Observations on the Phosphatase Content of Blood and Bone Marrow Cells in Normal and Pathologic Hemopoiesis

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PHOSPHATASE can be demonstrated in tissues by means of reliable histochemical methods. Such a method was described for alkaline phosphatase in 1939 by Gomori,1a and in the same year by Takamatsu.2 Two years later Gomori1b described his method for demonstrating acid phosphatase. The principle on which these methods are based is to incubate slices of tissue with glycerophosphate; the phosphate is then liberated by phosphatase. The phosphate is converted through calcium chloride into calcium phosphate, and through a soluble cobalt or lead compound into the corresponding phosphate. This is precipitated with ammonium sulfide as black-brown cobalt sulfide or black lead sulfide. The former is used for demonstrating alkaline phosphatase, the latter for localizing acid phosphatase.

Previous studies of blood and bone marrow by this technic have been few in number. Wachstein3 and Dalgaard4 found alkaline phosphatase in polymorphonuclear leukocytes, particularly in the nuclei of these cells in myeloid leukemia. Rabinovitch, Junqueira and Mendels5 studied acid phosphatase in bone marrow. They found that eosinophile granules in myelocytes and the nuclei of megakaryocytes contained much phosphatase in three normal individuals, in one case of pernicious anemia, and in one of myeloid leukemia. Rabinovitch and Andreucci6 studied the distribution of alkaline and acid phosphatase in normal human bone marrow smears fixed by means of formol vapor and by somewhat modified Gomori methods. For both enzymes a relation between staining intensity and cellular richness was found and both were predominantly nuclear in location. Specific neutrophilic granules were variable, and eosinophilic granules were consistently positive for acid phosphatase. Nucleoli were positive for the alkaline enzyme. Mitotic chromosomes were positive for both technics. Acid phosphatase reactions in cytoplasmic zones of lymphocytes, erythroblasts, plasmacytes and megakaryocytes were described. Gomori7 found a high phosphatase content in polymorphonuclear leukocytes in tuberculous tissue.

Plum7 studied alkaline phosphatase in blood cells using Wachstein’s modification of the Gomori-Takamatsu method. In normal individuals he found that about one-third of the neutrophile granulocytes in the blood were positive for phosphatase, and an even greater proportion of those in the bone marrow. Phosphatase was also present in young granulocytes and occasionally in the nuclei of immature erythrocytes; such phosphatase-containing nuclei also occurred in pernicious anemia. All granulocytes and their young forms contained phosphatase.

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in myeloma, polycythemia, Hodgkin's disease and in lymphatic leukemia, but not in myeloid leukemia.

**METHOD**

These investigations comprise determinations of phosphatases by the methods of Gomori-Takamatsu and Gomori. A study of the methods revealed that the quality of the ammonium sulfide solution affects the results decisively. The ammonium sulfide must be fresh and weakly alkaline. In old solutions polysulfides may arise, and these together with the excessive ammonia content dissolve the cobalt or lead sulfide produced in staining of the phosphatase; the sulfide then spreads out into the rest of the tissue. The preparations occasionally show a varying degree of black precipitate spreading over the entire preparation. The sediment can be easily distinguished from the cobalt or lead sulfide which, typically localized in the cell, shows where phosphatase is present. The sediment is perhaps due to the fact that the soluble cobalt or lead salt used in staining does not become wholly loosened from the preparation in washing. If an old or too ammoniacal solution of ammonium sulfide is then used, the precipitate outside the cells may dissolve and penetrate into the cells. Artefacts are produced in this way. In particular many nuclei, granules and cell cytoplasm, or entire erythrocytes, appear to contain phosphatase.

The most suitable time of incubation for blood smears and bone marrow smears has been found to be two hours in the case of alkaline and twenty-four hours in the case of acid phosphatase. As no other staining of the preparations is done, the hemoglobin-containing cells and parts of cells with black-brown cobalt sulfide or black lead sulfide are the only ones that are clearly discerned. Other cells are seen as unstained transparent formations or not at all.

