THE PATHOLOGY OF THE BONE MARROW IN SPRUE ANEMIA*

C. P. RHOADS, M.D., AND W. B. CASTLE, M.D.

(From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.; and the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) of the Boston City Hospital; the Department of Medicine and the Department of Tropical Medicine, Harvard Medical School, Boston, Mass.)

INTRODUCTION

The study of bone marrow by means of specimens removed during life has been applied frequently to forms of anemia other than those associated with tropical sprue. Ghedini,[1] Morris and Falconer,[2] and Zadek [3, 4] all studied the bone marrow of living patients with pernicious anemia. It remained for Peabody [5, 6] to apply more modern knowledge to the study of the finer structure of bone marrow. In his final paper he showed most clearly that in pernicious anemia the marrow was hyperplastic during relapse and tended to return to normal during remission. Although the existence of a relation between the macrocytic anemia of sprue and of pernicious anemia has become manifest, existing observations of the bone marrow in sprue with anemia are few in number and confusing. Ashford [7] briefly reported findings in specimens of tibial marrow studied during life by another worker. Both hyperplastic and aplastic histological pictures were found. No photographs are shown nor are any descriptions of the histological changes given. The author's conclusions as to the marrow pathology seem to be colored largely by existing hypotheses of marrow function based on the response of the peripheral blood to therapy. Mackie and Fairley [8] described the changes found mainly in the femoral and tibial marrows of a group of autopsied cases of sprue. They noted in certain instances hyperplastic marrow, such as is found in pernicious anemia. In others fatty marrow was found, with rare, circumscribed areas of activity. Still other marrows showed a type of gelatinous degeneration. These various mani-

* These observations were carried out in 1931 by the Commission of the Rockefeller Foundation for the Study of Anemia in Puerto Rico.

Received for publication June 1, 1933.

festations were considered to represent various stages, ranging from hyperplastic "active" marrow to atrophic "exhausted" marrow. The fact that the marrow of the long bones is constantly fatty in character under normal conditions and is the last to respond with hyperplasia was not emphasized. Their briefly reported histological studies added little to the descriptions of the gross picture as regards the character of the cellular reaction. However, the red femoral marrow of one patient was stated to have exhibited "production of numerous megaloblasts as well as nucleated red cells."

The most careful and detailed studies of the bone marrow in sprue are those reported by Krjukoff.[9] This worker studied biopsy material from the ribs of sixteen patients. He was impressed by the constancy with which he found a megaloblastic change of the marrow with the production of "lymphoid erythroblasts" of all sizes, megaloblasts and normoblasts. He does not mention any cases in which an inactive marrow was found. The uniformity of the pathological alterations found is in striking contrast to the observations of other investigators and conforms much better to what is now known to be the effect of adequate specific therapy.

The divergent results of these studies of sprue have, at least in part, been due to the fact that specimens from comparable areas of the marrow were not studied. Piney [10] has shown that only the ribs, flat bones, and vertebral column normally contain active cellular marrow in adult life. Peabody [5] has emphasized the fact that the marrow of a long bone, such as the tibia, is not necessarily homogeneous in structure and that the pathological process there does not necessarily correspond in extent and degree to that in the marrow of other bones. On the other hand, his own studies of pernicious anemia were carried out on specimens of tibial marrow because he felt that it was simpler in structure and hence presented alterations easier to interpret than the more complicated marrow of flat bones or sternum. After the clarification of the underlying cellular changes of the marrow in pernicious anemia as a result of Peabody's work, it appeared logical to us to examine a site of normally active marrow in sprue. It was felt that in this way early alterations might be observed more readily and conclusions drawn with more certainty as to the presence of hyperplasia or aplasia of the functional elements. For these reasons this communication is based largely upon examination of specimens of sternal bone marrow removed at operation.

Methods

Twenty-two patients with sprue anemia of a macrocytic type were studied. In certain instances specimens were removed both before and after remissions, in others during the height of the reticulocyte response to liver therapy. The eventual death of three patients made it possible to compare the postmortem pathological picture with that seen during life. In still another group abnormalities of the clinical course were correlated with the histopathological alterations of the marrow.

The procedure of removing the specimens of sternal marrow was carried out in the operating room under complete surgical precautions for asepsis. A longitudinal midline incision about 4 cm. long was made from the third to the fifth costal cartilages, and was extended down to the periosteum. Two incisions were made in the periosteum, each about 1 cm. long and at right angles to each other. The periosteum was carefully retracted to expose an area of bone about 1 cm. in diameter. With a small crown trephine the outer table of bone was removed as a button about 0.4 cm. in diameter. With a bone curette enough marrow was removed to ensure the inclusion in microscopic sections of actual marrow tissue. The bone button was replaced and hemorrhage from the bone stopped with bone wax. The periosteum was brought together with No. 0 plain catgut. One or two subcutaneous stitches of the same suture material were used to approximate the subcutaneous tissue and the skin was closed with interrupted silk sutures. A dry dressing applied with considerable pressure from heavy adhesive straps was kept in place for twenty-four hours.

The tissue was fixed immediately in Zenker's fluid. After the usual steps of dehydration the tissue was embedded in paraffin and cut at 6 microns without decalcification. Mallory's phosphotungstic acid hematoxylin, eosin-methylene blue and the Giemsa stain were employed. Certain blocks were cut serially.

