THE DISTRIBUTION OF HISTOCHEMICALLY DEMONSTRABLE GLYCOGEN IN HUMAN BLOOD AND BONE MARROW CELLS

By Max Wachstein, M.D.

RENEWED attention has recently been given to the glycogen content of the human blood. Wagner,17 confirming older observations in the literature,4,15 found glycogen only in the granulated leukocytes by chemical estimation, while the other formed elements in normal blood did not contain this substance. Increased amounts of glycogen were found in the blood of patients with myeloid leukemia, while in the blood of patients suffering from acute leukemia, as well as of those with chronic lymphatic leukemia, normal amounts of glycogen were present.14,18

The particular usefulness of the periodic acid-Feulgen technic for the histochemical demonstration of polysaccharides (McManus,11 Lillie,10 Hotchkiss9), prompted an examination of human blood and bone marrow smears.

TECHNIC

Either air-dried blood and bone marrow smears, or films fixed in absolute alcohol were placed into a 0.5 per cent solution of periodic acid in water for five minutes. Even slides several years old gave very satisfactory staining reactions. After washing in tap water, the slides were immersed in Schiff’s reagent, prepared according to Hotchkiss,9 for fifteen minutes. They were then rinsed in three changes of SO2 containing water, each time for two minutes, and then washed for five minutes under tap water. Harris’ hematoxylin was used as counterstain. After the usual dehydration, slides were covered with a resin under cover slips. In order to identify the stained substance as glycogen, alcohol-fixed blood and bone marrow films were first covered with saliva for 15–30 minutes at room temperature.2 After a short wash in distilled water, the above described staining technic was employed.

RESULTS

Normal blood smears. Only the polymorphonuclear leukocytes and platelets showed consistent staining. The cytoplasm of the polymorphonuclear leukocytes revealed a dark red to Bordeaux red uniform color (fig. 1). Occasionally it contained small dustlike and also somewhat coarse granules. The nucleus was always unstained. At least 90 per cent of all polymorphonuclear leukocytes gave this staining reaction. Eosinophiles in normal blood, as well as in films in which markedly enlarged amounts of these cells were present, showed faint staining of their cytoplasm. The granules remained unstained. Lymphocytes were mostly without staining. However, some showed small granules in the cytoplasm. Monocytes were either negative or showed only faint staining. The thrombocytes consistently gave a positive reaction. For the most part they showed a brilliantly stained center and a paler outer border.

From the Laboratories of the St. Catherine’s Hospital, Brooklyn, New York, and the Division of Pathology, Mt. Sinai Hospital, New York, New York.
Leukocytosis. In blood smears from patients suffering from marked infectious leukocytosis due to various causes, polymorphonuclear leukocytes, including the metamyelocytes, contained large amounts of cytoplasmic glycogen. The cytoplasm showed not only diffuse staining, but in some cells it appeared in coarser granules than commonly seen in the blood films of normal subjects.

Infectious mononucleosis. The atypical lymphocytes were negative with the exception of some which contained dark red granules as seen in normal lymphocytes.

Various anemias. In smears from patients with various anemias (erythroblastosis fetalis, Cooley's anemia, etc.) nucleated red cells, erythroblasts, as well as normoblasts, were negative.

Lymphatic leukemia. The lymphocytes found in blood smears of patients suffering from lymphatic leukemia showed the same staining reaction as those in normal blood smears.

Chronic myeloid leukemia. Myelocytes, metamyelocytes, and polymorphonuclear leukocytes showed the cytoplasmic polysaccharide reaction. Myelocytes took only a faint reddish color (fig. 2). The intensity of the reaction obviously increased with the maturation of the cells. Dark red granules and coarse stippling were fairly frequent in polymorphonuclear leukocytes.

Acute myeloid leukemia. Most of the myeloblasts did not contain glycogen, while some cells, obviously still quite immature, revealed occasional dark red granules or even a brim of red-staining cytoplasm around the large immature nucleus. More mature myeloid elements showed the usual amount of glycogen.
Blood smears from various animals. The polymorphonuclear (heterophile) leukocytes in the blood film of dogs, rabbits, guinea pigs and frogs showed considerable staining reaction. Only faint traces were demonstrable in the white cells of the rat and mouse.