**RESULTS**

**Alkaline Phosphatase**

In individuals with no pathologic symptoms, alkaline phosphatase in the form of cobalt sulfide can be demonstrated in the bone marrow and in the blood only sparsely in hemoglobin-containing blood corpuscles. Here and there a black-brown ring is seen in the center of the corpuscle; its size is about that of the nucleus of a normoblast or smaller. In the latter, the center of the ring is brown-gray or dark brown, and finally only a black-brown large or small rounded or angular formation is visible. In other blood cells, alkaline phosphatase cannot be demonstrated.

The picture is greatly altered if there is accelerated regeneration of blood. Alkaline phosphatase is then present in increased amounts—even in the hemoglobin-containing red blood cells—in a definite and regular manner. After acute blood loss of various kinds, more or less numerous black-brown rings and bodies of the type described above appear first in the bone marrow and shortly afterwards in the blood; in the same cell there may be several of these formations of varying size and shape. The rings also appear in the form of "strings of pearls" or in fragments. In some of the blood corpuscles, with a perfectly complete ring surrounding a grayish mass, delicate round points are seen outside the ring. Such points or droplets may also be observed in hemoglobin-containing cells which have a fairly large unstained nucleus without a ring.

At the same time, there occasionally appear outside the cells, in the bone marrow or especially in the blood, a few round gray-brown or black-brown bodies
of which the largest are of the size of a normoblast nucleus and may show a
definite nuclear structure but, in general, no distinct nucleoli.

If we compare the phosphatase-containing formations in the red blood cells
with those outside, noting that both of these can be visualized only because of
their phosphatase content but not by vital staining, there seems to be no doubt
whatever that the formations represent intracellular or expelled normoblast
nuclei in varying stages of disintegration. In red blood cells, punctate cobalt
sulfide is now and again also found outside unstained or phosphatase-containing
intact nuclei. These points may probably be interpreted as phosphatase-contain-
ing droplets in the cytoplasm. Occasionally the droplets are arranged so as to
form a “string of pearls” around an unstained nucleus. These “pearls” probably
represent a transition to the next stage, i.e., frequent phosphatase rings around
normoblast nuclei. In reality, a ring in the slide preparations corresponds to a
layer around the nucleus.

In a case of familial hemolytic icterus with persistent reticulocytosis, the
concentration of alkaline phosphatase was high in the red blood cells, especially
in the peripheral blood. Numerous “strings of pearls,” rings of varying thickness
and size around the nuclei, and stained nuclei and remnants of nuclei were seen
(figure 1).

However, alkaline phosphatase can not be demonstrated in young non-hemo-
globinized red blood cells. This applies also to all white blood cells, megakaryo-
cytes and blood platelets.

Cases of pernicious anemia without spontaneous reticulocytosis show no appre-
ciable phosphatase content in the bone marrow or in the peripheral blood.
If such a patient is given liver extract, an increase of alkaline phosphatase, as
described above in connection with accelerated regeneration of blood appears.
The reaction is mild in some cases; in others it is fairly intense. The phenomenon
is observed shortly before the appearance of reticulocytosis and is most pro-
nounced at the stage when reticulocytes increase rapidly. In some cases, especially
in the blood smears, numerous large or small intracellular fragments filled with
cobalt sulfide are seen in the red cells. These probably are disintegrating nuclei
(figure 2). At the same time a small number of extracellular nuclei with high
phosphatase content (figures 3a, b) are often visible. When the number of
reticulocytes begins to fall, the phosphatase almost completely disappears from
the blood corpuscles. Some alkaline phosphatase appears temporarily also in
polymorphonuclear leukocytes.

Increased alkaline phosphatase content is demonstrable in the bone marrow
and in the blood in certain pathologic conditions. In two cases of panhematopenia
(pannymelophthisis) abundant alkaline phosphatase was found in red and white
blood cells, particularly in the bone marrow.

Case 1. Thirty-eight year old woman. Blood: Hb. 3.55 Gm./100 ml; R.B.C. 1,820,000,
average diameter 7.1 μ; C.I. 89; W.B.C. 2,000 with 83 per cent lymphocytes; thrombocytes
32,700. Bone marrow: few cells, no pathologic forms, no megaloblasts. Alkaline phosphatase
in the blood slightly elevated.

Case 2. Sixty-three year old man. Blood: Hb. 2.20 Gm./100 ml; R.B.C. 850,000, average
diameter 7.0 μ; C.I. 1.33; W.B.C. 3.100 with 70 per cent lymphocytes; thrombocytes 3,500.