Results

The results of the microscopic examination of the bone marrow are summarized in the table and will be discussed below. In addition a few illustrative cases are briefly cited, and the microscopic picture described in detail.

TABLE I

Table Showing Blood Picture and Bone Marrow Histology in Macrocytic Anemia of Sprue

Case No.	Blood picture: Red blood cells in millions	Hemoglobin *per cent*	White blood cells in thousands	Reticulocytes *per cent*	Mean corpuscular volume in cu. μ	Icterus index units	Color index	Bone marrow: Biopsy or postmortem	Bone source	General cellularity	Megaloblasts and erythroblasts	Normoblasts	Fat	Comment
								Untreated Cases						
1	2.15	54	8.1	1.8	130	2	1.25	Biopsy	Sternum	++	++	++	++	Very early though definite megaloblastic preponderance (Fig. 1).
2	1.76	49	6.2	1.4	126	5	1.39	"	"	+++	+++	+	+	Moderate megaloblastic proliferation and preponderance (Fig. 2).
3	1.41	40	3.3	3.2	126	4	1.42	"	"	++++	++++	−	−	Intense megaloblastic proliferation and preponderance (Fig. 3).
4	1.42	48	2.2	6.6	140	40	1.69	"	"	++++	++++	−	−	" " " " "
5	0.98	28	2.2	0.8	143	25	1.43	"	"	++++	++++	−	−	" " " " "
6	2.51	55	3.3	4.8	101	2	1.10	"	"	++++	++++	−	−	" " " " "
7	1.96	46	9.8	1.4	114	2	1.17	"	"	++++	++++	−	−	" " " " "
8	1.92	42	4.8	2.0	102	5	1.10	"	"	++++	++++	−	−	" " " " "
9	2.67	66	7.4	2.2	119	3	1.24	"	"	++++	++++	−	−	" " " " "
10	1.15	34	5.1	3.8	131	30	1.48	"	"	++++	++++	−	−	" " " " "
11	2.52	63	8.1	3.2	128	3	1.25	"	"	++++	++++	−	−	" " " " "
12	3.12	73	7.5	1.6	107	2	1.14	"	"	++++	++++	−	−	" " " " "
13	1.25	29	1.8	1.0	104	18	1.16	"	"	++++	++++	−	−	" " " " "
14	1.68	55	5.0	4.6	134	5	1.63	"	"	++++	++++	−	−	" " " " "
15	2.40	54	1.9	0.4	113	40	1.12	"	"	++++	++++	−	−	" " " " "
16	1.46	40	3.0	0.1	121	5	1.37	"	"	+++	++	+	+	Moderate megaloblastic proliferation and preponderance.

17	1.05	29	2.9	8.8	157	12	1.38	Postmortem	Sternum and femur	++++	++++	–	–	Phagocytosis of red cells present (Fig. 6).
18	2.86	68	7.6	1.6	119	3	1.19	"	"	++++	++++	–	–	Sternal marrow red, femoral partly red. Lung abscess. Microscopically very loose structure.
19	0.97	19	7.3	4.5	103	30	0.98	"	"	++++	++++	–	–	Brick red marrow sternum and upper third of femur. Transfusion. Microscopic structure confused. Phagocyted erythrocytes.
								Treated Cases						
20	0.94	19	3.6	4.4	115	10	1.00	1st biopsy	Sternum	++++	++++	+	–	Before remission. Many megaloblasts, few normoblasts (Fig. 4), almost no fat cells.
20	2.97	60	3.3	4.2	100	2	1.01	2nd biopsy	"	++	+	+++	++	20 days after intramuscular liver extract. Predominant normoblasts (Fig. 5).
21	0.83	26	3.0	14.0	162	3	1.57	Biopsy	"	++	++	+	+	9th day oral liver extract. During reticulocyte rise. Megaloblasts separating into clumps. Normoblasts appearing.
22	1.40	29	2.3	21.6	114	6	1.04	1st biopsy	"	+++	++	++	+	9 days after liver extract intramuscularly. During reticulocyte rise. Decrease in megaloblasts. Increased normoblasts.
22	2.69	55	2.8	2.0	99	3	1.02	2nd biopsy	"	+	+	++++	+++	30 days after liver extract. Late remission. Marrow picture approaches normal, except for increased normoblasts.

Cellular Composition of the Sternal Bone Marrow in Untreated Cases of Sprue

CASE 1. *Clinical History:* The patient was a Puerto Rican housewife 47 years of age. There was a history of fairly typical sprue for one year, with loss of weight, gastro-intestinal disturbance, glossitis and anemia. Before the biopsy the patient had been treated for sixteen days with autolyzed yeast, to which she showed a slight reticulocyte response and slight clinical improvement. By the time of the biopsy the lingual and gastro-intestinal symptoms were somewhat improved without significant changes in blood values.

On the day of the biopsy, Aug. 26, 1931, examination of the blood showed: Red blood cells 2,150,000 per cmm.; hemoglobin 54 per cent (Sahli); color index 1.25; mean corpuscular volume 120 cu. μ; leukocytes 8100 per cmm.; reticulocytes 1.8 per cent; icterus index 2. The blood smear was consistent in appearance with the macrocytic anemia of sprue.

