. Aged 50.

History. For one month had suffered from the usual signs and symptoms of a severe anaemia.

Examination.

Haemopoietic system. R.B.C. 2,400,000. Hb. 55%. C.I. 1.2 (approx.) W.B.C. 4,500. Film. The usual features of pernicious anaemia in relapse were discovered and the differential white blood count was normal.

Other /

Other investigations revealed that the I.I. was 16, and there was also a histamine fast achlorhydria. All other examinations were negative.

Marrow examination. There was an intense megaloblastic reaction. He was treated with the usual injections of Anahaemin but as there was no response after ten days to this treatment a further marrow examination showed that there was now a normoblastic reaction. Davidson (1945) reported that in his three cases a normoblastic reaction was found shortly after a haemopoietic response had occurred but in my patient despite the normoblastic reaction no demonstrable change occurred in the peripheral blood, nor did one come about until considerable quantities of iron were added. There may prove to be another series of cases, which fail to manufacture mature red cells despite the change from megaloblastic to a normoblastic reaction in the marrow. My last example in this series of so-called refractory anaemia supports the conclusions arrived at from the study of the preceding patient.

Case N. E.W. Aged 70.

History. For many months had suffered from the usual symptoms of a severe anaemia.

Haemopoietic system. R.B.C. 1,400,000. Hb. 44%. C.I. 1.6. W.B.C. 5,400. Film. Red cells were well filled with haemoglobin and showed marked changes in size and shape. A few nucleated forms of the type megaloblast B. were seen. The differential white count was normal.

Confirmation /

Confirmation of the confident diagnosis of pernicious anaemia in relapse was forthcoming when a histamine fast achlorhydria, I.I. 9, and a negative barium series were found. A megaloblastic picture was also discovered in the marrow. She failed to respond to the usual therapy and after a fortnight's time a second sternal puncture revealed a normoblastic reaction in the marrow. A detailed myelogram showed that the normoblast A. constituted 20% of the total marrow count, and approximately 55% of the total of the nucleated red cells. This definitely indicated a hold-up in the maturation of the red cells due to lack of synthesis of haemoglobin. Ferrous sulphate was prescribed and within a few days a satisfactory response resulted. Some of these patients, therefore, would not pass satisfactorily out of the refractory stage without both liver and iron.

My experience with marrow examination in patients suffering from refractory anaemia makes it possible to extend Davidson's (1943) classification to include further sub groups. The classification of refractory anaemia would then read :-

A. Refractory anaemia with hypocellular normoblastic marrow.

(1) Secondary to the exposure of toxic substances.

(2) Idiopathic.

(a) Progressive hypoplastic anaemia with fatal termination.

(b) Chronic hypoplastic anaemia surviving for about two years.

(c) /

(c) Relapsing hypoplastic anaemia.

B. Refractory anaemia with hypercellular megaloblastic marrow.

(1) Occurring in pregnancy and the puerperium.

(2) Idiopathic.

(a) With hypercellular megaloblastic marrow responding to proteolysed liver or less satisfactorily to Anahaemin.

(b) With hypercellular megaloblastic and normoblastic marrow responding to proteolysed liver.

(c) Megaloblastic reverting to normoblastic with proteolysed liver but requiring iron to terminate the refractory phase.

C. With cellular normoblastic marrow and arrested myelocytic maturation.

TABLE X.

IRON DEFICIENCY ANAEMIA - ALL TYPES

Details of Myelograms

	White	Johnstone, K.	Sneddon	Mitchell, K.	Johnstone, E.	Buchan	Wilson	Dryberg	Baptie	Lawrie	Sunderland	Mitchell, L.
Haemocytoblast	1	4	-	7	10	4	7	2	1	5	1	-
Pro-erythroblast	2	4	-	5	8	5	4	1	2	9	10	3
Normoblast A.	4	10	2	7	6	9	2	4	1	8	3	3
Normoblast B.	9	5	6	21	15	18	2	3	5	15	6	18
Normoblast C.	56	19	11	20	24	28	21	19	44	26	25	24
Myeloblast	1	-	-	1	-	-	-	2	-	-	-	1
Promyelocyte	3	2	-	2	1	2	1	2	-	2	1	-
Myelocyte	3	9	2	4	2	4	6	14	-	1	13	3
Metamyelocyte	8	10	19	9	6	9	18	10	5	7	10	3
Band cell	4	6	6	1	4	6	20	16	11	4	6	3
Polymorph	9	25	50	20	22	15	19	26	27	19	13	29
Lymphocyte	-	6	4	-	1	-	-	1	4	4	2	12
Plasma cell	-	-	-	3	1	-	-	-	-	-	-	-

TABLE D.

NUTRITIONAL ANAEMIA

Details of myelograms.

	Sunderland	Baptie	Lawrie
Haemocytoblast	1	1	5
Pro-erythroblast	10	2	9
Normoblast A.	3	1	8
Normoblast B.	6	5	15
Normoblast C.	25	44	26
Myeloblast	-	-	-
Promyelocyte	1	-	2
Myelocyte	13	-	1
Metamyelocyte	10	5	7
Band cell	6	11	4
Polymorph	13	27	19
Lymphocyte	2	4	3
Plasma cell	-	-	1

TABLE A.

IDIOPATHIC HYPOCHROMIC ANAEMIA

Details of myelograms.

	Sneddon	Mitchell, K.	Johnstone, E.	Buchan	Wilson
Haemocytoblast	-	7	10	4	4
Pro-erythroblast	-	5	8	5	4
Normoblast A.	2	7	6	9	10
Normoblast B.	6	21	15	18	5
Normoblast C.	11	20	24	28	19
Myeloblast	-	1	-	-	-
Promyelocyte	-	2	1	2	2
Myelocyte	2	4	2	4	9
Metamyelocyte	19	9	6	9	10
Band cell	6	1	4	6	6
Polymorph	50	20	22	15	26
Lymphocyte	4	-	1	-	6
Plasma cell	-	3	1	-	-

VALUE OF MARROW EXAMINATION IN ANAEMIA DUE TO IRON DEFICIENCY.

It is generally agreed that the marrow is hyperplastic with a relative as well as absolute increase in the normoblast cell types in all iron deficiency anaemias, whether this anaemia is due to defective intake or absorption of iron or due to an undue loss of iron resulting from chronic haemorrhage. This is also true in a large group of cases of unknown origin.

