THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME III MAY, 1927 NUMBER 3

THE PATHOLOGY OF THE BONE MARROW IN PERNICIOUS ANEMIA *

FRANCIS W. PEABODY, M.D.

(From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.)

In spite of the enormous amount of attention that has been devoted to the subject, it must be admitted that very little is actually known about the pathogenesis of pernicious anemia. Cohnheim,[1] who, in 1876, was the first to describe the bone marrow, regarded the anemia as due to a primary disturbance of blood formation, and many authorities have since maintained the same point of view. Another conception of the disease, however, regards the bone marrow changes as of a compensatory nature, and as the result of an attempt on the part of the organism to make good the losses arising from excessive blood destruction. This theory has become the more generally accepted one, even though the evidence of increased blood destruction is not convincing. Recent investigations of pernicious anemia have been, for the most part, concerned with the cell types and chemical constituents of the peripheral blood, but there is reason to believe that in order to understand the pathology of this disease, and indeed that of the other diseases of the blood-forming organs, more attention must be paid to the bone marrow than is now customary among clinical hematologists. The present study is a contribution to the pathology of the bone marrow in pernicious anemia, and the observations indicate that the changes in the blood are largely the result, rather than the cause, of abnormal bone marrow function.

There are two main reasons why comparatively few attempts have been made to correlate the pathologic changes in the bone marrow

* Received for publication, February 1, 1927.

in pernicious anemia with the findings in the peripheral blood and the clinical condition of the patient. The first is that lack of definite knowledge of the normal anatomy and physiology of the bone marrow has made the interpretation of pathologic changes in the bone marrow almost impossible. This difficulty appears to have been met, to at least a large degree, by the recent work of Sabin and her associates,[2] which clarifies the situation in the normal, and opens the way to the investigation of the abnormal. The second reason is that, with few exceptions, all the studies of the bone marrow in pernicious anemia have been made on material obtained postmortem, and the pathologic changes represent the most advanced stage of an exceedingly complex process. This difficulty can also be surmounted if specimens of bone marrow are obtained at different phases in the relapses and remissions that are so characteristic of the disease. Such material can be secured by means of biopsies on the tibial marrow, and this paper deals with the pathology of the bone marrow at various stages in the course of pernicious anemia.

Puncture of the shaft of the tibia and withdrawal of small amounts of bone marrow for microscopic examination is by no means a new operation. It was apparently first performed on man in 1908 by Ghedini of Genoa,[3] who described his results in more than twenty cases, and recommended the method as a diagnostic procedure. Caronia [4] made bacteriologic examinations of the bone marrow in children with measles, and Morris and Falconer [5] studied the smears of tibial bone marrow in various pathologic conditions. More important use of the method was made by Zadek,[6] who compared the findings in the bone marrow and peripheral blood in a series of cases of pernicious anemia. He observed that the bone marrow, which is red in periods of relapse, becomes yellow and fatty during periods of remission, and he showed that the megalocytosis, which characterizes the blood picture in the relapse, corresponds to an increase of megaloblasts in the bone marrow.

All the above authors, who have studied the bone marrow intravitam, have concerned themselves, wholly or in large part, with smears rather than with sections of fixed tissue. The examination of smears of bone marrow is, however, unsatisfactory, for they do not necessarily give the correct idea of the relative number of different cell types present (thus megaloblasts may be so firmly adherent to one another, that only a small proportion appears in the smear), and

they give no indication of the actual structure of the bone marrow. As more is learned about the normal physiology of blood formation and the liberation of cells into the peripheral vessels, it becomes apparent that these physiologic functions can often be interpreted by the study of the anatomy of the bone marrow. It is thus evident that the elucidation of the pathologic physiology of the diseases of the hematopoietic system will be considerably aided by a better knowledge of the structure of the bone marrow, which can be acquired only from the study of sections of fixed tissue. The number of cases of pernicious anemia reported in this paper is relatively few (seven cases), but they suffice to throw light on the subject, and may serve as a basis for further work. It is also hoped that this method of study, namely, the study of the histology of the bone marrow at different stages of a disease, may be useful in the investigation of other pathologic conditions of the blood-forming organs.

There are certain obvious limitations to the value of an examination of small pieces of bone marrow from the tibia in the interpretation of the pathology of an organ which is so widespread in its distribution. The most important are, first, that the marrow of a long bone like the tibia is not entirely homogenous in structure, so that the specimen obtained by puncture may not be representative of the marrow as a whole, and second, that the pathologic process in the marrow of the tibia does not necessarily correspond in extent and in degree to that in the bone marrow of other regions. These points are admirably brought out in the colored illustrations in Sheard's [7] book, which show the variations in gross appearance of the marrow in different bones from a case of pernicious anemia. The evidence which has accumulated in this laboratory, from a study of bone marrow obtained intravitam and postmortem in a much larger group of cases of pernicious anemia than is reported in the present paper, seems to indicate that the pathologic process usually starts in the active marrow of the flat bones and vertebrae, and while progressing in them, spreads peripherally so that it involves the femur and later the tibia. Thus the process in the tibia is often less advanced than in the femur. Presumably the same course of extension to the periphery goes on in the arms. Within an individual long bone, the process generally begins at the epiphysis and spreads gradually to the center of the diaphysis. With the retrogression of the pathologic changes, during a clinical remission, the process is usually

reversed, and begins to clear up first in the tibia. Thus the marrow of the tibia, which may be involved only rather late, is, nevertheless, a more sensitive index of the extent and degree of the pathologic process than is, for instance, the marrow of the femur. Bearing in mind all the limitations that one must accept in examining small specimens of marrow from the tibia, there yet remains much to be learned from them, and this is especially true when two or more specimens can be obtained from a single case at different periods in the course of the disease.

Technic of Tibial Puncture

The operation of tibial puncture must, of course, be carried out under the strictest surgical precautions. I am greatly indebted to Dr. Robert C. Cochrane for all the specimens of bone marrow obtained during life. The soft tissues over the middle of the anterior or mesial aspect of the tibia were carefully infiltrated with a 2 per cent novocain solution over an area sufficient to permit a longitudinal incision about 4 cm. in length down to the periosteum. After elevation of the periosteum, the marrow cavity was entered by means of a small drill or a trephine which removed a 6 mm. cylinder of bone. Then small pieces of the marrow, 1 to 3 mm. in diameter, were removed by means of a small sharp bone curette, the cavity of which was rather well recessed and straight-sided. The specimens were immediately fixed in Zenker's solution, later embedded in paraffin, sectioned, and stained with eosin and methylene blue. Hemorrhage into the tissues sometimes complicated the interpretation of the histology. In sewing up the wound it was found best to use no sutures except in the skin, and to apply a tight bandage to prevent oozing. No untoward results have been observed in a series of eighteen operations, and the fact that the discomfort to the patient was not great is indicated by there having been two tibial punctures on several patients and three punctures on one patient. It is appropriate, however, to express deep appreciation for the coöperation of the patients who willingly submitted to these operations.

The description of the clinical cases and the observations on the condition of the bone marrow follow. The nomenclature of cells in the bone marrow is according to the terminology established by Sabin.[2]

CASE 1. A. F., a man 33 years old, with a history typical of pernicious anemia extending over two years, and apparently in his third relapse. Physical examination, laboratory tests and hematologic studies were all characteristic of the disease.

May 18, 1925, examinations of blood showed: hemoglobin, 15 per cent; red blood cells, 0.6 million per c.mm.; leucocytes, 5400 per c.mm.; serum bilirubin, 1.5 mg. per 100 cc.

Biopsy of tibial marrow, May 19, 1925. The tissue was very cellular, and the fat, which is normally present in the marrow of the tibia, had been completely replaced by cells. The histologic picture was as complex as that of the bone marrow obtained postmortem in pernicious anemia, and was, in general, similar to it in character. The most striking feature was the enormous hyperplasia of megaloblasts. These cells, which have large vesicular nuclei with a definite chromatin network, and basophilic cytoplasm, were found in clumps, cords and columns, and sometimes as separate cells. Some of this separation of individual cells was unquestionably due to shrinkage in the process of fixation. For the most part, the megaloblasts were adherent to one another and the appearance of the tissue was suggestive of a tumor. Rapid multiplication of megaloblasts was indicated by the great number of mitoses, sometimes as many as six or eight being found in a single field with the oil immersion lens. Fig. 1 is a photograph under low power to show the general character of the tissue and the distribution of the megaloblastic hyperplasia. Fig. 2 is a photograph under high magnification, and Figs. 3 and 4 are drawings to illustrate the megaloblasts and their mitoses. In addition to the megaloblasts, the marrow contained many normoblasts and cells intermediary between megaloblasts and normoblasts. There were relatively few mature erythrocytes. Giant cells were present in moderate number, while myelocytes and leucocytes were fairly common. A few myelocytes were found in mitosis. In contrast to the usual findings in bone marrow obtained postmortem from cases of pernicious anemia, there was scarcely any phagocytosis of red blood cells by clasmatocytes.[8] The venous sinusoids, which are frequently such a prominent feature in early or slight degrees of bone marrow hyperplasia, were narrow, compressed and recognized only with great difficulty.