Bone Marrow Biopsy: The tissue appeared only moderately cellular and contained quite a large number of bone spicules. It was red-brown in color. The microscopic appearance is illustrated in Figure 1. A considerable amount of fat is present. The number of cells is distinctly greater than that observed in normal sternal marrow. Groups of from six to eight megaloblasts are seen with round nuclei containing rather heavy masses of chromatin and occasionally in mitosis. Certain cells of this general type are larger, with a considerable amount of rather pale, basophilic, cytoplasm. The latter is the type of cell that is present in such large numbers in the bone marrow in pernicious anemia in relapse. Smaller cells, diffusely scattered, with round nuclei containing dense masses of chromatin are numerous. These have a heavily basophilic cytoplasm that varies greatly in amount, though it is never so great as in the megaloblast. These cells are considered to be erythroblasts of varying degrees of maturity. A considerable number of normoblasts is present, many more than are seen in the marrow of pernicious anemia in relapse or in marrows from patients with sprue of a more severe degree. Megakaryocytes are present in about normal numbers, as are cells of the granulocytic series. Many sinusoids are closed.

Comment: Subsequent heavy dosage with liver extract administered intramuscularly failed to effect a reticulocyte rise or improvement in blood values. When ferric ammonium citrate was administered, however, a distinct reticulocyte rise appeared and subsequently a return to normal blood levels resulted. In this marrow the frequent occurrence of normoblasts suggests a deficiency of iron

as well as of liver extract, which is confirmed by the fact that both liver extract and iron were required to bring about improvement. In contrast to the findings in the bone marrow of Case 2, the histological changes are slight. It is conceivable that this is the earliest change, since the illness of the patient was short, and the blood values were at a relatively high level.

CASE 2. *Clinical History:* The patient was a male journalist 60 years of age, who had suffered from gastro-intestinal disturbance, glossitis, and weakness for ten years. For the ten days preceding the biopsy the patient was treated with 6 gm. of ferric ammonium citrate daily without effect.

On the day of the biopsy, Sept. 8, 1931, the blood examination showed: Red blood cells 1,760,000 per cmm.; hemoglobin 49 per cent (Sahli); color index 1.39; mean corpuscular volume 126 cu. μ; white cells 6200; reticulocytes 1.4 per cent; icterus index 5. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: The marrow did not appear remarkable at operation. Some increase in cellular over bony tissue was present and the color was distinctly reddish. The microscopic appearance is illustrated in Figure 2. A moderate amount of fat is present, up to thirty cells per high power field. Between the fat cells are masses of cellular tissue. The vast majority of the cells present are megaloblasts, some arranged in groups and others scattered diffusely throughout. They are larger, in general, than are those seen in Case 1 and have considerably more cytoplasm. Mitoses are frequent. Many erythroblasts of varying degrees of maturity are seen. The relative number of normoblasts is strikingly decreased. A few cells of the granulocytic series are present. The marrow is similar to that seen in pernicious anemia in relapse, except for the lack of complete replacement of fat by cellular tissue. The sinusoids are open and contain many adult red cells. The uniformity of the cellular picture is most striking. Megakaryocytes are sharply reduced in number.

Comment: The administration of ferric ammonium citrate effected no improvement. Liver extract given orally brought about a slight reticulocyte rise and by subsequent parenteral administration restoration of normal blood values was slowly attained. This result could perhaps have been prognosticated from the changes present in the marrow. That the response was a slow one might be associated with the fact that although the cell type was uniform, complete replacement of fat had not taken place. This marrow is intermediate in

type between that of Case 1, where megaloblastic activity was mild and early, and much fat present, and that of Case 3, in which a picture quite similar in all respects to that of pernicious anemia was seen.

CASE 3. *Clinical History:* The patient was a Puerto Rican housewife, 60 years of age, who had suffered from malnutrition for one year, and during the past three months from glossitis, diarrhea, and edema of both feet. There had been considerable loss of weight.

On the day of the biopsy, Aug. 26, 1931, the blood examination was as follows: Red blood cells 1,410,000 per cmm.; hemoglobin 40 per cent (Sahli); color index 1.4; mean corpuscular volume 125 cu. μ; white blood cells 3300 per cmm.; reticulocytes 3.2 per cent; icterus index 4. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: Grossly the marrow appeared dark red and hyperplastic. Definite replacement of bone spicules had taken place. Little fat was seen. As shown in Figure 3, microscopic examination bears out this impression. Almost no fat is present, averaging fewer than one fat cell per high power field. The tissue is rather vascular and extremely cellular. Masses of large megaloblasts are present. These cells are somewhat irregular in size and outline. The nuclei are rounded or oval and contain rather little chromatin, that present being clumped in irregular masses. The cytoplasm is slightly basophilic and irregular in outline. Smaller clumps of cells of somewhat different character are seen. These cells have round nuclei with heavy masses of chromatin. The cytoplasm varies in amount and is deeply basophilic. These cells are considered to be erythroblasts. Very few normoblasts are seen. A moderate number of megakaryocytes is present, as is some myelopoiesis with all stages in the development of the granulocytic cell series.