Scott (1939) reported the detailed myelograms of twenty-three patients with iron deficiency anaemia of an idiopathic type, and concluded that the marrow is highly cellular with a relative increase in the erythroblasts using this term to denote any nucleated red cell. The predominant cell was a small mature polychromatic normoblast with an irregular outline and only a small rim of slatey-grey cytoplasm round the pyknotic nucleus. An analysis of the marrow findings in twelve cases of iron deficiency anaemia are given in Table X. A study of this Table shows that there is always an increase in the numbers of haemocytoblasts and pro-erythroblasts and the normoblast B. and C.

In Table D the increase in normoblast C. in nutritional anaemia is well shown. In Table A the haemocytoblasts, normoblast B. and normoblast C. are chiefly affected in the increase in the cells in idiopathic /

idiopathic hypochromic anaemia.

CLASSIFICATION OF IRON DEFICIENCY ANAEMIA.

A. Physiological Iron Deficiency.

(1) In the first year in both sexes.

(2) Puberty in the male.

(3) Complicating or following pregnancy, lactation and menstruation.

B. Pathological.

(1) Blood loss.

(2) Lowered iron content of the food, possibly aggravated by achlorhydria, gastric operations, etc.

C. Idiopathic.

Sternal marrow examinations were made in patients suffering from iron deficiency anaemia secondary to menstruation, lactation, blood loss, and lowered iron content of the diet. A normoblastic picture was always found. (Table X). A normoblastic picture was always present also in the idiopathic group, and it is only in this group and in the group of nutritional iron deficiency cases that any help can be gained from a study of the bone marrow. Examples of these two groups are now given in detail.

(1) Iron deficiency anaemia in the diet (nutritional anaemia). There are three such cases in my series, two occurring in old men and one in a middle-aged man, and these have added interest because of the rarity of this condition in males. This condition is perhaps becoming more frequent /

frequent as old men living alone, attempt to keep themselves alive on present day ration foods.

Case S. H.S. Aged 73.

History. For five years he had tried to live on the present day rations, having no extra food apart from bread. He noticed a gradual loss of strength, malaise, etc.

Examination.

There was no discoverable blood being lost from any part of his body. His finger nails were spoon-shaped.

Alimentary system. The X-ray of the stomach was negative but there was a histamine fast achlorhydria.

Haemopoietic system. R.B.C. 2,800,000. Hb. 40% C.I. 0.7. W.B.C. 2,800. Film. Red cells showed marked hypochromia. The B.T. and C.T. were normal. The marrow was normoblastic. The normoblastic reaction in the marrow helped in the diagnosis as it pointed to a diagnosis of iron deficiency anaemia in the absence of all other known causes of iron deficiency. It also ruled out aplasia of the marrow as well as replacement of the marrow by various tissues such as carcinoma, early leukaemia, etc.

Case T. A.B. Aged 59.

History. Similar to case S.

Examination.

Apart from a test meal showing a histamine fast achlorhydria, nothing abnormal was detected except in the haemopoietic system.

Haemopoietic /

Haemopoietic system. R.B.C. 2,300,000. Hb. 38%. C.I. 0.8. W.B.C. 6,400. Film. Red cells were badly filled with haemoglobin, and a few normoblast B. and C., together with an occasional myelocyte, were seen.

Sternal marrow. Throughout the section there were areas where the fat had been dissolved away and what remained showed a normoblastic reaction.

Following these findings a provisional diagnosis of aplastic anaemia was made and the patient treated with iron therapy. Fourteen days after commencement of this treatment a brisk reticulocyte response resulted, and a further sternal puncture revealed a hypercellular normoblastic marrow. It is evident from such an experience that an aplastic anaemia must not be diagnosed without awaiting the result of iron therapy followed by a second marrow examination.

Case U. B.L. Aged 42.

History. For many weeks he existed on a diet of milk, tea and biscuits. He gradually became tired and breathless on exertion.

Examination.

All his investigations were negative except for the haemopoietic system.

Haemopoietic system. R.B.C. 2,560,000. Hb. 50% C.I. 1 /

C.I. 1. W.B.C. 5,200. Film. The red cells showed variations in size and shape and were moderately well filled with haemoglobin. An occasional normoblast C. was present.

Marrow examination. There was an intense normoblastic reaction.

An iron deficiency anaemia, based upon above findings, was diagnosed.

(2) Details of patients belonging to the idiopathic group.

Idiopathic iron deficiency anaemia was first recognised by Dameshek (1931) and his findings were confirmed by Young and Osgood (1935) and Scott (1938 and 1939). These authors reported a normoblastic reaction in the marrow. This was also my experience and the details of the myelograms of these patients are set out in Table A . In order to save tiresome repetition the details of one patient representative of this group are given.

Case V. J.J. Aged 34.

History. She had suffered from signs and symptoms of anaemia which got better at times then relapsed. She also had a sore tongue, gastro-intestinal disturbances, parasthesias, pallor, spoon-shaped nails and fits of depression.

Examination.

She was not suffering from any discoverable haemorrhage /

haemorrhage. The rest of the examination was negative, except for the haemopoietic system.

Haemopoietic system. R.B.C. 3,200,000. Hb. 50% C.I. 0.78. Reticulocytes 0.5%. W.B.C. 3,700. Film. The redcells showed extreme anisocytosis and poikilocytosis. Many of the red cells were well filled with haemoglobin but the majority exhibited extreme pallor.

Marrow examination. There was extreme hyperplasia of all the red cell elements. The white cells appeared normal.

The main value of marrow examination in this patient was that it enabled any aplasia of the marrow to be excluded and the normoblastic reaction supported the already suspected diagnosis of idiopathic iron deficiency anaemia.

THE VALUE OF THE EXAMINATION OF THE MARROW IN HAEMOLYTIC ANAEMIA.

The marrow in haemolytic anaemia however caused is intensely normoblastic, due to the proliferation of the normoblasts in response to the increased blood destruction. Usually the raised icteric index, persistent reticulocytosis, etc., gives ample proof that an haemolytic process is at work without the need for confirmatory evidence of a normoblastic marrow. Sometimes, however, the haemolytic process results in a macrocytic high colour index anaemia, which is easy to confuse with pernicious anaemia. Details of such a patient, representative of this group, are given in the section devoted to pernicious anaemia. (Case B.). As was shown in this case the normoblastic reaction of the marrow ruled out any suggestion of pernicious anaemia.

In another patient the value of marrow examination was shown in a slightly different way.

Case W. E.E.D. Aged 54.