Two months later, on July 20, 1925, the blood examinations were as follows: hemoglobin, 13 per cent; red blood cells, 0.8 million per

c.mm.; leucocytes, 1200 per c.mm.; serum bilirubin, 0.57 mg. per 100 cc. On July 22, a second tibial puncture was performed, and it is of interest, that shortly afterwards a spontaneous remission set in.

Biopsy of tibial marrow, July 22, 1925. The tissue was very similar to that obtained on May 18, but there were occasional cells containing globules of fat, and there was a definite increase in the number of normoblasts and mature red blood corpuscles. The megaloblastic hyperplasia was still the predominant feature, however, and there were many mitoses of megaloblasts. There were also more cells of the leucocyte series. The venous sinusoids remained compressed and obscure.

On August 7, the hemoglobin was 34 per cent, and the erythrocyte count was 1.5 million per c.mm. On September 2, "great clinical improvement" was noted. On September 28, the hemoglobin was 52 per cent and the red blood cell count was 2.4 million per c.mm. During the following months the patient lived on a diet containing large amounts of liver, and a striking clinical remission took place. On March 12, 1926, the blood examinations were as follows: hemoglobin, 86 per cent; red blood cell count, 5.5 million per c.mm.; leucocytes, 6200 per c.mm.

Biopsy of tibial marrow, March 12, 1926. A large part of the specimen was composed of fat cells, and was without evidence of myeloid hyperplasia. The intersinusoidal capillaries in this area were filled with normal erythrocytes, but it was impossible to determine whether this was a true hyperemia or was the result of hemorrhage during the operation. Another part of the specimen consisted of well filled fat cells, separated from one another by small groups of myeloid cells which apparently were within intersinusoidal capillaries. The capillary endothelium was moderately hypertrophic and hyperplastic. Within the capillaries were a few megaloblasts and erythroblasts, very many normoblasts and many mature erythrocytes. There was also a moderate number of cells of the leucocyte series. The venous sinusoids were widely distended with blood, and the conical openings of the intersinusoidal capillaries into them were easily made out in many places. The whole picture resembled that of the early stage of simple marrow hyperplasia.[9] Fig. 5 shows the general character of the more cellular part of the specimen, and Fig. 7 shows the predominance of normoblasts in the islands of myeloid cells.

Summary of findings in Case 1

At the height of a severe relapse (May 19, 1925), the marrow was characterized chiefly by the complete replacement of fat by myeloid cells, and by the great hyperplasia of megaloblastic tissue with numerous mitoses. There were many normoblasts, but relatively few mature erythrocytes, and the venous sinusoids were narrow and compressed. Two months later (July 22, 1925), just before the onset of a clinical remission, the marrow was similar except for the presence of a few cells containing fat, and a relative increase of normoblasts and mature red blood cells. Ten months after the first examination (March 12, 1926), during a remission in which the erythrocyte count was normal, the cellular hyperplasia had almost completely disappeared and the marrow consisted largely of fat cells. In the small capillary spaces, between some of the fat cells, were many erythrocytes and normoblasts, but the more primitive cells (megaloblasts) were comparatively few in number. The venous sinusoids had become widely distended with blood.

CASE 2. C. H., a woman 46 years of age, who had been under observation for four years as a typical case of pernicious anemia. There had been several relapses followed by periods of moderate remission. On March 12, 1926, she was in the hospital during a severe relapse and the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 4400 per c.mm.; serum bilirubin, 0.9 mg. per 100 cc.

Biopsy of tibial marrow, March 12, 1926. The tissue showed a great hyperplasia of myeloid cells and relatively few cells containing fat globules. Fig. 8 is a photograph to show the general character of the tissue, and the displacement of the fat. The histology resembled that seen in Case 1 during the relapse. There was a striking hyperplasia of megaloblasts with a general tendency on the part of the cells to adhere to one another and to lie in clumps and columns. Rapid cell growth was indicated by the many mitoses seen in the megaloblasts. There were many normoblasts, but few mature red blood cells, few giant cells and few leucocytes. The venous sinusoids were often outlined by pigment granules in the endothelium, but they were compressed and indistinct. Abnormal phagocytosis of erythrocytes or of pigment was not observed.

Immediately after the above observation was made, the patient

began to live on a diet containing much liver, and a prompt clinical remission set in with rapid rise in the red blood corpuscles. On April 29, 1926, the erythrocytes had risen to 3.5 million per c.mm., and a second biopsy was performed on the tibial marrow. The other blood examinations showed: leucocytes, 8500 per c.mm.; serum bilirubin, 0.18 mg. per 100 cc.

Biopsy of tibial marrow, April 29, 1926. The tissue consisted largely of cells well filled with fat. It had considerably more fat than has normal vertebral marrow. Fig. 6 is a photograph, under low magnification, to show the relation of fat to cellular areas (compare with Fig. 8). The small cellular areas between the fat globules were chiefly composed of erythrocytes in the intersinusoidal capillaries, but there were also a great many normoblasts, some of which showed definite mitoses. The normoblasts were often in large clumps which filled the spaces between the fat cells, as shown in Fig. 9. Megaloblasts were not a prominent feature but there were a few small groups and a few scattered single cells. Mitosis were rare among the megaloblasts. Leucocytes and giant cells were few in number. The venous sinusoids were outlined by pigment in the endothelium, and they were comparatively wide and distinct.

Two months later, on June 29, 1926, the hemoglobin had risen to 92 per cent; and the erythrocyte count to 4.9 million per c.mm.

Summary of findings in Case 2

During a severe relapse (March 12, 1925), the fat of the bone marrow was almost entirely replaced by myeloid cells, and there was a striking hyperplasia of megaloblasts with many showing mitoses. Many normoblasts were present, but mature red blood cells were not particularly numerous. Early in the development of a rapid remission (April 29, 1926), the bone marrow was characterized by a great increase in fat deposits and by large numbers of normoblasts and mature erythrocytes, but at this time, only a few megaloblasts were observed. The venous sinusoids were much more distended and distinct during the remission than during the relapse.

CASE 3. V. D., a man 48 years old, with symptoms of anemia for one year, together with the physical and laboratory findings typical of pernicious anemia. On July 15, 1925, the hemoglobin was 43 per cent, and the red blood cell count 2.4 million per c.mm. On August

20, the hemoglobin was 31 per cent, and the red blood cell count 1.0 million per c.mm. On September 2, the blood examinations showed an erythrocyte count of 1.1 million per c.mm.; leucocytes, 4100 per c.mm.; reticulocytes, 1.1 per cent; and the serum bilirubin, 0.8 mg. per 100 cc. The patient was thus having a relapse.

Biopsy of tibial marrow, September 2, 1925. The tissue contained a considerable amount of fat, about the amount found in normal vertebral marrow. The general character is shown in Fig. 11. There were many rather large islands of megaloblasts arranged in clumps or columns, and many single megaloblasts. Megaloblasts and erythroblasts were frequently in close relation to the fat cells, and their position suggested that they arose from the endothelium lining the intersinusoidal capillary spaces. Mitoses of megaloblasts were found in moderate numbers. There were very many normoblasts, and several definite mitoses were observed among them. Mature erythrocytes were present in moderate numbers. Phagocytosis of red cells was not seen. Giant cells were rare, and there were few myelocytes or leucocytes. The sinusoids were compressed and indistinct.

In the subsequent months there was no striking change in the patient's condition, but he gradually failed in health. On Jan. 18, 1926, the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 3500 per c.mm.; serum bilirubin, 0.66 mg. per 100 cc. On this day a second tibial biopsy was performed. The patient left the hospital soon after, and died at home on Feb. 26, 1926. The second biopsy was thus made almost at the end of the terminal relapse.

Biopsy of tibial marrow, January 18, 1926. The tissue contained about as much fat as was present in the first biopsy. There was a striking hyperplasia of the megaloblasts, rather more than in the first biopsy, the cells lying in clumps which were so large that they sometimes filled the spaces between the fat cells. Fig. 10 shows the amount of fat present and the extensive hyperplasia of megaloblasts. Mitoses were common among the megaloblasts, as many as five having been observed in a single field of the oil immersion lens. There were very many normoblasts but comparatively few mature red blood cells. For the rest, the tissue was similar to that observed on September 2.

Summary of findings in Case 3

Two specimens of tibial marrow were obtained during the course of the prolonged terminal relapse, the first about six months and the second about one month before death. The two specimens resembled each other closely and both contained a considerable amount of fat. In this connection it may be noted, however, that sections from other cases show that even at death the replacement of fat by myeloid hyperplasia is frequently much less complete in the tibia than in the femur. The marrow from both biopsies showed great hyperplasia of megaloblasts, but this process was somewhat more extensive in the second specimen. Normoblasts were present in great numbers, but there were comparatively few mature erythrocytes in either piece of tissue.