Bone marrow: very few cells, plasma cells 3 per cent, no megaloblasts. Alkaline phosphatase in the blood normal.

No signs of increased blood destruction was observed in these cases.

Phosphatase is seen in the hemoglobin-containing red blood cells, not only as numerous rings around the nuclei (figure 4) and in the nucleus itself, but also in the form of large and small extranuclear drops, especially at the periphery of the cell. Many of the erythrocytes are in process of marked disintegration. In the polymorphonuclear leukocytes and metamyelocytes, the entire cytoplasm or cell appears blackened by phosphatase; the cell can then be transformed into a round black disc which is shrivelled and partly disintegrating (figures 5a, b).

The observation of an increased alkaline phosphatase content in blood cells in a number of persons organically normal but showing evidence of neurasthenia or neurocirculatory asthenia (effort syndrome) is of unusual interest. The blood and bone marrow cell counts by the usual technic were normal. At times a relative lymphocytosis or eosinophilia could be demonstrated. No normoblasts were present in the peripheral blood. A slight rise in the number of reticulocytes (up to 6 per cent) without signs of increased blood destruction was occasionally observed. The serum alkaline phosphatase was within normal limits.

The diagnosis of NCA rests chiefly on a typical history. Patients principally complain of lack of physical endurance and of sudden attacks of fatigue. Heart action is easily accelerated by exertion, mental excitement, hot baths and coffee or alcohol. Palpitation also occurs after meals and at night on waking up. Extrasystoles are common and occasionally there is paroxysmal tachycardia. Frequent symptoms are dyspnoea, sleeplessness, giddiness, headache and a tendency to sweating. Intestinal cramps and flatulence are features. The patients are very susceptible to infections, especially to those of the pharynx. Physical examination reveals little if any signs.

In many of such cases the preparations stained for alkaline phosphatase—bone marrow smears, blood smears or both—showed a definitely increased number of dark rings of phosphatase in the red cells around the unstained or cobalt sulfide-containing nuclei. The rings surrounded either the entire periphery of the nucleus or only part of it. At the same time, phosphatase-containing nuclei or fragments of nuclei were present in the red cells (figure 6). Nuclei varying in phosphatase content were also found outside the cells. At times the polymorphonuclear leukocytes and metamyelocytes contained a varying degree of phosphatase, again only in the cytoplasm (figure 7). Occasionally phosphatase-containing megakaryocytes were seen with stained cytoplasm and unstained nuclei.

Moreover, residua of nuclei containing alkaline phosphatase were occasionally observed in the red blood cells of the bone marrow and peripheral blood in patients with rheumatoid arthritis. Alkaline phosphatase was also seen at times in the polymorphonuclear leukocytes and metamyelocytes of the patients.

In certain pathologic conditions, alkaline phosphatase appears only in polymorphonuclear leukocytes and metamyelocytes, but not in other blood cells.

Among the diseases, one group with the process wholly or partly localized to
the hemopoietic system should be mentioned. Phosphatase is often present in leukocytes in myeloid leukemia (figure 8), in lymphatic leukemia, polycythemia (figure 9), myeloma (figure 10) and in Hodgkin's disease (figure 11). In some cases of Paget's disease and of carcinoma of the liver, and especially in bone tumors, phosphatase-containing leukocytes also appear. All conditions mentioned above are characterized by some degree of malignancy. The phosphatase is then always first found in the cell cytoplasm. With a lower phosphatase concentration the cobalt sulfide becomes localized to the periphery of the cell and especially to the area inwards from the lobules of the nucleus. Gradually the entire cytoplasm becomes brown-black and in its center the nucleus is of a brilliant gray-white color. When the phosphatase content increases, the nucleus disappears gradually, and finally the leukocyte is transformed into a black irregular disc, frequently shrivelled and in process of disintegration. Moreover, in polymorphonuclear leukocytes, the same changes are often seen in metamyelocytes but not in the youngest leukocytes.

Illustrations

All figures are microphotographs with about 1500X magnification.

Staining: Gomori-Takamatsu technic. Phosphatase appears in the cells as black cobalt or lead sulfide.

Fig. 1.—A case of hemolytic icterus. A ring of alkaline phosphatase surrounds the nucleus of a red cell in the blood.

