13966

Serial Bone Marrow Studies in Pernicious Anemia. III. Occurrence of Protoporphyrin in Human Bone Marrow.

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The occurrence of preëxisting protoporphyrin in red blood cells of the peripheral blood[1,2] and in the megaloblasts and erythroblasts of the bone marrow has been demonstrated.[3] In the normal blood red fluorescing erythrocytes, which were called "Fluorescyten," were reported.[4] These cells became increased in proportion to the erythrocytic regeneration.[5] They were found to be numerous in the blood of patients with pernicious anemia during their response to liver therapy.[6] Subsequently it was shown by chemical methods that the increase of protoporphyrin in the blood was simultaneous with the increase in the number of "Fluorescyten."[7,8] Later it was concluded that the "Fluorescyten" were younger red blood cells,[9] and that the increase of protoporphyrin concentration of the blood was directly proportional with the number of reticulocytes present.[10,11] An increased protoporphyrin content of the bone marrow was found following hemorrhagic anemia in rabbits.[12]

In the preceding communications, data were presented showing that during the early phases of liver induced remission in the bone marrow of patients wtih pernicious anemia there was a marked normoblastic stimulation. The rapidity of morphological and quantitative changes which are characteristic of the normoblastic transformation of a megaloblastic marrow is suggestive of a change in cellular chemistry, which might be manifested also in variations of the protoporphyrin content. This assumption has prompted the present investigation.

Materials and Methods. One hundred and thirty-three bone marrow specimens were examined. Ninety-six marrow specimens were obtained from 12 patients with Addisonian pernicious anemia before and at frequent intervals during their response to liver therapy. Thirty-seven additional marrow specimens were obtained from patients with iron deficiency anemias, posthemorrhagic anemias, hemolytic anemias, sprue and leucemias.

The technic for obtaining marrow samples and for the determination of the total nucleated elements and of the percentage of reticulocytes has been described in detail in the preceding communication.

The following technic was used for the determination of the presence of protoporphyrins:

About 0.4-0.5 cc of the fresh sample of washed and packed bone marrow cells was placed in a 15 cc centrifuge tube. Ten cc of a mixture of 1 part glacial acetic acid and 2 parts of ethyl acetate were added. The

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contents of the tube were thoroughly mixed by shaking. The mixture was allowed to stand overnight and then centrifuged for 15 min. The clear, dark colored supernatant fluid was decanted into a second 15 cc centrifuge tube and 5 cc of 5% HCl added with thorough mixing. This mixture was centrifuged 5 min to separate the two layers. The upper layer of ethyl acetate was floated off carefully by addition of distilled water. The 5% HCl solution was then placed before a source of ultraviolet light (3200-4000 A) and the protoporphyrin estimated as 0, ±, +, 2+, 3+ or 4+ by visual comparison of the fluorescence of the solution with standard protoporphyrin solutions.

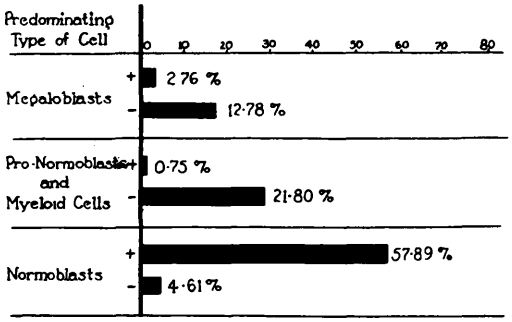

FIG. 1.

The highest incidence of protoporphyrin was coincidental with the preponderance of immature normoblastic red cells in 133 sternal marrow specimens.

Observations. The different marrow samples showed variations in cellularities and in the amount of protoporphyrin. There was no direct relationship between the total number of nucleated elements and the amount of protoporphyrin, but it appeared to be related to the presence of certain types of cells. As Fig. 1 shows, protoporphyrin occurred most consistently in marrow samples which consisted predominantly of immature normoblastic red cells. The included data were derived from the examination of 133 sternal bone marrow specimens, 96 of which were obtained from 12 cases of pernicious anemia before and during treatment with liver extract. Twenty-six sternal marrow samples were obtained from patients with secondary type of iron deficiency anemias showing normoblastic stimulation. Twenty-three bone marrow samples revealed the presence of protoporphyrin and in 3 instances no detectable amount was present. One hyperplastic normoblastic bone marrow of a case of sickle cell anemia, and one case of phosphorus poisoning with jaundice were strongly positive. Two bone marrows of sprue with a preponderance of pronormoblastic cells failed to show the presence of protoporphyrin. In 2 marrows from cases of aplastic anemia, there was only a small amount of detectable protoporphyrin present in one. From 5 cases of leucemias with hyperplastic myeloid or lymphoid marrow protoporphyrin could not be demonstrated in 4 instances.

The bone marrow of 12 patients with pernicious anemia was examined, showing a preponderance of megaloblastic cells. These specimens were negative for protoporphyrin with a few exceptions, although some of them were examined repeatedly. Serial sternal marrow samples were taken at 24-hr intervals after the beginning of the liver treatment. One typical chart (Fig. 2) illustrates that within 24-48 hr after the injection of 60 U.S.P. units of liver extract (Lederle) there were marked fluctuations in the number of nucleated marrow cells, the majority of which were of the normoblastic series.