History. Four months before admission to hospital she began to feel tired and run down and her own doctor treated her with injections of liver, in the belief that she was suffering from pernicious anaemia. She appeared to improve following these injections but relapsed from time to time, despite the continuation of different liver injections. Her symptoms gradually increased /

increased until she was admitted to hospital for further investigation.

Examination. She was not losing blood from any discoverable source.

Alimentary system. There was a histamine fast achlorhydria. Her spleen was enlarged two fingers breadth below the costal margin.

Haemopoietic system. R.B.C. 2,100,000. Hb. 35%. C.I. 0.7. W.B.C. 4,000. Film. The red cells showed marked irregularity in size and shape and a few polychromasia. Scattered throughout the film were normoblast A., B. and C. The differential white cell count was normal. The reticulocyte percentage was 7%.

Special investigations. The I.I. was 21 and urobilinogen was frequently found.

This patient had always been well before the onset of the illness and denied that she had ever been jaundiced. Furthermore, the fragility of her red cells was always within normal limits. The diagnosis rested between an atypical pernicious anaemia with a strong haemolytic element or an acquired familial haemolytic jaundice. A sternal puncture revealed a hypercellular intensely normoblastic reaction. This was not the appearance of a pernicious anaemia responding to liver but that of a reactive marrow secondary to blood destruction. A diagnosis of familial haemolytic jaundice was finally made and liver therapy abandoned. The marrow examination, taken in conjunction with other clinical /

clinical signs and symptoms, was of help in arriving at the diagnosis as well as helping in the determination of treatment.

THE VALUE OF THE EXAMINATION OF THE MARROW IN HAEMORRHAGIC DISEASES.

Wiseman, Doan and Wilson (1940) reported an increase in the megakaryocytes in essential thrombocytopenia, and various morphological changes in these cells have been described by Seeliger (1924) and Rohr and Coller (1938), such as toxic granulation, decrease in the granulations and even vacuolization of the cytoplasm, but Markoff (1938) thought that these changes were part of the normal maturation of the megakaryocytes. He also denied that the numbers were increased. These conflicting marrow findings deprive marrow examination of much of its importance in the diagnosis of this type of purpura.

Symptomatic thrombocytopenia due to the replacement of the various cells in the marrow by such tissue as carcinoma, leukaemia, Gaucher's cells and Niemann-Pick cells, can often be demonstrated by examination of the marrow which is usually performed as a routine in the diagnosis of the primary disease rather than for the incidental purpura. A sternal puncture may fail to reveal scattered foci of cells such as occur in metastases unless the needle is actually inserted in the deposit so negative findings are not trustworthy.

Marrow examination can be of value in confirming the /

the diagnosis of a simple symptomatic purpura due to a toxic degeneration of the vascular endothelium, by helping to eliminate the other forms of purpura. The following case illustrates this value of marrow examination.

Case Y. M.C. Aged 38.

History. Fourteen days prior to admission she had a mild attack of influenza which was followed during convalescence by a generalized purpura. She also complained of pain in the face and thighs. She was continually being sick and also suffered from diarrhoea.

Examination.

There were massive haemorrhagic areas all over her body, particularly on the face. She was drowsy but able to give details of her history. Temp. normal.

Abdomen. The spleen was not palpable. There was some tenderness in the epigastrium.

Haemopoietic system. R.B.C. 4,200,000. Hb. 100%. C.I. 1.2 (approx.). W.B.C. 40,000. Platelets 500,000. B.T. and C.T. normal. Film. Normal.

A sternal puncture revealed a normoblastic marrow and so helped to eliminate all the forms of symptomatic purpura. A diagnosis of simple symptomatic purpura due to an unknown toxin was made. Before any further investigations could be carried out she died. The post mortem examination revealed a deep seated influenzal bronchopneumonia which was no doubt responsible for the purpura.

Haemophilia can usually be diagnosed quite easily and accurately from the clinical features of this disease without recourse to marrow examination. However, a few cases of haemophilia occur spontaneously and in the absence of a family history of the disease. In such cases the accuracy of the diagnosis of haemophilia is always a little doubtful, so any evidence which supports such a diagnosis is welcome. Creveld (1942) reported that the megakaryocytes in the marrow were increased in numbers in three patients with haemophilia which he had studied. No other reports appear in the literature which throw light on the marrow appearance in this disease. I have studied a patient suffering from spontaneous haemophilia and there was a definite increase in the megakaryocytes in his marrow and this gave valuable evidence in support of the diagnosis, and also supported Creveld (1942) findings.

Case X. F.F. Aged 43.

History. Since early childhood he had suffered from repeated haemorrhages from slight abrasions. His nose frequently bled and his joints often became swollen and painful. There was no family history of haemophilia. He was admitted because of haemoptysis.

Examination. /

Examination.

Chest. In the right apex there was an active tuberculous focus.

Abdomen. There was slight enlargement of the spleen.

Joints. Both his knee joints were swollen and limited in their movements.

Haemopoietic system. R.B.C. 4,800,000. Hb. 95%. C.I. 1 (approx.). W.B.C. 7,200. Film. Normal. The B.T. was normal but the C.T. was markedly prolonged, and the actual time of clotting varied from day to day.

Other investigations. The capillary resistance test was normal. The fibrinogen content of the blood was within normal limits. The prothrombin time was normal.

The co-existence of haemophilia with pulmonary tuberculosis resulting in a severe haemoptysis with survival is extremely rare. Spontaneous haemophilia is also uncommon. Any corroborative evidence of the diagnosis of haemophilia would be welcome. A sternal puncture revealing as it did an increase in the megakaryocytes gave this evidence. Details of the myelogram are given in full.

Haemocytoblast /

Haemocytoblast	1	per	cent
Pro-erythroblast	2	"	"
Normoblast A.	7	"	"
Normoblast B.	8	"	"
Normoblast C.	11	"	"
Myelocyte	6	"	"
Metamyelocyte	25	"	"
Band cell	20	"	"
Polymorph	16	"	"
Megakaryocyte	4	"	"

CRITICAL CONSIDERATIONS OF OPINIONS AS TO THE VALUE OF THE EXAMINATION OF THE MARROW IN DISEASES OF THE BLOOD FORMING ORGANS.

Conflicting opinions are expressed by other workers concerning the value of the evidence which can be secured from a careful examination of marrow smears, in arriving at a diagnosis in the various diseases of the blood forming organs. Similar differences of opinion are expressed about its value in assessing prognosis, and estimating the response of the marrow to treatment. The views of the various leading authorities are now summarized.

Reich (1935) and Dameshek, Henstell and Valentine (1937) thought the examination of the marrow useful in

(1) differentiating the various types of anaemia.