Fig. 2.—A case of pernicious anemia. Six days after injection of liver extract. Large numbers of intracellular alkaline phosphatase-containing fragments of nuclei in the red blood corpuscles in the blood.

Fig. 3. a and b.—Same case as in figure 2. An expelled normoblast (a) and macroblast (b) nucleus in the bone marrow.

Fig. 4.—A case of pancytopenia. Clearly defined ring of alkaline phosphatase in a red blood cell in the bone marrow.

Fig. 5. a and b.—Same case as in figure 4. Polymorphonuclear leukocytes with high alkaline phosphatase content in the bone marrow.

Fig. 6.—A case of neurocirculatory asthenia. Remnants of nuclei containing alkaline phosphatase in the red blood cells in the blood.

Fig. 7.—A case of neurocirculatory asthenia. Alkaline phosphatase containing polymorphonuclear leukocytes in the bone marrow.

Fig. 8.—A case of myeloid leukemia. Very high alkaline phosphatase content in polymorphonuclear leukocytes in the blood. Cells are small and in process of disintegration.

Fig. 9.—A case of polycythemia. Cytoplasm of metamyelocyte in the blood shows high alkaline phosphatase content.

Fig. 10.—A case of myeloma. High alkaline phosphatase concentration in cytoplasm of polymorphonuclear giant leukocyte in the blood.

Fig. 11.—A case of Hodgkin's disease. Cytoplasm of polymorphonuclear leukocytes in the blood shows high alkaline phosphatase content.

Fig. 12.—A case of acute nephritis. High acid phosphatase content of the nuclei of young red cells in the bone marrow.

Fig. 13.—A case of polycythemia. Nucleus of a young red cell and a polymorphonuclear leukocyte in the blood shows high acid phosphatase content.

Fig. 14.—A case of "achrestic" anemia. Very high acid phosphatase content in young red cells in the bone marrow.

Fig. 15.—A case of rheumatoid arthritis. Young red cells, polymorphonuclear leukocytes and metamyelocytes in the bone marrow contain very much acid phosphatase.
Acid Phosphatase

In normal individuals, slide preparations incubated for acid phosphatase give, as a rule, a negative result. When blood formation is accelerated, acid phosphatase may in exceptional cases be seen in red blood cells in the same position and pattern as in the case of alkaline phosphatase, but usually only sparsely around and in the fairly large nuclei. However, acid phosphatase has not in such cases been demonstrated in young non-hemoglobinized red blood cells. This also applies to all white blood cells, megakaryocytes and blood platelets.

If a patient with pernicious anemia is given liver extract an increase of acid phosphatase occasionally occurs in the same manner as described above for alkaline phosphatase.

Acid phosphatase, as stated already, can seldom be demonstrated in blood cells. An exception is true polycythemia. At a definite stage of this disease the cells contain only acid phosphatase. Alkaline phosphatase, as described above, appears in polymorphonuclear leukocytes and metamyelocytes. Acid phosphatase is in addition demonstrable in myeloblasts + myelocytes which cannot be differentiated by the method used. The phosphatase is then localized chiefly to the cell cytoplasm. However, it may also penetrate into the nuclei so that the whole cell becomes a dark disc which is often shrunken and in process of breaking down. The presence of acid phosphatase in the youngest red blood cells is of special interest. In the bone marrow the nuclei of these cells are darkened by lead sulfide and the cytoplasm stains to a weak degree. But some cells are quite black-brown, and many of these shrunken and disintegrating (figure 13). In the peripheral blood the same changes are observed as in the bone marrow, but they are less pronounced. A great number of large nucleated red blood cells with their highly phosphatase-containing nuclei are shrunken and in process of disintegration. In a case treated with nitrogen mustard both alkaline and acid phosphatase disappeared from the blood cells and the bone marrow.

In this connection a case of “achrestic” anemia may be described.* In addition to severe slightly hyperchromic anemia the patient showed some leukopenia and granulocytopenia. The stomach had retained the power to secrete hydrochloric acid. The anemia responded weakly to vitamin B12. The bone marrow was found to contain many cells, especially young erythrocytes, and megablasts. Acid phosphatase could be demonstrated in large amounts in the youngest nucleated red blood cells of the bone marrow, particularly in their nuclei. Some of the cells were shrunken and in process of breaking down. In addition there was also much acid phosphatase in all types of leukocytes such as myeloblasts + myelocytes, metamyelocytes and polymorphonuclear leukocytes. Generally the phosphatase is localized to the cytoplasm of the cells but it may also fill the whole cell; in that event the cells are usually shrunken and disintegrating. Similar, though weaker changes can be demonstrated in the peripheral blood cells (figure 14).