Before liver extract was administered the megaloblastic cells were predominate in the marrow and characterized by finely dis-

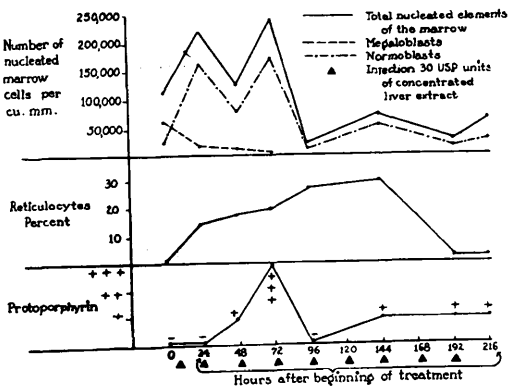

FIG. 2.

Illustrates the serial bone marrow studies in a case of pernicious anemia before and at frequent intervals after the first liver injection. The chart shows that the appearance of protoporphyrin was observed simultaneously with the proliferation of immature normoblastic cells.

tributed chromatin in the nucleus and blue cytoplasm. Twenty-four to 48 hr later these cells were replaced by numerous normoblasts with a distinctly coarse chromatin nuclear pattern and blue cytoplasm. About the same time there was an increase in the amount of protoporphyrin. This was followed by a rapid rise in the number of reticulocytes in the marrow.

It should be emphasized, that a simple comparative method which was used in this work for the protoporphyrin determinations provided only the roughest kind of quantitative data as to the relative amount of protoporphyrin of the different marrow samples. After these observations, however, more accurate quantitative determinations are in progress with the use of the Coleman Photofluoro-meter.

Comment. The present observation that the immature red blood cells of normoblastic origin in the bone marrow exhibit an increased amount of protoporphyrin is well in accord with the other observations that an increased amount of protoporphyrin was directly correlated with the number of reticulocytes,[10] or young erythrocytes.[9] It is noteworthy that contrary to the findings of others,[3] we found little or no protoporphyrin in the megaloblastic marrow.

It has been noted that patients with pernicious anemia in relapse had a low protoporphyrin content of the blood and that the values became elevated during the early stages of liver induced remission.[13-15] Increased amounts of protoporphyrin in the urine and feces of patients with pernicious anemia has also been reported.[13, 18-20]

By perfusion of surviving livers with protoporphyrin solution it was shown that a varying fraction of the protoporphyrin was converted into coproporphyrin.[21] This was not confirmed by other workers and it was shown that the porphyrin formed under these circumstances is not coproporphyrin, but one of the pseudodeutero-porphyrins.[22,23] Nevertheless, coproporphyrin I has been found to be excreted normally and in increased amounts in the urine and feces obtained from pathological cases showing increased hematopoietic activity.[16,17,19,24,25] It is unlikely that the coproporphyrin I is a hemoglobin derivative, since all the respiratory pigments are Type III compounds. Therefore, it was assumed in accordance with the theory of the dualism of the porphyrins that there is a simultaneous construction of Type I and Type III porphyrins in direct proportion. The coproporphyrin I excretion was regarded as an index of blood regeneration.[25,26]

It is likely that protoporphyrin accumulates at the site of hemoglobin formation as an intermediate substance. It has also been suggested[7,12,14] that the increased amount of protoporphyrin is simply an expression of mild iron deficiency.

In the present study we found an increased amount of protoporphyrin concomitant with a large number of newly formed normoblastic cells. The relationship between the amount of protoporphyrin and the number of reticulocytes, however, was not clear. In some instances the increase of protoporphyrin preceded the rise in the number of reticulocytes. It is possible that during the marked normoblastic proliferation there are red cells containing improperly formed respiratory pigment which, even though they were not

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reticulocytes, might contain protoporphyrin. In this connection it is of interest that the amount of non-respiratory iron porphyrin compounds was increased in immature red cells.[27] Furthermore, during liver-induced remission in patients with pernicious anemia, there has been observed a precipitous fall in serum iron values[28] which preceded, but was almost reciprocal with, the rise in reticulocytes.[29]

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Summary. The occurrence of protoporphyrin was studied in 133 sternal bone marrow specimens. Protoporphyrin was most regularly present in marrow samples containing predominately normoblastic young red blood cells.

Ninety-six sternal bone marrow specimens from 12 patients with Addisonian pernicious anemia before and after liver extract injection were obtained by serial sternal punctures at 24-hr intervals. The appearance of protoporphyrin was coincidental with the increase of immature red cells of normoblastic type in the marrow.

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13967

Blood Iodine in Dogs Receiving Thyroxin or Phlorhizin.

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Since an increase in the basal metabolic rate may be due to various causes, supplementary tests have long been sought which would aid in differentiating thyroid disease from other conditions in which a rise in metabolism occurs. The intimate connection between iodine metabolism and the thyroid suggests that blood iodine determinations might be of particular value for this purpose. Extensive use of microdeterminations of iodine in balance studies and blood analyses has enabled several investigators to extend our knowledge of iodine metabolism and of the course of thyroid disease.[1,2] The same studies also indicate some of the limitations to be expected of blood iodine determinations. Known cases of hyperthyroidism, following a period of mobilization and loss of thyroid iodine, may show normal blood iodine levels but retain their high metabolic rate. Thus there appears to be no immediate relationship between the concentration of iodine in the blood and the basal metabolic rate at a given time. Occurrence of high values for blood iodine in non-thyroid conditions has also been mentioned.[2]

It therefore appeared of interest to study the relationship between basal metabolic rate and blood iodine in dogs receiving injections of thyroxin or phlorhizin. The calorigenic response to thyroxin is presumably due to the injected hormone itself. Phlorhizin, on the other hand, although it produces its principal effect in both normal and thyroidectomized dogs, sharply increases the metabolic rate only if the thyroid is present.[3,4] Can involvement of the thyroid in this experimental condition be detected by determining blood iodine?

In the following experiments normal adult

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