(2) establishing the diagnosis in doubtful cases of leukaemia.

(3) differentiating aleukaemic lymphatic leukaemia from agranulocytosis.

(4) in finding tumour cells in the bone marrow.

(5) in observing the direct results of therapy.

Hynes (1939) wrote that the study of the bone marrow is now essential in all the pure diseases of the blood. From it we may learn what are the pathological changes in the marrow, whereas blood merely reflect and distort these changes. Sternal puncture gives a clearcut and distinctive picture in leukaemia, pernicious anaemia, haemolytic anaemia and secondary anaemias /

anaemias.

Kendel and Leroy (1939) thought that examination of the marrow was of relatively little use in diagnosis.

Falconer and Leonard (1941) commented thus :- "It is interesting to note that the diagnosis arrived at after thorough study of each case before sternal aspiration was rarely changed by data subsequently obtained by study of the marrow cells."

Mendell, Meranze and Meranze (1942) thought that marrow examination was indicated in all cases suggesting disorders of the blood forming organs or reticulo-endothelial system.

Davidson (1941), wrote that the scene of interest in haematology has shifted from the peripheral blood to the bone marrow and urged the examination of the bone marrow in the hope of making a correct diagnosis in certain anaemias in which a similar peripheral picture may be the result of an entirely different pathological process in the bone marrow. He added that diseases such as cancer, lymphadenoma, multiple myelomatosis and the lipoidoses can be diagnosed by establishing the findings of the specific cellular elements in the bone marrow. It may also be of value in excluding the presence of disease, such as lymphatic leukaemia, when there is a lymphocytosis in the peripheral blood, such as occurs in whooping cough, and infectious mononucleosis.

Propp and Schwind (1944) considered that most cases could be diagnosed without recourse to sternal puncture.

These /

These workers either confine the examination of the marrow to a much too restricted field, or fail to give a detailed description of what they consider the value of marrow examination to be in all the various diseases of the blood forming organs. The preceding account of my cases establishes that examination of the marrow can give valuable assistance in a much wider field than is suggested by the writings of some of these authors.

The evidence presented in this thesis shows that the chief value of a study of the marrow is the gaining of information as regards the morphology of the marrow cells as well as their respective numbers in disease. This information should be correlated with the other clinical features in order to establish an accurate diagnosis. Information gained from this examination can also be useful in forecasting the prognosis and assessing the efficacy of therapy. This value of marrow examination can be summarized as follows :-

Diseases primarily affecting the red cell series.

(a) Haemopoietic factor deficiency anaemia. Sternal puncture has a great value in unmasking early pernicious anaemia with atypical signs and symptoms. (Cases C and D). It is in this type of case that marrow examination serves one of its most useful purposes and this fact is not stressed by any writings on this subject. It was of almost equal value in ruling out pernicious /

pernicious anaemia when this diagnosis had been suspected on clinical grounds. (Cases F, G. and H). Prompt examination of the marrow in patients with proved pernicious anaemia, who have relapsed whilst receiving injections at home, eliminates serious marrow pathology at an early stage in the investigation, and allows treatment with other liver preparations to be instituted. (Case I). Finally, patients with an initial macrocytic anaemia due to such conditions as leucopoenic myelosis, macrocytic haemolytic anaemia or anaemia secondary to infiltration of the marrow with carcinoma or lymphadenoma can have these conditions diagnosed as soon as they are admitted to hospital by examination of the marrow, (Cases A and B), thereby reducing the length of stay in hospital.

Abnormal leukopoiesis found in the majority of marrows in patients suffering from lack of the haemopoietic factor gives little help in diagnosis.

(b) Refractory anaemias. The marrow should be examined when there is lack of response to adequate liver therapy whether or not the initial diagnosis of pernicious anaemia was confirmed by sternal biopsy. There are a group of cases which fail to respond to liver injections given at home but when they are readmitted to hospital and the injections repeated immediately respond satisfactorily. (Case I). In a sense these are examples of refractory anaemia, but are /

are not included in the classification of this condition. If, after ten days of liver therapy, there is a failure to respond, a further sternal puncture must be performed. If the picture is still megaloblastic a true refractory anaemia can be diagnosed. (Cases J. and K.). Study of other patients with refractory anaemia demonstrated that the marrow may have a dimorphic appearance. (Case L.). Others with a megaloblastic picture, after the marrow has become normoblastic, require iron to bring about a satisfactory haemopoietic response. (Cases M. and N.). Study of the marrow cytology has enabled the classification of refractory anaemia to be clarified (Davidson, 1943) and further extended by experience gained in this work.

(c) Iron deficiency anaemias. In many instances the evidence for diagnosing an iron deficiency of unknown origin is often incomplete and a marrow examination, if it reveals a normoblastic marrow, gives valuable confirmatory evidence of this condition, and is of particular value in ruling out aplasia of the marrow. (Case V.). The demonstration of a normoblastic reaction in the marrow is of value in confirming iron deficiency anaemia in males. (Cases S., T., U.). The demonstration of an aplastic condition does not necessarily mean that the red cells will not react satisfactorily to treatment. (Case S.).

(d) /

(d) Haemolytic disease. An intense normoblastic reaction is always found in this disease. Sternal puncture is of value in differentiating the macrocytic blood picture sometimes produced by an haemolytic condition from that of a true pernicious anaemia. (Case B.) The finding of a normoblastic reaction was of value in ruling out pernicious anaemia. (Case W.) The improvement in this patient's health was attributed to the benefit gained from liver injections after a mistaken diagnosis of pernicious anaemia instead of to the natural remissions characteristic of familial haemolytic jaundice.

Sternal biopsy is of little value in the routine examination of haemolytic disease.

Diseases primarily affecting the white cell series.

(a) The myeloid series. The value of marrow examination in this condition can be demonstrated in three distinct groups of cases.

(1) To confirm a confident diagnosis of chronic leukaemic myelosis. (Case Z.)

(2) To unmask the diagnosis of the aleukaemic form. (Cases A 1 and A 2).

(3)a. To differentiate lymphadenoma from leukaemic myelosis. (Cases A 3 and A 4).

b. To differentiate leukaemoid states from leukaemic myelosis. (Case A 5).

It is of minor importance in confirming chronic leukaemic myelosis complicating pernicious anaemia.