CASE 4. B. C., a woman who gave her age as 48 years, but who appeared to be considerably older. She had been "weak" for four months, and reported having numbness of the fingers for one month. Physical examination and laboratory tests were typical of pernicious anemia. Blood examinations were as follows: Aug. 1, 1925, hemoglobin, 18 per cent; erythrocytes, 0.8 million per c.mm.; leucocytes, 5500 per c.mm.; August 20, hemoglobin, 25 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 4800 per c.mm.; August 31, hemoglobin, 42 per cent; erythrocytes, 2.3 million per c.mm.; leucocytes, 6900 per c.mm.; September 30, hemoglobin, 63 per cent; erythrocytes, 3.5 million per c.mm.; leucocytes, 8100 per c.mm. A biopsy performed on September 2 was, therefore, done at a time when the patient was making rapid spontaneous improvement.

Biopsy of tibial marrow, September 2, 1925. The fat of the marrow had been entirely displaced and the tissue had a solid appearance. Mature red blood cells were the most prominent feature, and it is possible that they may have been due to hemorrhage, but their relation to the islands of myeloid tissue suggests that they were true components of the marrow. Megaloblasts were found in a limited number of small clumps and as scattered individual cells. Mitosis of megaloblasts was infrequent. There were a great many normoblasts in large and small groups. Giant cells were few in number, and leucocytes were fairly numerous. The sinusoids were compressed.

On Oct. 27, 1925, the blood examinations were as follows: hemoglobin, 71 per cent; red blood cells, 3.5 million per c.mm.; leucocytes, 9100 per c.mm. The second biopsy was performed on Oct. 28, 1925, at a time of marked clinical improvement, and just before the patient left the hospital. She was then in a rather prolonged remission of moderate degree. The patient was subsequently fed on liberal amounts of liver, and about eight months later the erythrocyte count was 4.6 million per c.mm.

Biopsy of tibial marrow, October 28, 1925. This tissue was taken from somewhat higher up in the tibia than was the first specimen. It contained about as much fat as normal vertebral marrow. There were a few small clumps of megaloblasts and scattered individual cells, but they were not a prominent feature. Mitoses of megaloblasts were found rarely. There were many normoblasts, but only a moderate number of mature erythrocytes. Cells of the leucocyte series were very numerous and giant cells were not uncommon. The sinusoids were somewhat more distinct than in the tissue from the former biopsy. The appearance of the tissue resembled that of normal active marrow from a vertebra, except that there were rather more megaloblasts.

Summary of findings in Case 4

The tissue obtained on Sept. 2, 1925, soon after the onset of a spontaneous improvement, contained no fat, and the cells consisted largely of erythrocytes and normoblasts. Megaloblastic hyperplasia was not a prominent feature. The specimen is to be compared with that of Case 2, taken on April 29, 1925, which was also obtained during a remission, and which differs from Case 4 chiefly in that it contained more fat. Both sections showed a predominance of normoblasts and mature red blood cells, and in both the megaloblastic hyperplasia was relatively slight. Two months later (Oct. 29, 1925), after the patient's still further improvement, the bone marrow showed an increase of fat, and a cell picture which resembled that of normal active vertebral marrow except for the moderate increase of megaloblasts. There were no longer such large numbers of erythrocytes in the marrow and it is probable that they had passed out into the capillaries. It may also be that after the relapse was over, the part of the marrow near the epiphysis (this specimen was taken from high up on the tibia) continued to function

as normal marrow. Unfortunately no specimen was obtained during the more complete remission that took place eight months later.

CASE 5. J. S., a man 61 years old, who had run a characteristic course of pernicious anemia for about eighteen months, most of which time he had been under observation. In August, 1925, he entered the hospital, shortly after the onset of his second relapse, with physical examination and laboratory tests wholly typical of pernicious anemia. The results of blood examinations were as follows: March 26, 1925, hemoglobin, 72 per cent; erythrocytes, 3.9 million per c.mm.; leucocytes, 8200 per c.mm.; June 12, 1925, hemoglobin, 82 per cent; erythrocytes, 3.3 million per c.mm.; leucocytes, 5800 per c.mm.; Aug. 25, 1925, hemoglobin, 30 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 5500 per c.mm.; Sept. 2, 1925, erythrocytes, 1.3 million per c.mm.; leucocytes, 4900 per c.mm. During these months he was, therefore, slowly going into a severe relapse.

Biopsy of tibial marrow, September 2, 1925. A part of the section consisted of fat cells with mature erythrocytes filling the intersinusoidal capillary spaces (hemorrhages?). The rest of the section showed much hyperplasia of myeloid cells between the fat cells, (there was about as much fat here as in normal vertebral marrow) and in one area the fat was completely displaced. The hyperplastic areas were composed chiefly of megaloblasts, growing in columns and islands, and often filling the space between the fat cells. There were many mitoses of megaloblasts. Fig. 12 shows a clump of megaloblasts between fat cells and one megaloblast undergoing division. Just below and to the left, there is another megaloblast in the same phase of mitosis, but it does not show clearly in this focus. There were a great many normoblasts, a moderate number of mature red blood cells, and many cells of the leucocyte series. The sinusoids were compressed and indistinct.

In the subsequent weeks the patient continued to fail gradually. The blood examinations on Dec. 7, 1925, were as follows: erythrocytes, 0.7 million per c.mm.; leucocytes, 9200 per c.mm.; serum bilirubin, 1.21 mg. per 100 cc. He died on the same day.

Tibial marrow at necropsy (two hours after death). The tissue showed the typical, extremely confused structure usually found at necropsy. The fat had been almost entirely replaced by myeloid

cells. The prominent feature was the hyperplasia of megaloblasts, the cells being single, in clumps, or in columns. There were many mitoses among the megaloblasts. Normoblasts were common. Giant cells were rare. There were many myelocytes and leucocytes. Throughout the tissue, and so numerous that there were often six or eight in a high power field, were clasmatocytes (endothelial cells) which had phagocyted erythrocytes, normoblasts, and occasionally leucocytes. The number of ingested red blood cells was enormous. The red cells within the phagocytes usually retained their normal appearance, and there were few phagocytes containing hemosiderin. Some of the sinusoids were broad and well defined, but most of them were compressed and difficult to distinguish.

Summary of findings in Case 5

On Sept. 2, 1925, during a severe relapse which took place three months before death, the bone marrow contained a considerable amount of fat, but between the fat cells there was an active hyperplasia of megaloblasts, with many normoblasts. On Dec. 7, 1925, the bone marrow, taken two hours after death, showed such an extensive increase in myeloid cells that the fat had almost completely disappeared. Hyperplasia of megaloblasts was the predominant feature, but there were many normoblasts and cells of the leucocyte series. Of considerable interest was the appearance of great numbers of clasmatocytes which had phagocyted erythrocytes and normoblasts.

In addition to the above five cases in which two or more specimens of bone marrow have been obtained at different times, brief mention will be made of two additional cases in which only one specimen has been obtained, but from which, nevertheless, certain impressions may be formed.

CASE 6. M. H., a man 55 years old, came under observation in the first relapse of typical pernicious anemia. The symptoms had lasted about six months. Blood examinations on Jan. 25, 1926, were as follows: hemoglobin, 50 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 2040 per c.mm.; reticulocytes, 1.6 per cent. Liver feeding was begun on January 28, and on February 12 the blood examinations showed: hemoglobin, 50 per cent; erythrocytes, 1.8 million per c.mm.; leucocytes, 3900 per c.mm.; reticulocytes, 9.1 per cent.

Biopsy of tibial marrow, February 12, 1926. The specimen consisted of fat tissue and showed no evidence of increased vascularity or cellular hyperplasia. It was normal, atrophic, fatty marrow.

Summary of findings in Case 6

This specimen was taken shortly after the onset of what subsequently proved to be a very rapid remission following a first and relatively short relapse. If this sample represents the general character of the tibial marrow, then the presence of a normal fatty marrow can be explained either by the fact that, at least in the first relapse, the pathologic process does not necessarily extend to the marrow of the tibia, or by the assumption that the pathologic process can disappear completely, and very rapidly, during a period of clinical improvement.

CASE 7. A. H., a woman 64 years old, with a history of pernicious anemia of two years duration. She entered the hospital during a relapse with an erythrocyte count of about 1.0 million per c.mm. For three weeks she ate considerable amounts of liver, and at the end of this time (May 26, 1926) the blood examinations were as follows: hemoglobin, 57 per cent; erythrocytes, 2.6 million per c.mm.; leucocytes, 6000 per c.mm.; reticulocytes, 4.4 per cent.