Comment: The picture is one of diffuse megaloblastic hyperplasia, similar in nature to that seen in pernicious anemia in relapse. As might be expected with the type of bone marrow change described, a prompt reticulocyte response followed the intramuscular injection of liver extract. This response was followed by eventual restoration of normal blood values.

In the three cases just described different degrees of the same fundamental histological change have been observed. The presence of such definite variations in the intensity of the process, even in the sternal marrow, is considered to be a partial explanation of the failure of many investigators to find active marrow in the long bones of

certain autopsied cases of sprue anemia. Presumably, in such instances, the pathological change has failed to extend to the long bones, though strikingly present in the sites of most active blood formation where the earliest changes would be expected to appear. In no case examined, and many, as may be seen from the table, had anemia of an extreme degree, was the sternal bone marrow atrophic. This is in agreement with the observations of Krjukoff and quite opposed to the statements of those who have confined their studies to the marrow of the long bones.

The Effect of Therapy on the Cellular Composition of the Sternal Bone Marrow in Sprue

Hitherto the marrow in cases of sprue anemia has not been studied before and after treatment capable of producing reticulocyte rises and restoration of normal blood values. Two cases of the present series were subjected to sternal punctures at different stages of the disease and the histological pictures compared. The changes were similar in both cases.

CASE 20. *Clinical History:* The patient was a male Puerto Rican, 35 years of age, who entered the hospital complaining of weakness of six months duration. For four months he had suffered from watery diarrhea and glossitis. The day before the first biopsy he received a transfusion of 200 cc. of whole blood and an injection of an effective extract derived from 100 gm. of liver.

On the day of the first biopsy, Aug. 6, 1931, a blood examination showed: Red blood cells 940,000 per cmm.; hemoglobin 19 per cent (Sahli); color index 1.0; mean corpuscular volume 115 cu. μ; white blood cells 3600 per cmm.; reticulocytes 4.4 per cent; icterus index 10.

First Bone Marrow Biopsy: In the gross the tissue was soft, deep red, and had definitely replaced a large part of the bony trabeculae. Histologically, as shown in Figure 4, all resemblance to normal marrow structure is lost. No fat is present. The tissue is an almost solid mass of large, pale cells, with nuclei containing scattered masses of chromatin. Their outline is irregular, the cytoplasm moderately basophilic, and the cells are in close apposition. These cells are considered to be megaloblasts. Scattered diffusely throughout in large numbers are somewhat smaller cells with round nuclei containing dense and heavy chromatin. The cytoplasm of these cells is small in amount and is somewhat more deeply basophilic. Many mitoses are present. Nucleated red cells are almost entirely absent. Myelo-

poiesis is slight. Only a limited number of megakaryocytes are present. The vascular channels are obscured by the enormous cellular overgrowth. The whole picture is quite consistent with that seen in severe pernicious anemia.

The effect of liver extract on this patient was entirely similar to that occurring in Addisonian pernicious anemia. In response to the single injection of liver extract derived from 100 gm. of liver the reticulocytes reached a peak of 38 per cent on the fifth day and the blood values rose rapidly, as similar therapy at ten day intervals was maintained.

Twenty days after the first biopsy and after remission had occurred, though complete restoration of the blood levels to normal had not taken place, a second biopsy was performed. On the day of the second biopsy, Aug. 26, 1931, the blood examination showed: Red blood cells 2,970,000 per cmm.; hemoglobin 60 per cent (Sahli); color index 1.02; mean corpuscular volume 100 cu.μ; white blood cells 3300 per cmm.; reticulocytes 4.2 per cent; icterus index 2.

Second Bone Marrow Biopsy: Histologically, the picture is quite different from that of the first biopsy, as shown by a comparison of Figure 4 with Figure 5. Fat cells are present up to ten to twelve per high power field. A few large pale megaloblasts with irregular nuclei are present in groups of three or four. The solid background of these cells seen in the first specimen is completely replaced by sheets of normoblasts that greatly outnumber any other cell type present. Vascular channels are open, myelopoiesis is proceeding, and a fair number of giant cells may be seen. Clearly, the administration of effective therapy caused a maturation of megaloblasts to normoblasts with the production of a bone marrow morphology more nearly approaching the normal and so resembling the transition observed during the treatment of pernicious anemia.

Phagocytosis of Erythrocytes by Bone Marrow Cells in Sprue

The presence of erythrocytes in the cytoplasm of large cells of the bone marrow in pernicious anemia has been described frequently in specimens of marrow removed at autopsy. At a time when current views associated pernicious anemia with increased blood destruction, based upon the observations of the increased serum bilirubin content and an output of bile pigment greater than normal in the

excreta, Peabody considered that phagocytosis might be of significance in the production of the anemia. In an early paper Peabody and Broun [11] gave a detailed description of the phagocytic picture in pernicious anemia, as seen in tissue fixed postmortem, and compared it with postmortem material from other types of disease. Although phagocytosis occurred, especially in infectious diseases, it was apparently greater in cases of pernicious anemia where death occurred in an acute stage. Later observations of biopsy material, however, failed to reveal erythrocytes in the cytoplasm of large marrow cells and led to the conclusion that the cytological appearances, which were supposed to indicate the phagocytosis of red blood cells during life, were really postmortem, or at least terminal phenomena.