(b) Lymphoid /

(b) Lymphoid series. The valuation of the examination of the marrow smears in this disease was shown to be intimately linked to the starting point of this disease. If the condition does involve all the lymphocytic tissue simultaneously throughout the body then the marrow will reflect this process with an increase in the lymphocytes at the earliest stages. Whether the process does in fact start simultaneously throughout the body is still unsolved, but the case presented in this thesis did support the view that an early involvement of the bone marrow occurs. (Case P). Little reliance can be placed upon a marrow lymphocytosis of less than 30% in the diagnosis of chronic lymphocytic leukaemia. (Case Q.). In doubtful cases with a marrow lymphocytosis of about 30% a further sternal biopsy should be performed in about three weeks' time when clearcut findings are usually present. (Case Q.). A figure of over 40% is pathognomonic of leukaemia. (Case P.).

Post mortem studies on cases of lymphosarcoma cell leukaemia have proved that the bone marrow can remain unaffected but that this is not always the case, is proved by my patient in whom a marrow, infiltrated with lymphocytic cells, was found. These lymphocytic cells had the morphological appearance of lymphosarcoma cells. (Case O.).

(c) Monocytic series. This disease can masquerade in an aleukaemic form as was clearly demonstrated by Case A 6. /

Case A 6. The only possible method of arriving at this diagnosis was by finding the marrow infiltrated with the various types of monocytes, as these cells were only identifiable in the marrow smear.

The haemorrhagic diseases.

Marrow examination is of little value in the diagnosis of essential thrombocytopenia.

Symptomatic thrombocytopenia following upon the replacement of the marrow by various cells can often be demonstrated by marrow biopsy, but failure to find the incriminating cell may be due to the aspiration of a piece of marrow unaffected by the disease.

Marrow examination also helps to eliminate all these forms of symptomatic thrombocytopenia when the purpura is suspected to be due to damage to the capillary endothelium by an unknown toxin. (Case Y.).

Valuable confirmation of the diagnosis of spontaneous haemophilia is forthcoming when a definite increase in the megakaryocytes is found in the marrow of a suspected case. (Case X.).

Tropical diseases.

Marrow examination is said to be of value in demonstrating the parasites of malaria when they cannot be found in the peripheral blood. I have had the opportunity of performing a marrow examination in two cases of suspected chronic malaria in soldiers recently repatriated from malarial districts, but I found no parasites in the red cells of the marrow, nor were any parasites found in the peripheral blood. Both patients recovered on anti-malarial therapy. It is difficult to assess the value of this method in the diagnosis of malaria.

In kala-azar, Leishman-Donovan bodies have been demonstrated in the marrow cells and Murray (1946) reported that the frequency of positive results depends upon the time spent in the examination of the films for the parasites. If a painstaking examination is made the proportion of positive results is very high and becomes of great value as a diagnostic procedure. I have had no opportunity to study kala-azar.

S U M M A R Y.

(1) This thesis opens with an historical review of the literature dealing with the discovery of the marrow and the realisation of the value of the study of this tissue, in widening the knowledge of the diseases of the blood forming organs.

(2) My technique of sternal puncture is described and compared with that of other authorities.

(3) The nomenclature used by different writers in their description of the marrow cells is discussed and the reasons for my choice in terminology given. Each cell found in normal and abnormal marrow is minutely described so that when any cell is referred to subsequently the reader will have no doubt as to which cell I am referring.

(4) An historical review of the discovery and development of the various methods used in staining the cells is given and my technique described.

(5) The value of myelograms is discussed.

(6) The importance of the examination of the marrow in the various diseases of blood forming organs is described in detail, and the wider application of this method of examination is urged in the diagnosis of these diseases.

(7) The value which I place on the importance of marrow examination as compared with other authorities is summarized.

REFERENCES

INTRODUCTION AND NOMENCLATURE

ARINKIN. M.I. 1929. Folia Haemat. 38 233.

COHNHEIMJ, cit. MERWE, C.F. 1936. Folia Haemat. 45 108. 218.

CORONIA , G., 1922. Pediatria, Napoli, 30 607.

DAVIDSON, L.S.P., DAVIS, L.J., INNES, J., 1941. Edin. Med. Journal 48 680.

" " " " 1945 ibid 50 231

FALCONER, H., and LEONARD, M.E., Ann. Int. Med. 1941 15 446

GHEDINI, G., 1908. Clin. Med. Ital. 47 724

" 1910 Wien Klin. 23 1840

" 1911 ibid. 24 736

HOLMES, W.F., and BROWN, G.O., Proc. Soc. Exper. Biol. and Med. 30 1306.

HYNES, M., 1939. Lancet 1 1373-1379.

JOPPICH, G., and LIESSENS, P., 1937, Monatschr.k. Kinderh 71 382

KRUMBHAAR, E.B., and CUSTER, R.P., 1935, Amer. Journ. Med. Sci. 189 630

MANDELL, T.H., and MERANZE, D.R., 1942. Ann. Int. Med. 1180-1196

MEUTENS, E., 1945. Amer. Journ. Med. Sci. 210. 630-634

NEUMANN, 1868. Centralbe. Med. Wiss. 6 689

PROPP, S., and SCHWIND, J.L., 1944. Ann. Int. Med. 21 605.

REICH, C., and KOLB, E.N., 1942. Amer. Journ. Med. Sci. 204 496.

REICH, C., Journ. Lab. and Clin. Med. 1934. 20 286.

ROBIN /

ROBIN, C., 1849, Gaz. Med., Paris, 12 992.

SEYFARTH, C., 1913, Deutsch. Med. Wchnschr. 46. 1380.

SCHLEICHER, E.M., and SHARP, E.A., Journ. Lab. and Clin. Med. 1937 22 949

SCOTT, R.B., 1939. Quart. Journ. Med. 32 127-173

TURKELL, H., and BETHELL, F.H., 1943. Journ. Lab. and Clin. Med. 28 1246-1251

TUSCHINSKI, M.D., and KATLAVANDO, B.N., 1937. Folia Haemat. 46 235

VOGEL, P., ERF, L, and ROSENTHAL, N., 1937. Amer. Journ. Path. 7 437

VOGEL, P., and BASSEN, F.A., 1939. Amer. Journ. Dis. Child. 57 245

WELLER, G.L., 1932. Journ. Lab. and Clin. Med. 22 753

YOUNG, R.H., and OSGOOD, E.E., 1935. Arch. Int. Med. 55 189-203

ZANATY, A.F., Lancet 1937. 2 958-962.

STAINING METHODS.

GIEMSA, 1910. Munchen. Med. Wchnschr. 51 2479.

JENNER, 1899. Lancet 1 37.