Biopsy of tibial marrow, May 26, 1926. The specimen showed essentially a fatty, normal, aplastic, tibial marrow. There were a few rather large endothelial cells with vesicular nuclei, lying between the fat cells and forming the walls of intersinusoidal capillaries. These resemble the hypertrophied endothelial cells which are characteristic of the earliest stage of marrow hyperplasia,[9] but they were so few in number that it is impossible to say that they were not within the normal limits.

Summary of findings in Case 7

In this case, as in Case 6, it is unfortunate that no biopsy was performed before the onset of the remission. Here again, it cannot be determined whether the normal appearing marrow was the result of a rapid clearing up of the pathologic process after the onset of a remission, or whether it merely indicated that even after the disease had lasted two years, the tibial marrow had not become involved. The fact that in Case 2 a remission of even greater degree and

rapidity was accompanied by a striking change, but not by a disappearance of the pathologic process, may be taken to suggest that in Cases 6 and 7 the marrow of the tibia had never been affected.

Discussion

The essential lesion of the bone marrow in pernicious anemia, and that which dominates the histologic picture during clinical relapse, is an hyperplasia of the myeloid cells in which the megaloblasts play the chief part. The development of the process can be studied in a simple fatty marrow like that of the tibia more easily than in complex active marrow like that of the vertebrae, but the lesion seems to be the same wherever it occurs. The megaloblasts develop from the endothelial cells of the intersinusoidal capillaries which, in an a atrophic marrow, are collapsed and almost invisible between the fat cells.[2, 9] They are formed within the lumen of the capillary, and where active proliferation is taking place, the capillary may be entirely filled by one, two or more rows of megaloblasts. This is illustrated in Fig. 12. In the more active stages of the pathologic process, as seen at necropsy, and in tissue taken at biopsy during a relapse, the proliferation of megaloblasts is very rapid. This is indicated by the extraordinary number of mitoses, and by the tendency of the cells to remain adherent to one another in columns and clumps, rather than to separate off as individual cells. Coincident with the hyperplasia of megaloblasts there is also a limited development of the more highly differentiated forms of the red blood cell series, namely erythroblasts, normoblasts, and erythrocytes. These cells also proliferate in the capillary spaces between the fat cells, and, since the marrow is confined within a rigid shaft of bone, their multiplication goes hand in hand with a disappearance of the globules of fat. It is evident, however, that even after the globules of fat are displaced, the fat cells remain in their normal position in the marrow, for specimens taken at biopsy show that when the myeloid hyperplasia recedes, the fat cells take up globules of fat again, and fill the space of the marrow cavity. The fat cells are practically invisible during the period of myeloid hyperplasia, but they are a constant element in the structure of the marrow, and serve an important subsidiary function by compensating for the proliferation and retrogression of the true blood-forming cells. The number and types of leucocytes vary in the bone marrow in pernicious anemia,

but many are found in the stages of most active hyperplasia, while giant cells are almost always abnormally decreased.

The study of bone marrow obtained by biopsy at different stages of pernicious anemia throws light on the relation of the pathologic process in the bone marrow to the clinical course of the disease, and the observation of Zadek [6] that the marrow hyperplasia disappears during clinical remissions has been confirmed by this investigation. In general, the more active the disease and the more profound the relapse, the greater is the pathologic hyperplasia of the bone marrow. This is, to some extent, indicated by the relation of cellular hyperplasia to the amount of fat in the marrow. Thus in the terminal stage, as shown in tissue obtained at necropsy, there is usually a complete or nearly complete replacement of fat by myeloid hyperplasia (see, for instance, Case 5). A similar but sometimes less marked condition is found during a serious relapse. In a severe relapse, Case 1 showed complete disappearance of fat, and the marrow in Case 2 contained extremely little fat. On the other hand, considerable fat may be present at autopsy in the marrow of a peripheral bone like the tibia, and in Case 5, the marrow contained a good deal of fat during the progress of the terminal relapse. The first specimen in Case 4, obtained after the onset of a remission, contained no fat. The relationship is thus by no means constant, and the displacement of fat, although an index of the cellularity of bone marrow, does not necessarily run parallel to the clinical course of the disease.

Of much greater significance in relation to the clinical course of the disease than either the amount of fat or the degree of cellularity of the bone marrow are the types of cells of which the marrow hyperplasia is composed. Thus the evidence indicates that severe relapses are characterized by a predominance of rapidly proliferating megaloblasts, while in remissions or during periods of clinical improvement the megaloblastic hyperplasia becomes less evident, and more mature cells of the red blood cell series, normoblasts and erythrocytes, become the prominent feature in the marrow. Essentially the same observations were made by Zadek.[6] Cases 1 and 2 show the change in cell type very clearly, for specimens of bone marrow were obtained, first in relapse and then at the height of a remission, or during the development of it. The first specimen from Case 4, taken soon after the onset of a remission, showed many normoblasts and erythrocytes,

but comparatively few megaloblasts. In Case 3, on the other hand, there was a slight increase in megaloblastic hyperplasia as the relapse progressed, and in Case 5 the marked hyperplasia of megaloblasts, seen during the course of the terminal relapse, was found to be still further increased at necropsy. Although the megaloblastic hyperplasia seems to be the essential feature of the pathology of the bone marrow in pernicious anemia, it cannot be stated that the lesion is necessarily specific for this disease.

Cases 6 and 7, in which normal, fatty marrows were obtained early in the development of clinical remissions, suggest, without definite proof, that pernicious anemia may exist for a considerable time and even present the picture of a serious relapse, without involvement of the marrow of the tibia. It is quite possible that for indefinite periods the disease may be limited to those parts of the marrow that are normally active. At necropsy an involvement of the marrow of the femur is, of course, practically constant.

The study of specimens of bone marrow taken at different stages in the course of pernicious anemia also furnishes evidence on the long disputed question of whether the anemia is primarily due to an increased destruction of red corpuscles, or whether it is the result of a primary disorder of blood formation in the bone marrow. At present the most widespread opinion appears to be that the disease is a hemolytic type of anemia, and that the bone marrow undergoes a compensatory hyperplasia as the result of the blood destruction. The histology of the marrow, however, does not tend to support this theory. The evidence of Zadek,[6] together with that presented above, shows that the characteristic megaloblastic hyperplasia is most highly developed in severe relapses, and disappears, completely or in large part, during the remissions. This, in itself, might be interpreted as meaning that the hyperplasia recedes as soon as the hemolytic process ceases. It is, therefore, significant to observe further that the megaloblastic hyperplasia begins to decrease, and the cytology of the marrow becomes more normal, very early in the development of a clinical and hematologic remission, and at just the period when one might expect a compensatory hyperplasia to be most marked. In Case 1, just before a remission started, there was a slight increase of fat (indicating a less cellular marrow) with an increase of cells more mature than megaloblasts, and after a complete remission had taken place the marrow showed only slight signs of

the previous hyperplasia. In Case 2, there was a striking decrease in megaloblastic hyperplasia early in the course of a rapidly developing remission. In Case 4, the marrow taken soon after the onset of a remission showed little megaloblastic hyperplasia. On the other hand, Case 5 illustrates that as a relapse progresses the opposite condition will be found, namely that the megaloblastic hyperplasia increases.

Such histologic evidence, however, does not prove that the pathologic condition of the marrow is the cause of the anemia, in spite of the fact that it suggests that the decrease in megaloblastic hyperplasia precedes the improvement in the hematologic picture. It is, therefore, interesting to correlate what is known about the pathologic histology of the marrow with some of the characteristic changes of the red blood corpuscles in the peripheral blood. In a severe relapse, with an erythrocyte count of 1.0 million or less per c.mm., the number of young cells in the blood, as shown by a count of reticulocytes, is usually relatively increased, but absolutely normal or decreased. Thus a reticulocyte count of 2 per cent of the total red blood cells, which is common under such circumstances, actually means that no more young cells are being put out by the bone marrow per day than is the case in a normal person with a red blood cell count of 5.0 million per c.mm. and approximately 0.5 per cent reticulocytes. This is so in spite of the fact that the active bone marrow, in the patient with a relapse of pernicious anemia, is an organ many times larger than that in the normal subject. The extensive hyperplastic marrow delivers fewer young cells in a unit of time than a normal marrow. There is cellular hyperplasia with functional inefficiency. This becomes particularly clear if one compares the situation with that in congenital hemolytic jaundice, a disease which is probably of a primary hemolytic nature. Here the marrow continues for months and years to put out so many young cells that the percentage of reticulocytes may be 15 to 30 or more of a red blood cell count between 4.0 and 5.0 million per c.mm. In addition to this, it is during the development of a remission that one often finds the large numbers of reticulocytes in the peripheral blood in pernicious anemia, and it has been seen that at exactly this period, when the bone marrow is beginning to function more effectively, the megaloblastic hyperplasia is beginning to disappear. Such histologic and hematologic evidence, therefore, indicates that

the megaloblastic hyperplasia of pernicious anemia produces a bone marrow with diminished functional capacity, and it leads to the belief that this type of anemia is the result of the pathologic lesion in the bone marrow.