* A complete report of the case, treated at the Second Medical Clinic of the University, will be published at a future date.
In some patients suffering from rheumatoid arthritis and in some with acute nephritis acid phosphatase is present in blood cells. Occasionally it is very abundant in the nuclei of the younger normoblasts of the bone marrow and less in their cytoplasm. Finally the whole cell is changed into a dark-brown disc of which many are shrunken and breaking down. At the same time acid phosphatase may be present in large amounts in polymorphonuclear leukocytes and in their juvenile forms (figures 12, 15). In these the phosphatase is localized to the cytoplasm, but finally spreads to the whole cell, which is then shrunken and disintegrates. Occasionally large amounts of acid phosphatase are found in young megakaryocytes. In these cases there is also acid phosphatase in the cells of the peripheral blood. In other cases there is much less acid phosphatase and then only in the nuclei of the youngest red blood cells of the bone marrow.

The patients reported above were all women; most of them had had rheumatoid arthritis, only a few spondylarthritis. These diseases lasted over a period of years from one and a half to twenty-two. Their ages varied from 43 to 67 years, but one was 29. All were afebrile. The sedimentation rate was usually high. One of the patients had hypertension but the others had no pathologic changes in the vascular system. In no case was the spleen enlarged. The blood picture as well as the bone marrow cells in routine preparations was normal in all patients with the following exceptions:

A woman of 46 who had had rheumatoid arthritis for a year and a half received gold treatment which resulted in dermatitis; in this case the number of bone marrow cells was greatly increased. Of these cells 161 were basophilic and 60 were orthochromic normoblasts as calculated per 200 white blood cells. In 2 more cases the number of nucleated red blood cells was also slightly increased in the bone marrow. One of these patients had had rheumatoid arthritis for twenty-two years. She had renal amyloidosis and fairly severe hypochromic anemia and leukocytosis.

The alkaline and acid phosphatase concentration of the serum was determined in a number of the cases studied. Increased values were only rarely noted. No correlation was observed between the phosphatase content of the blood cells and the serum phosphatase.

Summary of Results and Discussion

These investigations comprise a large number of phosphatase determinations by the methods of Gomori-Takamatsu and Gomori. Alkaline and acid phosphatase was studied in slide preparations from blood and bone marrow of normal individuals and in a wide variety of pathologic conditions. A certain regularity was discovered in the occurrence of phosphatases. Thus they were never demonstrated in lymphocytes, monocytes and thrombocytes, or in eosinophilic and basophilic granules. Mature erythrocytes were always negative for phosphatase. The presence of a particular phosphatase was found to be associated to the cellular reaction; thus alkaline phosphatase was found in orthochromic normoblasts, neutrophilic polymorphonuclear leukocytes, in metamyelocytes and in older megakaryocytes. Acid phosphatase was demonstrated in basophilic normoblasts,
myeloblasts and myelocytes, in metamyelocytes and polymorphonuclear leuко-
ocytes, and in younger megakaryocytes. Neutral cells contained both alkaline
and acid phosphatase; acid cells, acid; and alkaline cells, alkaline phosphatase.
The distribution of phosphatase between the nuclei and the cytoplasm was also
regular. Phosphatase was found in red cells chiefly in the nuclei and in leukocytes
in the cytoplasm. Diffusion of phosphatase from one cell into another is scarcely
possible in thin preparations in which the cells are generally apart from each
other. In one and the same disease similar localization of phosphatase has usually
been observed. At present there are not many cases of the diseases here studied;
it is possible that in later investigations somewhat different results may be ob-
tained. What has been stated above seems to show that the methods used and
the results obtained can be considered reliable.

With the exception of Gomori, whose staining technic has been used in these
investigations, previous workers have obtained results which vary widely and
conflict in many respects with those here reported. The dissimilarity of results
may be partly due differences in fixation and in the methods used. As pointed
out in connection with the description of methods, special attention must be
given to the ammonium sulfide solution. If an ammonium sulfide solution w'hich
is too
or too alkaline is used for staining, the distribution of phosphatase
resembles in many respects that described by some previous investigators.