LEISHMAN, 1901. Brit. Med. Journ. 1 635 and 275.

MALACHOWSKI, 1891. Centralbe. Clin. Med. 12 601.

NOCHT, 1900. Ibid. ibid. 28 114.

REUTER, 1901. Munchen. Med. Wchnschr. 48 1261.

ROMANOWSKI, 1891. Centralbe. F. Bakt. I. Abt. 10 163.

UNNA, 1891. Ztschr. f. Winshrsch. Mikr. 8 475.

WRIGHT, 1902. Journ. Med. Research. 7 138.

MYELOSIS.

FORKNER, C.E., 1936. Leukaemia and allied disorders, New York.

LEIBOWITZ, A., 1938. Virchows. Arch. Berl. 124.

KLIMA, R., 1938. Berl. u. Wien.

SCOTT, R.B., 1939. Quart. Journ. Med. 32 127.

STERNE, E.H., SCHIRO, MOLLE. W., 1941. Amer. Journ. Med. Sci. 202 167.

WOOLLEY, P.B., 1944. Lancet 1 85.

LYMPHOCYTIC LEUKAEMIA.

BANTI, G., 1904. Zentralbl. f. alleg. Path. u. path. anat. 14.

BEATTIE, J.M., and DICKSON, W.C., 1943. A Textbook of Path. Heinemann 4th ed.

BETHELL, E., 1943. 118 95-99.

BOYD, 1945. A Textbook of Path. 4th ed. London, 1943.

CAIRD, A., 1940. Edin. Med. Journ. 47 278.

DIBBLE, and DAVIE. 1943. Churchhill, London, 2nd ed.

FLEISHER, 1881. Virchows. Arch. Berl. 73 124.

FREIDMANN, 1945. Radiology 44.

GULLAND, 1925. Brit. Med. Journ. 2 108.

ITALO, 1930. Proc. Roy. Soc. Med. 25 1400.

ISRAELS, R., 1935. Arch. Path. Chicago 20 155.

KLIMA, 1938. Berl. u. Wien.

KRACHI, R.R., and GARVER, H., 1935. J.A.M.A. 104 697.

KUNDAT, 1893. Wien. Klin. Wchnschr. 6 211.

LIVINGSTONE, 1932. Minerva. Med. 2 560.

MALLORY, 1914. W.B. Saunders & Co., Phil.

MOORE, 1945. Textbook on Pathology. London.

McCALLUM, 1940. Textbook on Pathology. 7th ed.

NAEGELI, 1913. Leukaemia. u. Pseudoleukaemia. Wien.

NEUMANN, 1870. Centralbl. Med. Wien. 6 684

SCOTT, R.B., (1939, Quart. Journ. Med. 32 127.

STERNBERG, 1904. Ztschr. f. Heilkunde 25 170.

WARREN, S.L., 1929. Amer. Journ. Med. Sci. 178 490.

WIERNER and KAZNELSON, 1925. Folia Haemat. Leipzig, 32 233.

WISEMAN, 1935. Ann. Int. Med. 1303.

1943. J.A.M.A. 118 (2) 100-106.

ZANATY, 1934. Virchows. Arch. f. Path. Anat. 270 356

REFRACTORY ANAEMIA.

BOMFORD, R.R., and RHOADS, C.P., 1941. Quart. Journ. Med. 10. 175.

DAVIDSON, L.S.P., DAVIS, L.J., and INNES, J., 1943. Edin.Med. Journ. 50 226,355,431.

THOMSON, W.P., RICHTER, M.N., and EDSALL, K.S.,1934. Amer. Journ. Med. Sci. 187 77.

RHOADS, C.P., and MILLER, D.K., 1938. Arch. Path. 26 648.

IRON DEFICIENCY

SCOTT, R.B., 1939. Quart. Journ. Med. 32 127-173.

DAMESHEK, W., 1931. Amer. Journ. Med. Sci. 182 41.

YOUNG, R.H., and OSGOOD, E.E., 1935. Arch. Int. Med. 55 200-203.

HAEMORRHAGIC DISEASES.

CREVELD, S.V., 1942. Acta. Paediat. 29 37.

MARKOFF, N., 1936. Deutsch. Arch. f. Clin. Med. 164 113

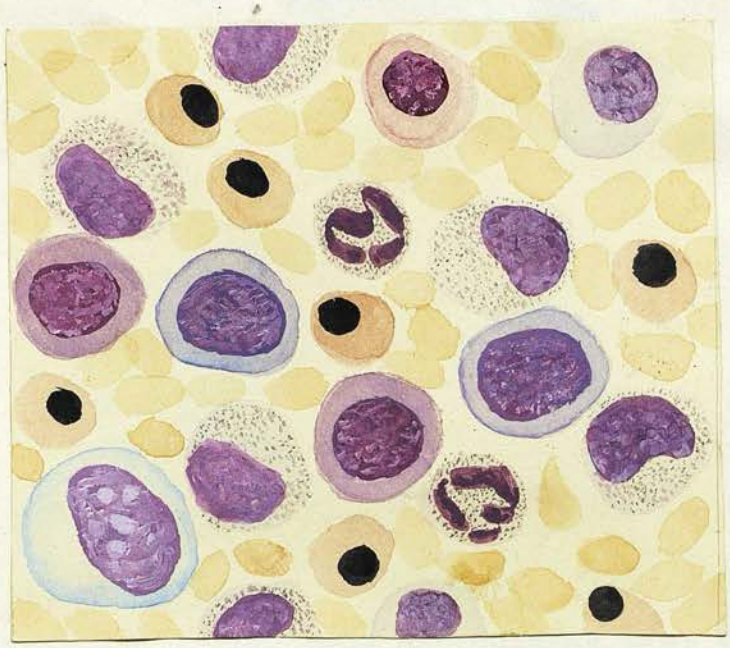
ROHR, K., and KOLLER, F., 1936. Klin. Wchnschr. Berl. 15 1549.

SEELIGER, S., 1924. ibid. 3 731.

WISEMAN, B.K., DOAN, C.A., and WILSON, S.J., 1940. J.A.M.A 115 8.