Study of postmortem specimens of bone marrow from two cases of sprue anemia in Puerto Rico revealed the same phenomenon, so frequently observed in marrows of patients dying of pernicious anemia. In both instances erythrocytes are seen in the cytoplasm of large bone marrow cells, as shown in Figure 6. In both of these specimens the architecture of the marrow is seriously distorted, as compared with tissue removed at biopsy. In the latter, closely packed masses of cells, uniform in size and shape and easily identifiable, with well marked vascular channels and stroma structure are seen. In the postmortem specimens the structure is extremely loose and almost unrecognizable. There is an enormous variation in size and shape of the cells. Some are very large and have an irregular, slightly basophilic cytoplasm, which contains large numbers of erythrocytes. Since this was not observed in any of the specimens removed at biopsy the conclusion is unavoidable that it is a postmortem change. In neither case was the body kept more than eight hours before the tissue was fixed, although lack of facilities for refrigeration and the heat of the climate may have accelerated a postmortem alteration. Peabody states that the cells that ingest erythrocytes in the bone marrow of pernicious anemia are clasmatocytes. He quotes the observation of Rich [12] that clasmatocytes may ingest red cells when grown *in vitro*. From the observations here presented no conclusion as to the nature of the phagocytic cell can be drawn. Only further evidence, based on supravital and tissue culture study of material removed at autopsy, can be expected to solve the problem.

Discussion

The material presented indicates that, as concerns the fundamental histopathological picture of the bone marrow during exacerbation, during remission and at postmortem, the changes accompanying the macrocytic anemia of tropical sprue are similar to those found in pernicious anemia, as described by others.

Peabody confirmed the previous observation of Zadek that during remission the bone marrow became less cellular; and for the first time accurately described the microscopic appearances both during remission and in relapse. He considered the hyperplasia occurring in relapse to be an extensive proliferation of primitive marrow cells, chiefly megaloblasts, associated with a relative decrease in the other elements, including the fat cells. Remission was shown to be characterized by a picture more nearly normal, with few megaloblasts, a relative increase of normoblasts and mature red blood cells, and the reappearance of fat. The anemia of relapse was explained by the functional ineffectiveness of the marrow resulting from the failure of the megaloblasts to form mature erythrocytes. In the sternal marrow of nineteen patients with untreated sprue the same fundamental pathological change was found, an increase in number and size of the megaloblasts, a decrease in the amount of fat present and in the number of megakaryocytes and cells of the granulocyte series. The number of normoblasts was strikingly small in comparison to the number of megaloblasts. The differences in the bone marrow changes reported by previous observers can thus apparently be explained by the fact that only the marrow of the long bones was studied. Beyond question the extent of marrow involvement is greater in pernicious anemia than in certain cases of sprue, but the changes occurring in the normally active marrow of the adult, obtained here by sternal biopsy, present a uniform picture.

Furthermore, the effect of specific therapy in sprue anemia is folfowed by the same maturation of megaloblasts to normoblasts and restoration of normal morphology of the marrow, as occurs in pernicious anemia. This fact parallels the similarity of the blood changes in the two conditions. Although, as a rule, the effect of liver extract on the lingual and gastro-intestinal symptoms is as striking as in pernicious anemia, the hematopoietic response is frequently not so marked for a given blood level in sprue as in per-

nicious anemia. This is probably to be explained, at least partly, on the basis of the fact that in pernicious anemia the megaloblastic hyperplasia involves a greater amount of the normally inactive bone marrow. It is also clear that in sprue a combination of therapy with iron, as well as with liver extract, is necessary in many cases, a fact that correlates with the findings in the bone marrow of certain patients, as illustrated by Case 1. Despite this fact, the basic similarity of the blood pictures and of the changes in the active portions of the bone marrow in relapse and in response to similar therapy would seem to be strong evidence for a similar etiological mechanism. Observations have been made by Castle and Rhoads [13] that indicate the macrocytic anemia of sprue is mainly the result of a deficiency similar to that existing in pernicious anemia, although brought about in a somewhat different manner.

Conclusions

1. Observations on the bone marrow of sprue anemia made on tissue obtained at biopsy in different stages of the disease show that the changes are similar to those of pernicious anemia.

2. During relapse the essential change is a proliferation of megaloblasts and suppression of maturation to the normoblast stage.

3. During remission the marrow tends to return to normal with a great increase in the number of normoblasts and mature red cells in the marrow.

4. Phagocyted erythrocytes were observed in the bone marrows removed at autopsy but not in those removed during life.

The authors wish to acknowledge their indebtedness to Drs. W. R. Galbraith, George C. Payne, and to Herman A. Lawson for their cooperation in the conduct of these observations, as well as to Mr. Emil Bohnel and Mr. Louis Zetzel for their assistance in making the blood studies.

REFERENCES

1. Ghedini, G. Neue Beiträge zur Diagnostik der Krankheiten der hämatopoetischen Organe mittels Probepunktion des Knochenmarks. *Wien. klin. Wchnschr.*, 1910, **23**, 1840–1847.

2. Morris, L. M., and Falconer, E. H. Intravitam bone marrow studies. Part II. Survey of the clinical field. *Arch. Int. Med.*, 1922, **30**, 490–506.