The phosphatase in blood corpuscles may be classified in three main groups:
(1) that in the normoblast nuclei during the formation of erythrocytes, (2) that
in the blood cells in connection with accelerated malignant activity of the bone
marrow and (3) that occurring in blood cells in diseases which cannot be con-
sistently classified.

By studying hemopoiesis, especially during active regeneration of blood, it has
been shown that alkaline phosphatase plays a decisive part in the disappear-
ance of the normoblast nucleus. At the stage when a young red blood cell begins to
develop hemoglobin, alkaline phosphatase appears around the nucleus as a dense
layer. Gradually the nucleus is infiltrated with phosphatase, becomes pycnotic
and breaks down mostly within the cells; but it may also be extruded and dis-
solved in the bone marrow or the blood.

During accelerated regeneration of blood, the content of alkaline phosphatase
increases in nucleated hemoglobin-containing red cells, and alkaline phosphatase
also temporarily appears in polymorphonuclear leukocytes. If a patient with
pernicious anemia is treated with liver extract, alkaline phosphatase appears in
the blood cells in the same way as in accelerated blood formation. The reaction
is most marked when reticulocytosis sets in. In exceptional cases, the activity
of acid phosphatase in erythropoiesis seems to resemble that of the alkaline.

In some cases of disease abnormality in the disappearance of the normoblast,
nucleus is observed. Chiefly in peripheral blood, but also in the bone marrow,
there are numerous red blood cells with nuclei or remnants of nuclei containing
alkaline phosphatase, though the blood picture does not differ from normal.
Occasionally there may also be some amount of alkaline phosphatase at the
same time in the polymorphonuclear leukocytes, metamyelocytes and mega-
karyocytes. The people in whom this disturbance was noted suffered in most cases from a nervous affection called neurocirculatory asthenia or effort syndrome. In a few cases they had rheumatoid arthritis. In the latter, and in some cases of acute nephritis, acid phosphatase has been demonstrated in the bone marrow; it has occasionally been demonstrated in the peripheral blood cells also. The phosphatase is localized chiefly to the nuclei of the youngest red blood cells. Many cells are in process of breaking down. Occasionally a large amount of phosphatase is also present in young granulocytes, in polymorphonuclear leukocytes and young megakaryocytes. The cells containing much phosphatase are usually small and shrunken or actually breaking down.

In rare cases phosphatase occurs in very large amounts in connection with severe anemias. Thus the older normoblasts, polymorphonuclear leukocytes, metamyelocytes and megakaryocytes were found to be of very high alkaline phosphatase content in two cases of pancyclopenia (panmyelophthisis). Processes of breaking down were very frequently visible in the cells. Acid phosphatase, on the other hand, was present in very large amounts in bone marrow and blood cells in a case of “achrestic” anemia. The phosphatase was localized to the youngest red blood cells and to leukocytes and their juvenile forms. There was general cell destruction.

In leukemias, myeloma, Hodgkin’s and Paget’s diseases and in some malignant tumors of the skeleton and liver (in cases more or less malignant in character), alkaline phosphatase was observed only in polymorphonuclear leukocytes and metamyelocytes. In connection with true polycythemia much acid phosphatase was also present in the youngest red blood cells, especially in their nuclei, and in polymorphonuclear leukocytes and their juvenile forms. The phosphatase concentration was especially high in shrunken and disintegrating cells. The large amount of phosphatase in this last group may be a manifestation of accelerated tissue growth.

The fact that phosphatase occurs in tumor tissue deserves special attention. If skin is treated with methylcholanthrene, an accumulation of alkaline phosphatase is demonstrable after one day (Biese and Biese@). Several workers have studied phosphatases in tumors of widely varying types (Kabat and Furth,9 Gomori,10 Landow, Kabat and Newman,10 Wolf, Kabat and Newman,11 Greenstein12 and others). No simple correlation has been shown to exist between tumor growth and the concentration of phosphatase in tissue. For instance, the acid phosphatase content of the liver, high even under normal conditions, increases in cases of liver tumor while alkaline phosphatase does not increase (White and Edwards13). In hepatomas induced in rats with butter yellow, an enormous rise in alkaline phosphatase has been demonstrated. Gomori14 observed an increase in acid phosphatase in carcinoma of the stomach. It is further noteworthy that during wound healing a high concentration of alkaline phosphatase is present in the skin, notably in the leukocytes (Fell and Danielli14).