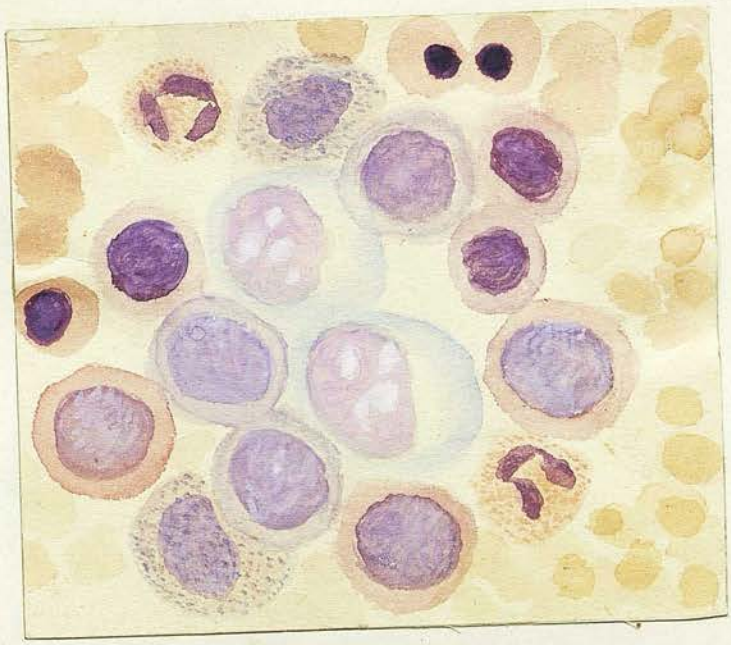
BONE MARROW

NORMAL FILM.

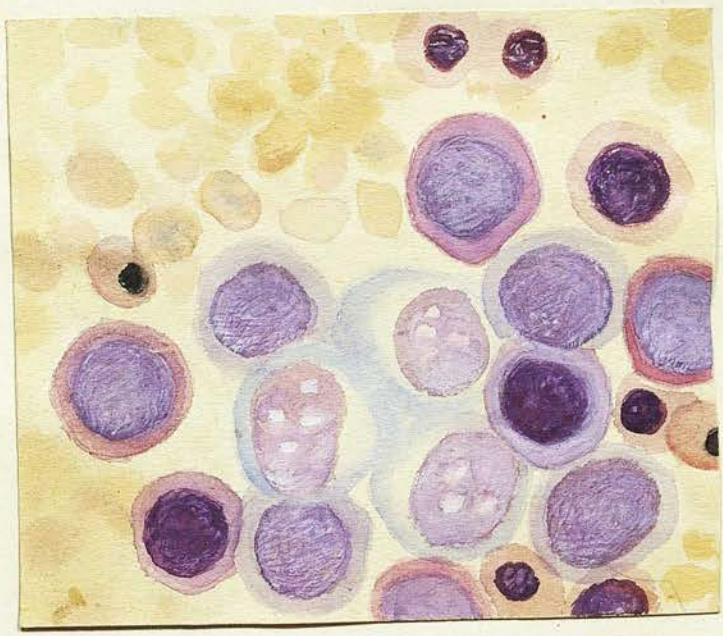


BONE MARROW.

MEGALOBLASTIC ERYTHROPOIESIS.



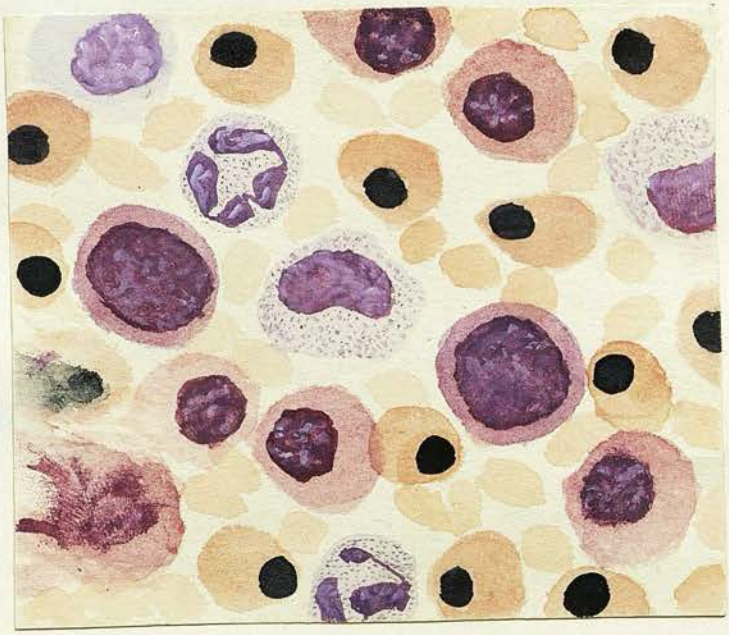
This film shows two myeloid cells in addition to primitive red cells, thus emphasising that there is often a myeloid proliferation in this disease.



This film shows islands of haemocytoblasts surrounded by pro-erythroblasts and megaloblasts A. and C.

BONE MARROW.

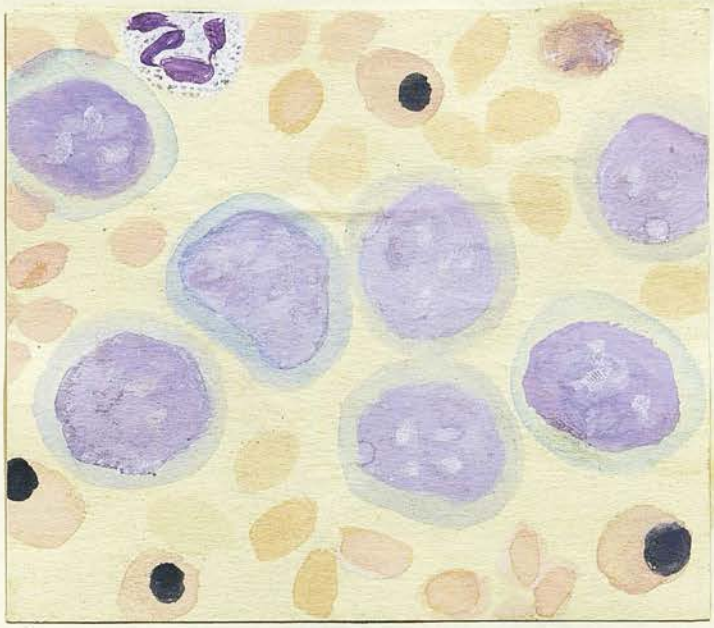
NORMOBLASTIC ERYTHROPOIESIS.