The histologic material now at hand, can only suggest why it is that the megaloblastic hyperplasia of pernicious anemia produces a bone marrow of diminished functional efficiency. At the height of a relapse, when the output of cells from the marrow is at its lowest, there is an extraordinarily rapid and extensive proliferation of megaloblasts, but the relative number of more mature cells in the bone marrow, normoblasts and erythrocytes, is usually diminished. During the progress of a remission on the other hand, when the marrow is hyperactive, there are fewer megaloblasts, but many more normoblasts and erythrocytes. The relapse is thus characterized by the rapid proliferation of primitive cells, and by a diminished tendency towards the differentiation of the more mature forms of the erythrocyte series, while the onset of a remission is marked by a resumption of a more normal process of cell differentiation. The cause of the anemia would thus appear to be an abnormal type of cell growth consisting in a development of the primitive megaloblasts, and a failure of differentiation of the more mature red blood cells that normally get into the peripheral blood. There is little to indicate whether this is to be regarded as a hyperplasia due to some extraneous toxin, or whether the process is similar to that of a tumor growth. The extraordinary clinical results that have been obtained recently in the production of remissions in pernicious anemia by the feeding of large amounts of liver [10] suggest that this organ possesses some factor which affects cellular metabolism, and promotes the differentiation of the more mature cell types.

In addition to the above, it is worth noting that the venous sinusoids, into which mature erythrocytes are normally discharged, are extremely narrow and compressed in specimens of highly cellular marrow, and it is conceivable that a decrease of the vascular bed is a secondary factor in preventing red blood cells from getting out of the marrow.

It is also worthy of note that the phagocytosis of erythrocytes, which is such a striking feature in the bone marrow obtained at necropsy in almost all cases of pernicious anemia (see Case 5, bone marrow at necropsy), has rarely been observed in tissue obtained at

biopsy, even when this was taken during a severe relapse. The most obvious explanation is that the phenomenon occurs only postmortem, but there are several points that cast doubt on such an hypothesis. Thus it must be remembered that while phagocytosis of red blood cells may be found in any type of marrow at necropsy, it is particularly constant and prominent in pernicious anemia. In addition to this, it has often been found in bone marrow 1 to 2 hours after death (the necropsy in Case 5 was performed 2 hours after death), and shows no tendency to be more marked if the autopsy is performed later. The question could be settled by the examination of tissue taken a few hours or days before death, but such material is not at hand. The phagocytic cells are clasmatocytes and, according to Sabin, they are derived from endothelial cells. In marrows obtained at biopsy, it is extremely hard to distinguish clasmatocytes from endothelial cells as they are obscured by the confused mass of myeloid cells (just as fat cells may be invisible in a very hyperplastic marrow), but when they have ingested erythrocytes and normoblasts, the clasmatocytes become enlarged and are easily seen. It is possible that clasmatocytes are actually increased in number from an early stage in the disease, but that they become phagocytic only in the terminal stage. If this is shown to be true it would be a fact of considerable importance, for it would indicate that the pathology of pernicious anemia is characterized by the proliferation of two derivatives of the endothelial cell, the megaloblast and the clasmatocyte, and it might be possible to go back one step further and consider whether the primary lesion is not associated with the endothelial cells. Rich [11] showed that clasmatocytes, grown *in vitro*, ingest red blood corpuscles with which they are brought in contact. Contact between clasmatocytes and erythrocytes seems to lead to phagocytosis. Is it possible that in the terminal stage of pernicious anemia the red blood corpuscles are not delivered to the circulation, but remain in the marrow, where they come in contact with large numbers of clasmatocytes by which they are ingested?

The fact that megaloblasts are not frequently found in the peripheral blood, even during a relapse when they are numerous in the bone marrow, and the comparative rarity of other immature forms in the blood stream, is best explained by Key's [12] observation that immature red blood cells tend to adhere to one another. Under such

circumstances they would not be easily displaced from the marrow into the circulating blood.

If pernicious anemia be considered as primarily due to a bone marrow lesion rather than to a hemolytic process, the question naturally arises as to how one can explain the excess of bilirubin which is found in the blood plasma. In the absence of more definite knowledge of the physiology of pigment metabolism, one can only suggest that it results from an excess of pigment over and above what the marrow can use in constructing erythrocytes. This is consistent with the fact that bilirubin is increased during relapse, when the marrow is inefficient, and falls soon after the onset of a remission. It is also in harmony with the conception of Whipple [13] who regards the disease as being due to the decreased formation of the stroma of red blood cells, rather than to the lack of the constituents of hemoglobin. The erythrocytes in pernicious anemia are, indeed, more than normally filled with hemoglobin. Reference may also be made again to that most definite type of hemolytic disease, congenital hemolytic jaundice, in which the amount of bilirubin in the plasma is many times greater than it is in pernicious anemia.

Conclusions

1. Observations on the structure of the bone marrow in pernicious anemia, made on tissue obtained at biopsy at different stages of the disease, show that the myeloid hyperplasia is most marked during relapse, and that the structure of the marrow tends to return to normal during remission.

2. During relapse the essential histologic lesion is a rapid and extensive proliferation of primitive cells (megaloblasts), with a relatively diminished tendency towards the differentiation of mature cells of the erythrocyte series. The bone marrow shows a cellular hyperplasia, but it is functionally inefficient.

3. Remissions are characterized by the presence of few megaloblasts and a great relative increase of normoblasts and mature red blood cells in the bone marrow.

4. The anemia of the relapse is explained by the functional ineffectiveness of the bone marrow, which results from the failure of the megaloblasts to differentiate towards mature erythrocytes. The blood picture of the remission is explained by the resumption of a

more normal type of cell development with an increased production of normoblasts and erythrocytes.

5. It is suggested that the striking clinical results obtained by the feeding of large amounts of liver in the production of prompt and marked remissions may be due to some factor in the liver which affects cell metabolism and promotes the development and differentiation of mature red blood cells.

I am greatly indebted to Dr. W. R. Castle for assistance in obtaining the pathologic material, to Miss E. Piotti for the drawings, and to Dr. Henry Jackson, Jr. for the photomicrographs.

BIBLIOGRAPHY

1. Cohnheim, J. Erkrankung des Knochenmarks bei perniciöser Anämie. *Virchows Arch. f. path. Anat.*, 1876, lxviii, 291.
2. Sabin, F. R. On the origin of the cells of the blood. *Physiol. Rev.*, 1922, ii, 38.
   Doan, C. A. The circulation of the bone-marrow. *Carnegie Institution of Washington, Contributions to Embryology No. 67*, 1922, xiv, 27.
   Doan, C. A. The capillaries of the bone marrow of the adult pigeon. *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 222.
   Doan, C. A., Cunningham, R. S., and Sabin, F. R. Experimental studies on the origin and maturation of avian and mammalian red blood cells. *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, No. 83, 1925, xvi, 163.
3. Ghedini, G. Per la patogenesi e per la diagnosi delle malattie del sangue e degli organi emopoietici, puntura esplorativa del midollo osseo. *Clin. med. ital.*, 1908, xlvii, 724.
   Ghedini, G. Neue Beiträge zur Diagnostik der Krankheiten der hämatopoetischen Organe Mittels Probefunktion des Knochenmarks. *Wien. klin. Wchnschr.*, 1910, xxiii, 1840.
   Ghedini, G. Die Technik der Knochenmarkspunktion. *Wien. klin. Wchnschr.*, 1911, xxiv, 284.
4. Caronia, G. Recherches sur l'Etiologie de la Rougeole. *Presse méd.*, 1923, xxxi, 877.
5. Morris, L. M., and Falconer, E. H. (*a*) Intravitam bone marrow studies. Preliminary report. Part I. Description of a marrow trephine and experimental studies. *Arch. Int. Med.*, 1922, xxx, 485.
   Intravitam bone marrow studies. Part II. Survey of the clinical field. *Arch. Int. Med.*, 1922, xxx, 490.
6. Zadek, I. (a) Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Schweiz. med. Wchnschr.*, 1921, li, 1087.
   Zadek, I. Blut- und Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Ztschr. f. klin. Med.*, 1922, xcv, 66.

7. Sheard, Arthur. Pernicious Anæmia and Aplastic Anæmia. New York, Plate I, facing page 23 and Plate II, facing page 81, 1924.
8. Peabody, F. W., and Broun, G. O. Phagocytosis of erythrocytes in the bone marrow, with special reference to pernicious anemia. *Am. J. Path.*, 1925, i, 169.
9. Peabody, F. W. A study of hyperplasia of the bone marrow in man. *Am. J. Path.*, 1926, ii, 487.
10. Minot, G. R., and Murphy, W. P. Treatment of pernicious anemia by a special diet. *J. A. M. A.*, 1926, lxxxvii, 470.
11. Rich, A. R. The formation of bile pigment from hæmoglobin in tissue cultures. *Bull. Johns Hopkins Hosp.*, 1924, xxxv, 415.
12. Key, J. A. Studies on erythrocytes, with special reference to reticulum, polychromatophilia and mitochondria. *Arch. Int. Med.*, 1921, xxviii, 511.
13. Whipple, G. H. Pigment metabolism and regeneration of hemoglobin in the body. *Arch. Int. Med.*, 1922, xxix, 711.