As regards the origin of the phosphatase in the blood cells it is of interest that no correlation was observed in my cases between serum phosphatases and the phosphatase in the blood cells. The phosphatases are assumed to arise from the
reticulo-endothelial tissue and considered most probably to belong to the globulins.

Can the presence of phosphatases and their activity in the different cases be consistently explained? When the development of a normoblast has advanced to the stage at which the cell no longer divides but its nucleus begins to break down, alkaline phosphatase can always be demonstrated in the nucleus and in its disintegration products. The quicker the new blood forms, the greater is the number of nuclei in which phosphatase occurs. The phenomenon seems most readily understandable if we assume that alkaline phosphatase is actively concerned in the destruction of the normoblast nucleus. In trying to explain this it must be remembered that enzyme is capable of splitting several organic compounds which play a part especially in the carbohydrate metabolism. The abundance of alkaline phosphatase in certain cases of panmyelophithsis in which a large number of bone marrow cells are destroyed and in process of breaking down might be understood if phosphatase is regarded as a cell-destroying factor. Too intense phosphatase activity may conceivably also be present in the cases in which the circulating red blood cells contain larger amounts of nuclear remnants than usual. The phosphatase concentration was especially high in shrunken and disintegrating leukocytes in cases related to accelerated or malignant growth of bone marrow. This seems also to suggest the possibility of phosphatase being able to destroy cells. The same applies to acid phosphatase as a possible cause of "achrestic" anemia. In this disease destruction of large numbers of young red blood cells is observed. In connection with true polycythemia the acid phosphatase concentration was especially high in shrunken and disintegrating cells. In rheumatoid arthritis the acid phosphatase was localized chiefly to the youngest red cells. Many of these were disintegrating. Thus the investigation has revealed many circumstances which indicate the possibility of phosphatases destroying cells or their nuclei. In some cases the activity of phosphatase is physiologic, as when the normoblast nucleus disappears during erythropoiesis. In other cases the presence of phosphatase is related to accelerated or even malignant growth of bone marrow or of tissues associated with blood formation. Finally there are cases in which the appearance of phosphatases in blood and bone marrow cells cannot at present be explained.

SUMMARY

The alkaline and acid phosphatase content of blood and bone marrow has been studied by the methods of Gomori-Takamatsu and Gomori in normal individuals and in a great number of different diseases. The presence of a particular phosphatase is related to cellular reaction. Alkaline phosphatase was found in orthochromic normoblasts, polymorphonuclear leukocytes, metamyelocytes and older megakaryocytes. Acid phosphatase was demonstrated in basophilic normoblasts, polymorphonuclear leukocytes and their juvenile forms and in young megakaryocytes. In red blood cells and young megakaryocytes phosphatase was present chiefly in the nuclei, in leukocytes and older megakaryocytes in the cytoplasm.
By studying erythropoiesis, especially during active regeneration of blood, it has been shown that alkaline phosphatase plays a decisive part in the disappearance of the normoblast nucleus. In certain diseases abnormality is observed in the disappearance of the normoblast nucleus (effort syndrome, rheumatoid arthritis). In others, acid phosphatase occurs in large amounts in the youngest normoblasts (polycythemia vera, rheumatoid arthritis, nephritis). At the same time phosphatase may also be found in polymorphonuclear leukocytes and their juvenile forms. In rare cases of severe anemia alkaline phosphatase (panmyelophthisis) or acid phosphatase (achretric anemia) is noted in large amounts in nucleated red cells and in leukocytes.

Only alkaline phosphatase is present in polymorphonuclear leukocytes and metamyelocytes in leukemias, myeloma, Hodgkin's and Paget's disease, and in some malignant tumors of the skeleton and the liver.

Several circumstances indicate that phosphatases might destroy cells or their nuclei. In some cases phosphatase activity is physiologic, in others phosphatase concentration is related to accelerated or malignant growth of bone marrow or tissue associated with blood formation. Finally there are cases in which there is as yet no explanation for the appearance of phosphatases.

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