3. Zadek, I. Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Schweiz. med. Wchnschr.*, 1921, **51**, 1087–1091.

4. Zadek, I. Blut- und Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Ztschr. f. klin. Med.*, 1922, **95**, 66–87.

5. Peabody, F. W. A study of hyperplasia of the bone marrow in man. *Am. J. Path.*, 1926, **2**, 487–502.

6. Peabody, F. W. The pathology of the bone marrow in pernicious anemia. *Am. J. Path.*, 1927, **3**, 179–202.

7. Ashford, B. K. The differential diagnosis of sprue and pernicious anemia. *Am. J. Trop. Med.*, 1932, **12**, 199–215.

8. Mackie, F. P., and Fairley, N. H. The morbid anatomy of sprue. *Indian J. M. Research*, 1928–29, **16**, 799–811.

9. Krjukoff, A. Anämie bei Sprue. *Folia hæmat.*, 1928, **35**, 329–352.

10. Piney, A. The anatomy of the bone marrow: With special reference to the distribution of the red marrow. *Brit. M. J.*, 1922, **2**, 792–795.

11. Peabody, F. W., and Broun, G. O. Phagocytosis of erythrocytes in the bone marrow, with special reference to pernicious anemia. *Am. J. Path.*, 1925, **1**, 169–183.

12. Rich, A. R. The formation of bile pigment from haemoglobin in tissue cultures. *Bull. Johns Hopkins Hosp.*, 1924, **35**, 415–416.

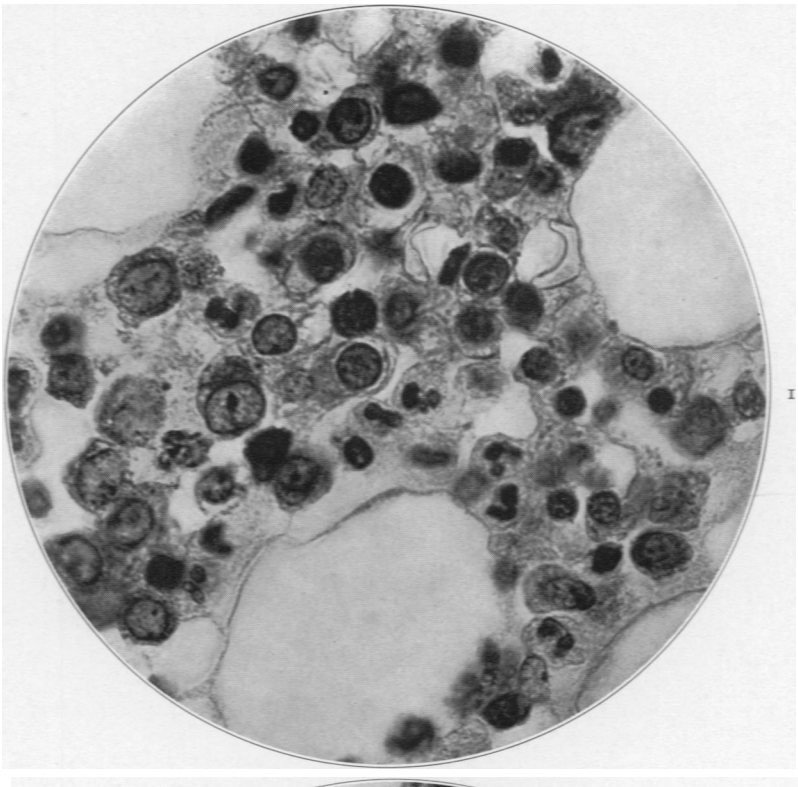
13. Castle, W. B., and Rhoads, C. P. Observations on the aetiology and treatment of sprue in Puerto Rico. *Tr. Assn. Am. Phys.*, 1932, **47**, 245–247. Abstr. *Lancet*, 1932, **1**, 1198–1199.

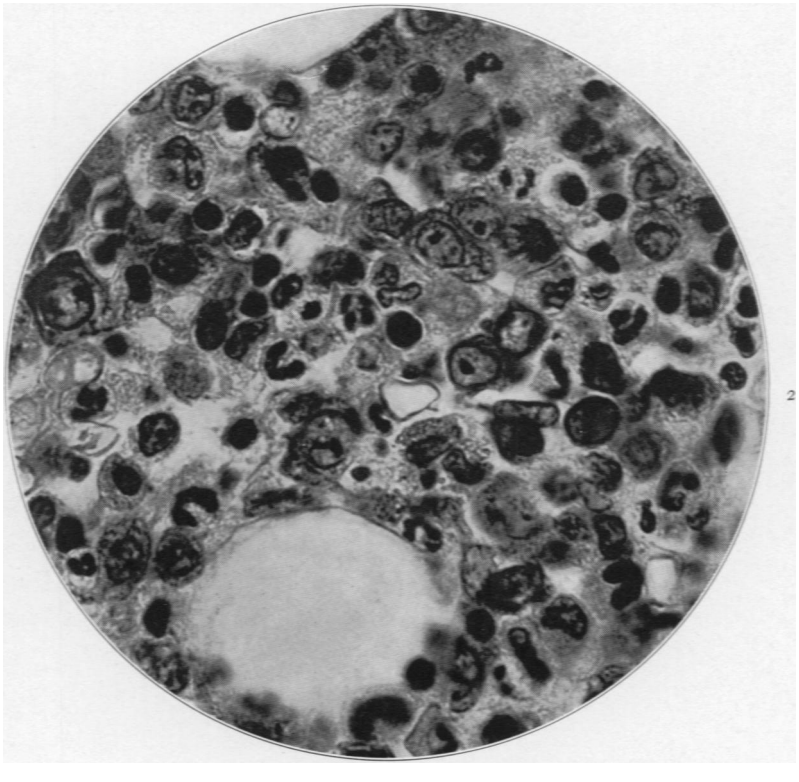
DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Case 1. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue of moderate degree and duration before remission produced subsequently only after addition of iron to liver extract therapy. Note presence of much fat, numerous normoblasts and moderate numbers of megaloblasts. Giemsa stain. × 1500.