DESCRIPTION OF PLATES

Plate 57

Fig. 1. Case 1. May 19, 1925. General character of marrow during severe relapse. Note complete absence of fat. × 500.

Fig. 2. Case 1. Same as Fig. 1, but under higher power to illustrate extensive hyperplasia of megaloblasts, with mitoses, and relative scarcity of normoblasts. × 1000.

Plate 58

Fig. 3. Case 1. May 19, 1925. Drawings of same material as Figs. 1 and 2. To illustrate hyperplasia of megaloblasts and numerous mitoses of megaloblasts. × 1250 (approx.).

Fig. 4. Case 1. Drawing similar to Fig. 3. × 1250 (approx.).

Plate 59

Fig. 5. Case 1. March 12, 1926. General character of marrow during a remission. Note large deposits of fat and small islands of myeloid cells between the fat globules. × 100.

Fig. 6. Case 2. April 29, 1926. General character of marrow taken early in a remission. × 200.

Fig. 7. Case 1. March 12, 1926. Marrow during remission. Island of cells between fat globules to show predominance of normoblasts. Very few megaloblasts. × 750.

Plate 60

Fig. 8. Case 2. March 12, 1926. General character of marrow during relapse. Hyperplasia of megaloblasts and displacement of fat. × 500.

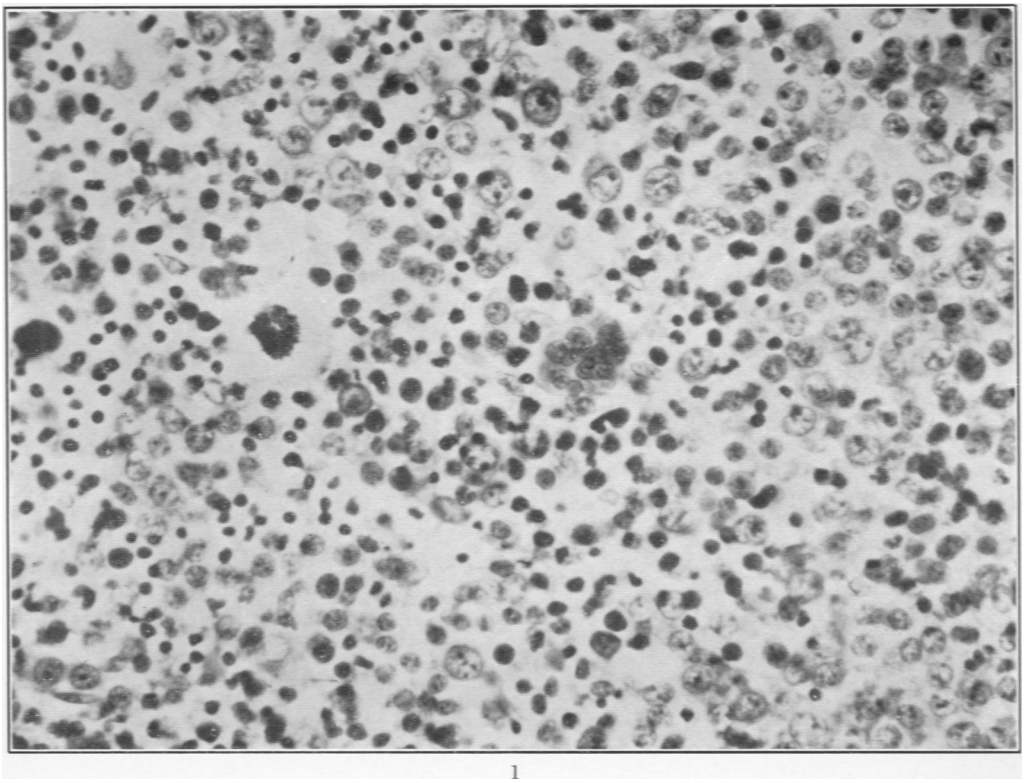
Fig. 9. Case 2. April 29, 1926. Masses of normoblasts and erythrocytes in the intersinusoidal capillaries between fat globules during a remission. Very few megaloblasts present. × 1500.

PLATE 61

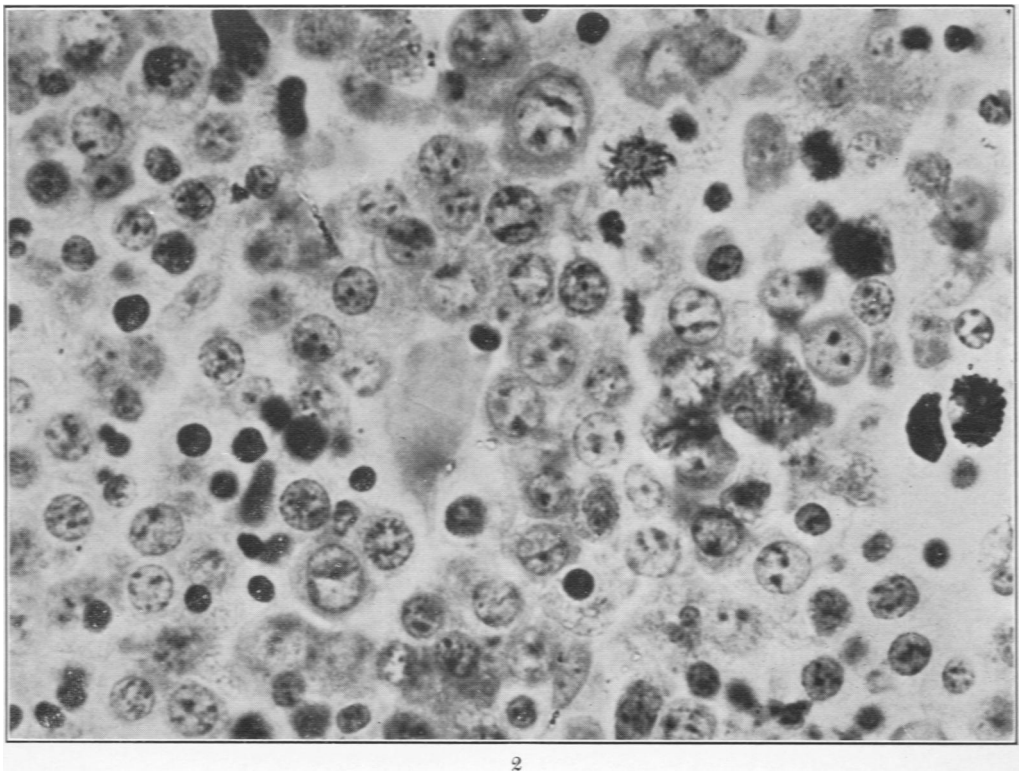
FIG. 10. Case 3. Jan. 18, 1926. To illustrate the extensive hyperplasia of megaloblasts and the presence of fat in the terminal relapse. × 500.

FIG. 11. Case 3. Sept. 2, 1925. General character of marrow as patient was going into a relapse. Note presence of fat containing fat cells, and also marked hyperplasia of megaloblasts. × 750.

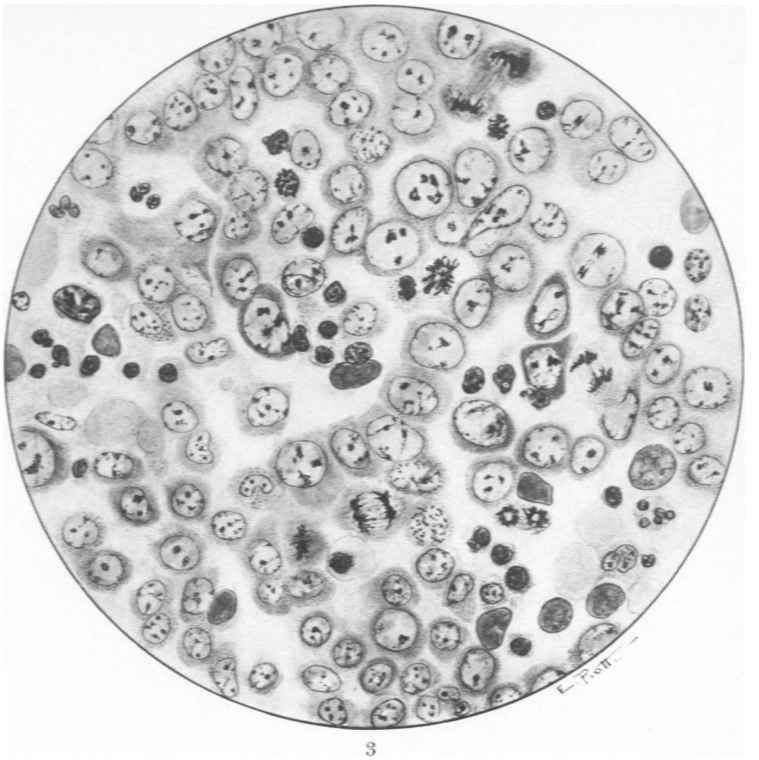
FIG. 12. Case 5. Sept. 2, 1925. Clump of megaloblasts developing in intersinusoidal capillary between fat cells. Note mitosis. × 1000.