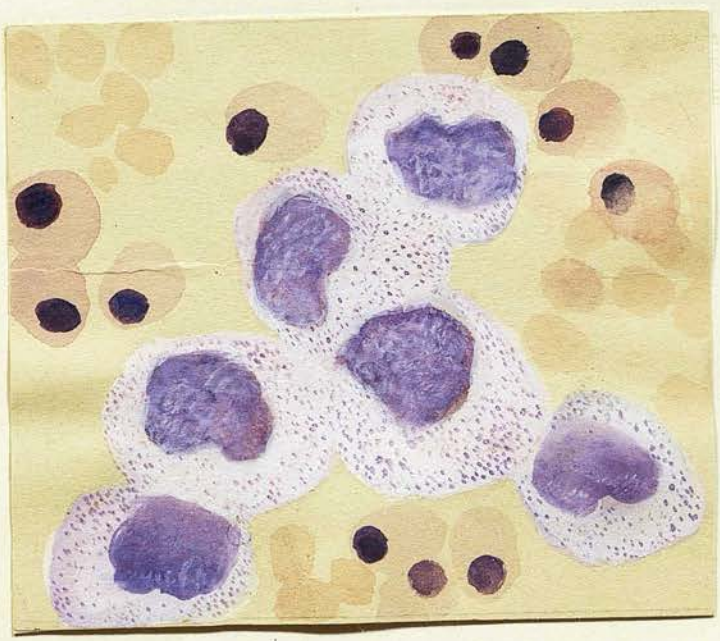
This film shows proliferation of normoblasts B. and C.

BONE MARROW.

LEUKOPENIC MYELOSIS.



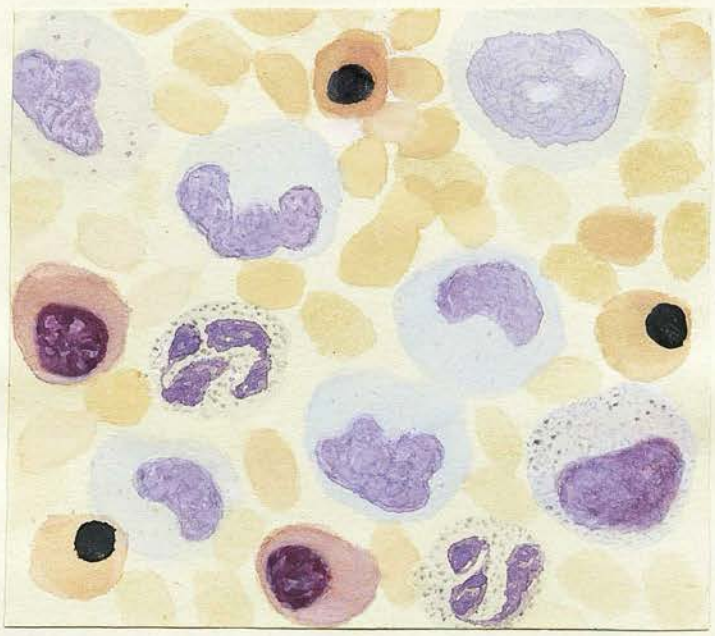
This film shows the invasion of the marrow with primitive myeloblasts in acute leukopenic myelosis.



This film shows the invasion of the marrow with promyelocytes and myelocytes in chronic leukopenic myelosis.

BONE MARROW.

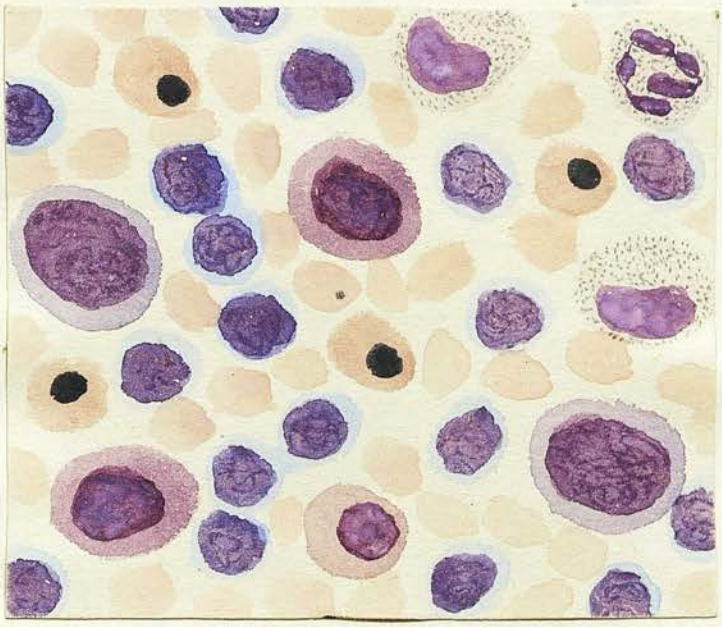
MONOCYTIC LEUKAEMIA.



This film shows the marrow invaded with various types of monocytes.

BONE MARROW.

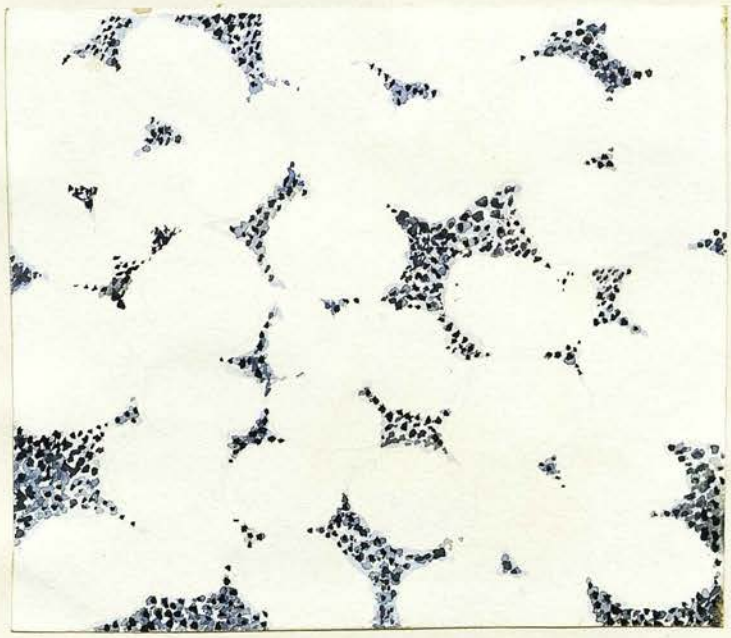
LYMPHOCYTIC LEUKAEMIA.



This film shows the marrow tissue invaded with small lymphocytes in chronic lymphocytic leukaemia.

BONE MARROW.

APLASIA.



This film shows aplasia of the marrow, but this does not necessarily mean that the marrow will prove to be aplastic, after treatment.