FIG. 2. Case 2. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue. Note presence of fat and moderate megaloblastic preponderance. Cells of the myeloid series are present in considerable numbers. Giemsa stain. × 1000.





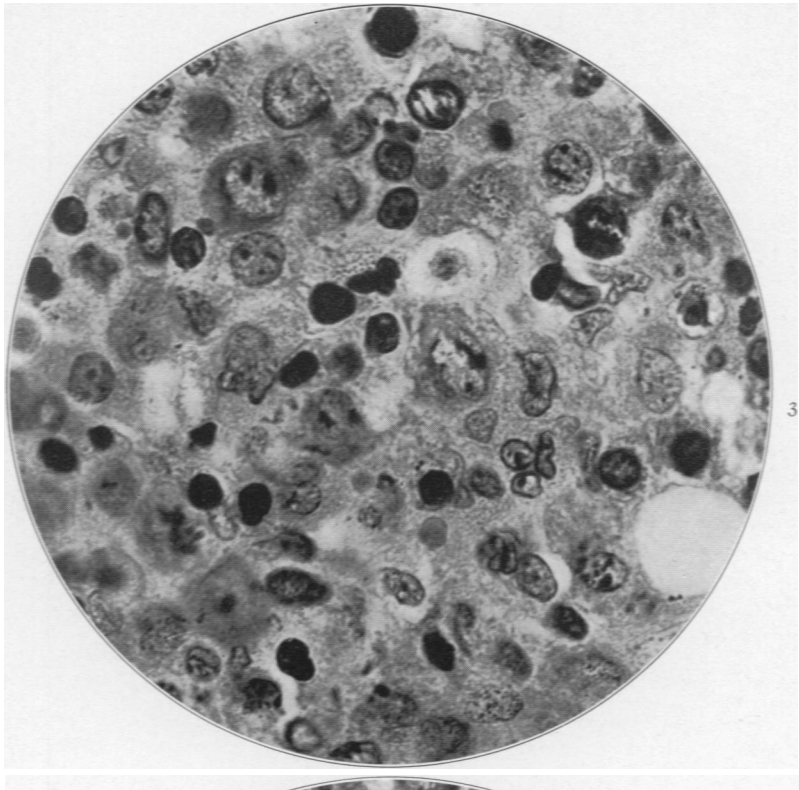
Rhoads and Castle

Bone Marrow in Sprue Anemia

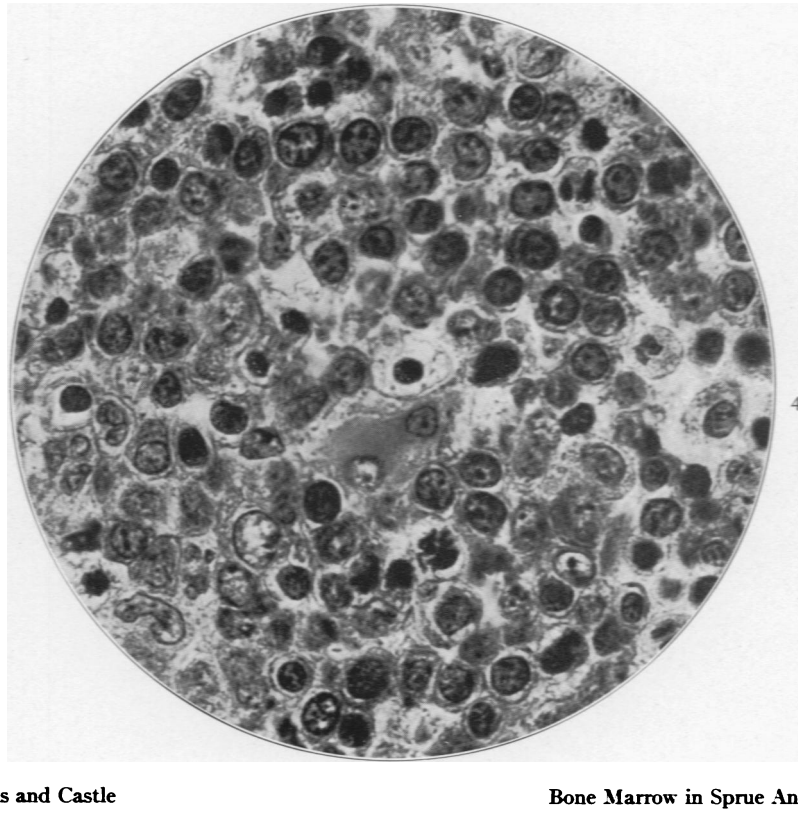
PLATE 131

FIG. 3. Case 3. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue. Note almost total absence of fat and intense megaloblastic proliferation with relatively few normoblasts and cells of myeloid series. Giemsa stain. × 1500.