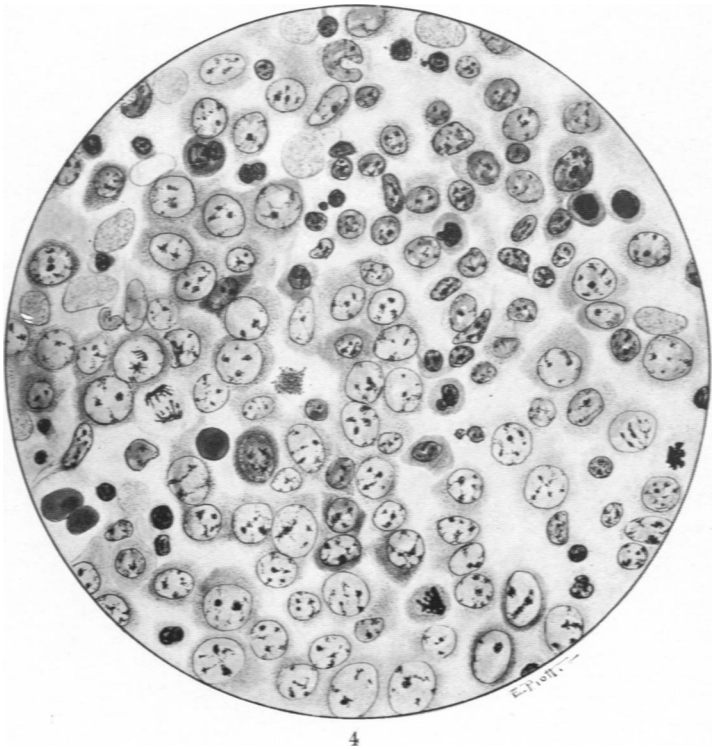
1



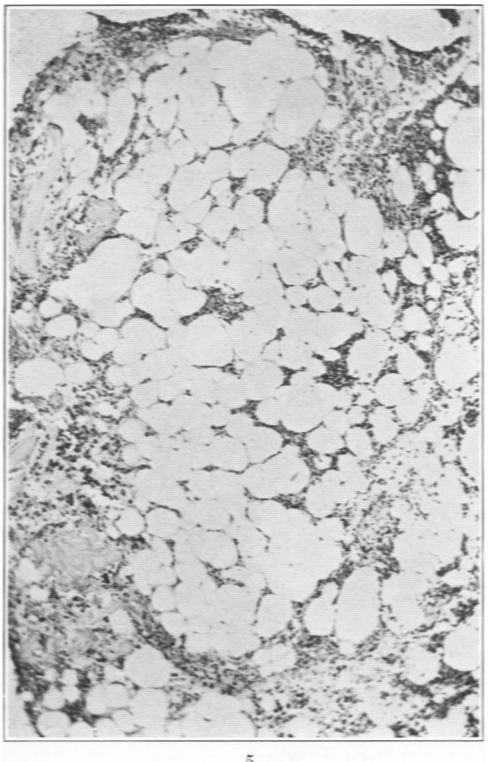
2



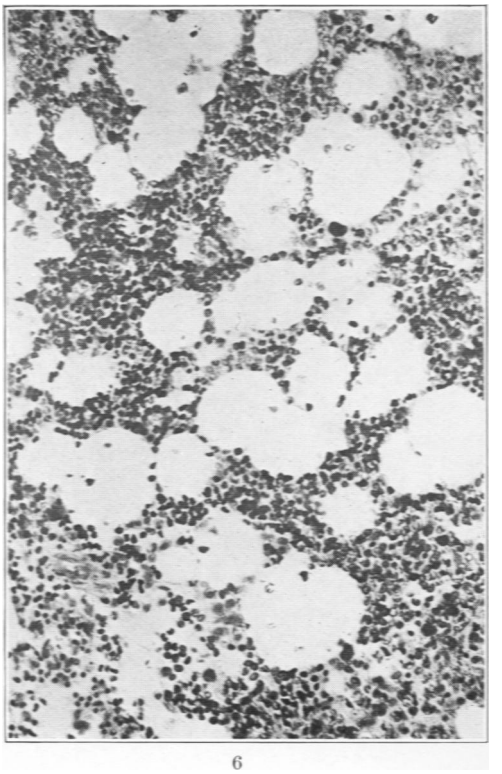
3



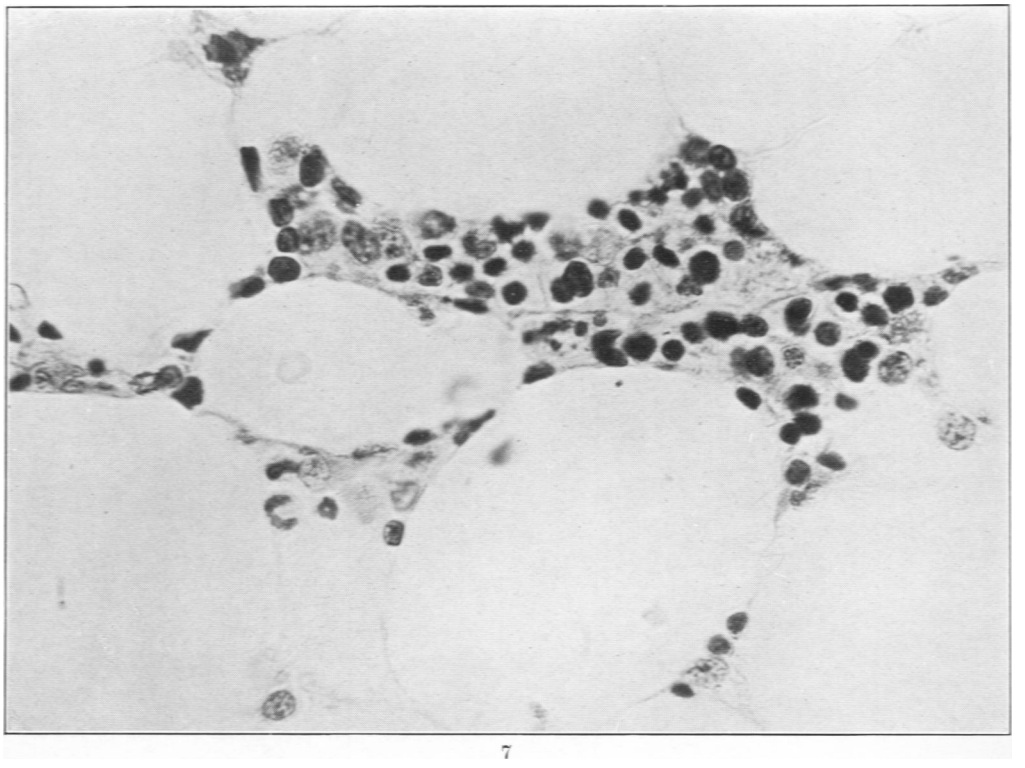
4



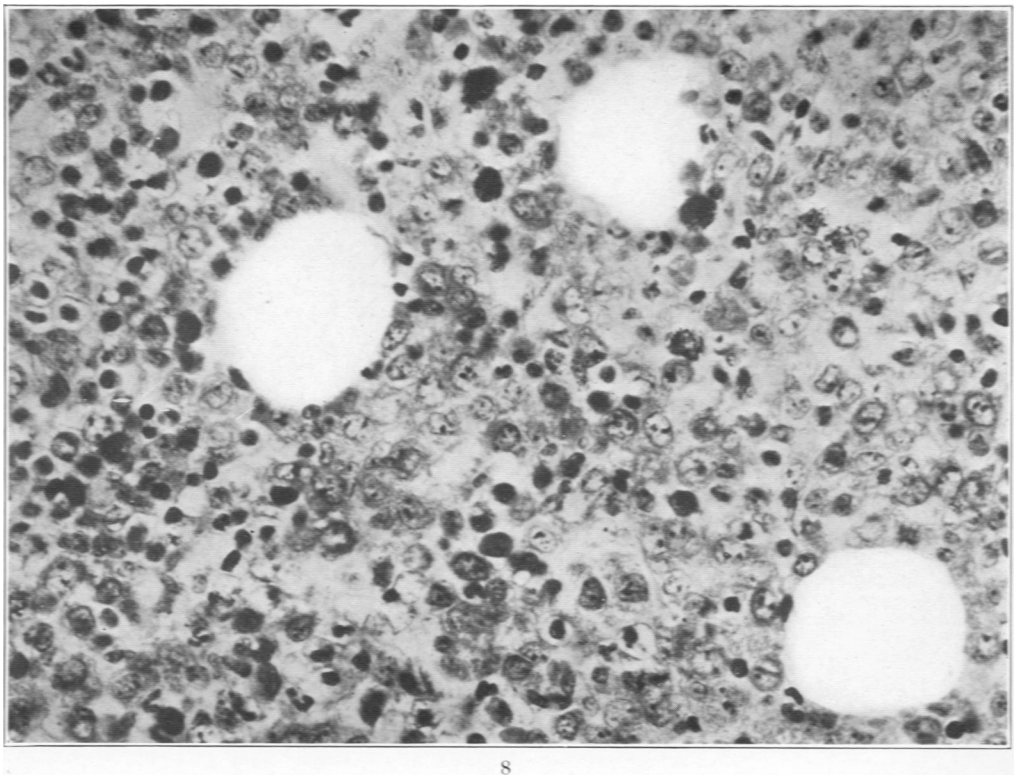
5



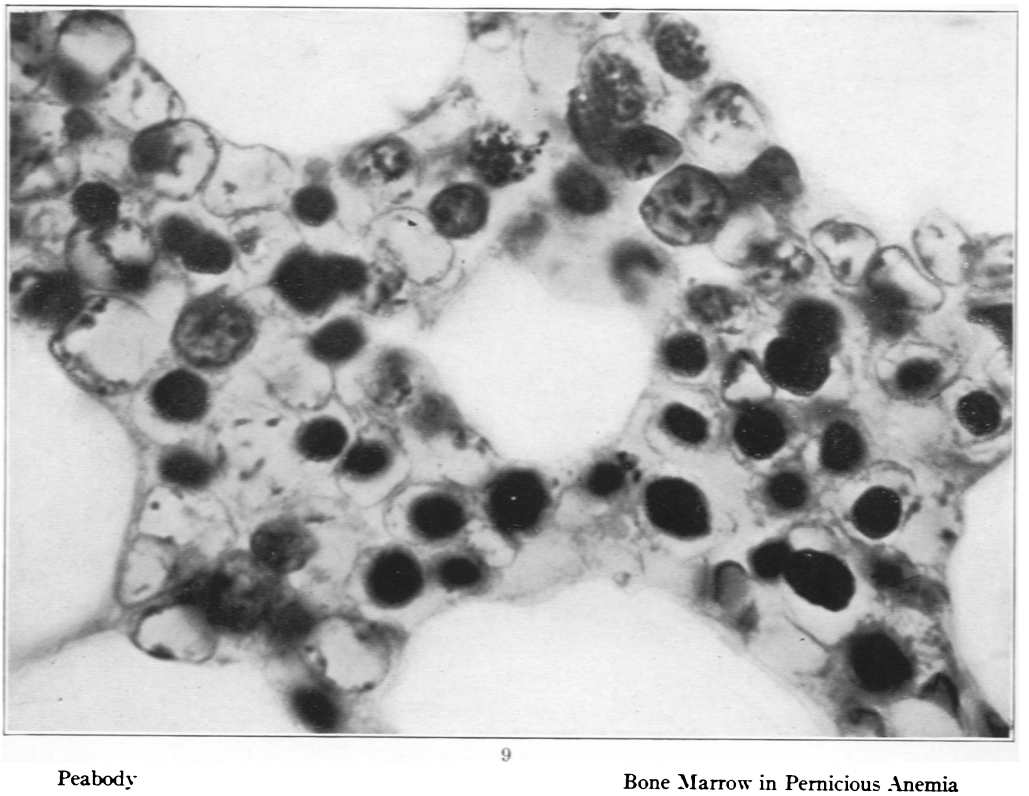
6



7



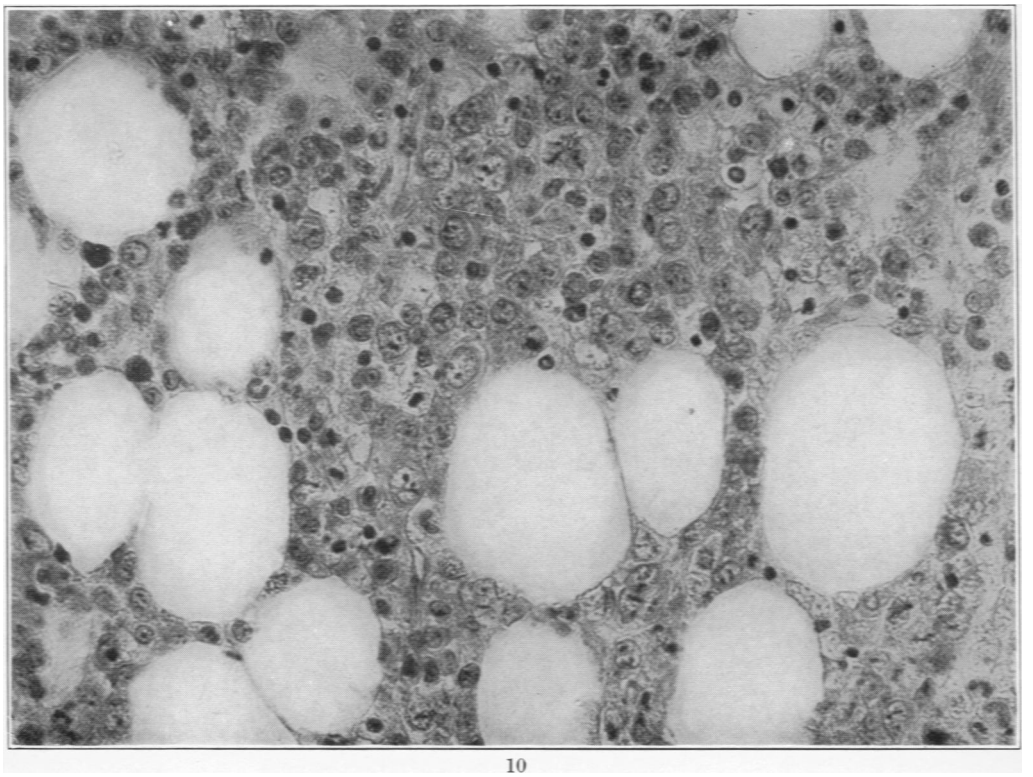
8



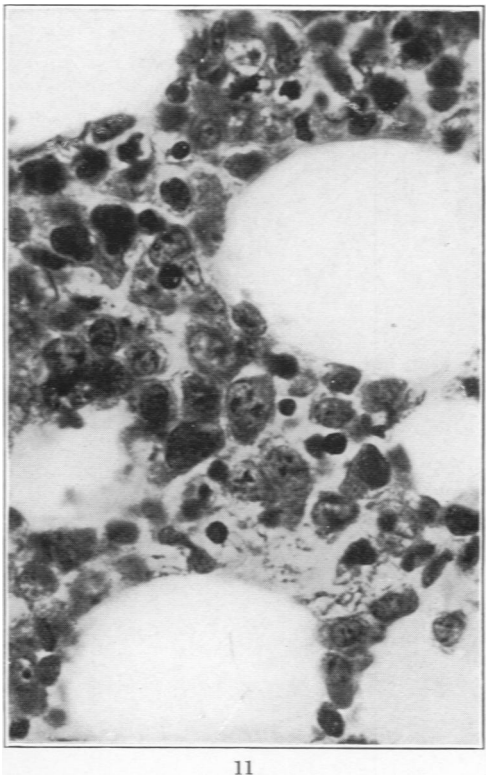
9

Peabody

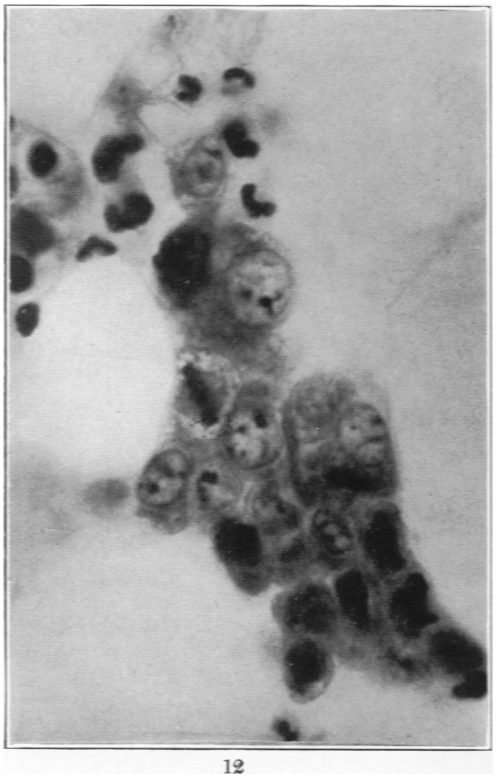
Bone Marrow in Pernicious Anemia



10



11



12

Peabody

Bone Marrow in Pernicious Anemia