FIG. 4. Case 20. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue before typical remission produced with liver extract. Note absence of fat with increased cellularity due largely to intense megaloblastic proliferation with occasional mitotic figures. Giemsa stain. × 1000.



3

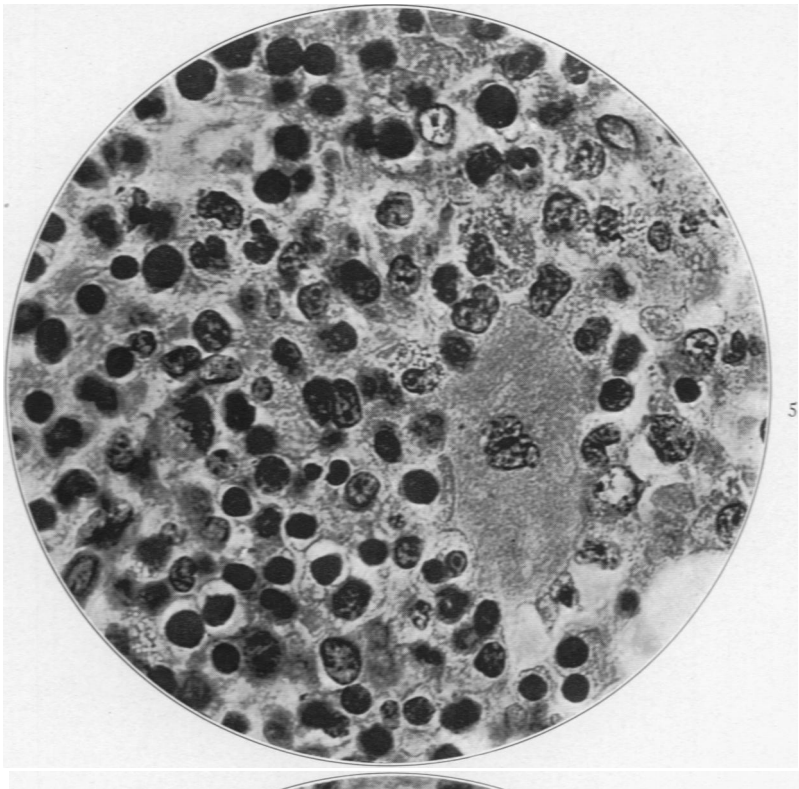


4

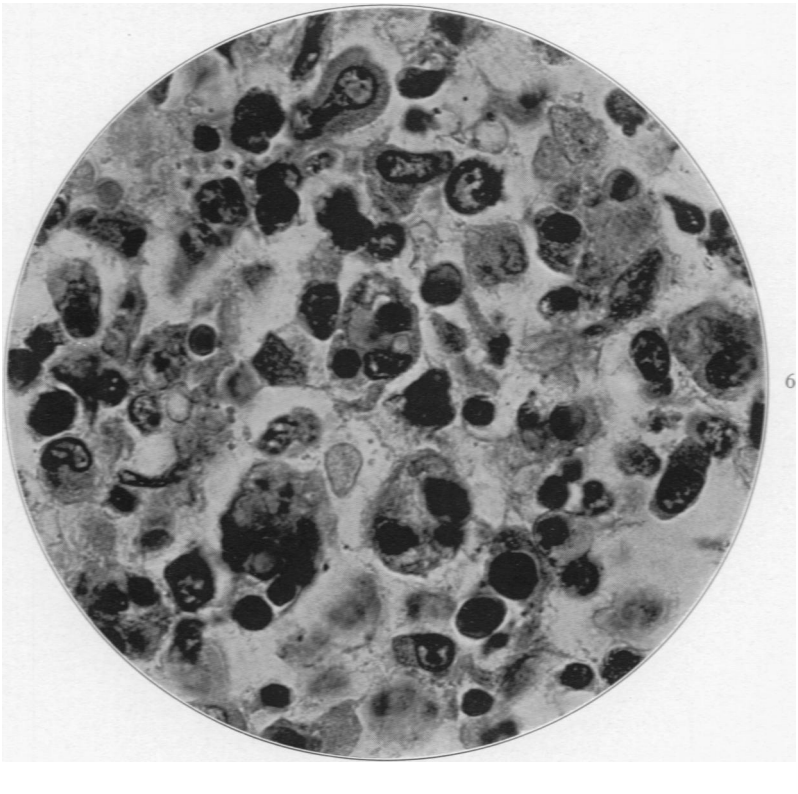
PLATE 132

FIG. 5. Case 20. Second sternal marrow removed at biopsy twenty days after injection of active liver extract, which produced a typical reticulocyte crisis. Note the decreased number of megaloblasts and the normoblastic preponderance. Giemsa stain. × 1000.

FIG. 6. Case 17. Sternal marrow removed at autopsy from patient dying of sprue with severe macrocytic anemia. Note loss of structural details and presence of phagocyted erythrocytes. Giemsa stain. × 1000.



5



6