Smears from lymph nodes. In films from tonsils and lymph nodes not involved by disease, the lymphocytes showed no trace of glycogen.

Bone marrow. In films from bone marrow of normal individuals, as well as of those with various abnormal conditions, a behavior of the myeloid elements similar to that seen in the blood films of patients with chronic myeloid leukemia was observed. Myeloblasts were mostly negative. As the myeloid cells matured, the staining reaction in the cytoplasm became more pronounced. Occasional myeloid cells showed coarse granules. Most nucleated red cells, including the typical megaloblasts of pernicious anemia, showed no staining reaction. A very occasional erythroblast revealed a faintly stained cytoplasm. Plasma cells as well as the atypical cells found in multiple myeloma, were mostly negative. A considerable proportion of the megakaryocytes gave a distinct reaction. The cytoplasm was coarsely stained. Only occasional megakaryocytes did not contain any stainable polysaccharides.

Comment

As far back as 1877, Ranvier demonstrated glycogen in the leukocytes of the frog with the help of iodine. Ehrlich, several years later, was the first to examine
films of human blood for its glycogen content. Since then a good number of papers dealing with the histochemical demonstration of glycogen have been published. The older literature has been extensively reviewed in Neukirch's and Girardin's contributions. So far, the following methods have been used for the demonstration of glycogen in blood cells.

1. **Iodine reaction**
   (a) Dried films were exposed to iodine vapors (Ehrlich and Lazarus). In normal blood films, leukocytes are unstained, red blood cells take a brownish hue and platelets are stained. The leukocytes in exudates, however, show a strong reaction.
   
   (b) Wet films were exposed to iodine vapors, according to Zollikofer. All neutrophilic leukocytes stain diffusely while some (about 20 per cent, according to Girardin) contain glycogen granules. As with Ehrlich's method, the platelets are distinctly and the red blood cells faintly stained.

2. **Best's carmine method**
   This method was modified by Neukirch for blood films. All polymorphonuclear leukocytes in normal blood give a diffuse to fine granular staining. In addition, the centers of the thrombocytes are stained. The other cells are unstained with the exception of occasional lymphocytes showing a few red granules. Neukirch found the eosinophilic granules positive in blood films and Arnold in bone marrow section, while Girardin, using Neukirch's method, found them consistently negative.

3. **The Bauer-Feulgen stain, as well as a silver technic**, have been used for bone marrow section of the normal rhesus monkey by Wislocki and Dempsey. Glycogen was demonstrable in polymorphonuclear neutrophiles and neutrophilic metamyelocytes, but not in any other cells. In the circulating blood, as observed in sections of blood contained in the heart, glycogen was seen only in polymorphonuclear leukocytes.

As is well known, substances giving any of the reactions described above, can only be considered as glycogen if they can be digested by amylase. It has been repeatedly demonstrated that the carbohydrate-like substance found in the cells of purulent exudates can be digested by saliva. According to Neukirch, however, with both the iodine or Best carmine technic, the staining reaction in the leukocytes of the blood, as well as that in the platelets, is not prevented by previous treatment with saliva. Dempsey and Wislocki, on the other hand, using the Bauer-Feulgen technic, found the stainable substance in leukocytes digestible by saliva.

Further examination of the nature of the substance giving the reaction with the periodic acid-Schiff technic was therefore undertaken. By using periodic acid, polysaccharides are oxidized to polyaldehydes. The aldehyde group reacts with Schiff's reagent. Low molecular compounds such as simple sugars and hydroxyamino acids can also react with this reagent, while the pentose component of nucleic acid does not react (Hotchkiss).

No staining reaction was seen in air-dried or alcohol-fixed films without previous treatment with periodic acid, thus excluding the possibility that the reaction was due to preformed aldehyde groups. Since the reaction occurred after twenty-four hours of alcohol fixation it could not have been caused by the alcohol-soluble plasmal. The substance was still present at room temperature (24-26°C), after immersion of the films up to 150 minutes in distilled water or saline solution. Therefore, it appears unlikely that the reaction was due to the presence of simple low molecular water-soluble substances.

In order to prove that the stainable substance was really glycogen, blood films were subjected to digestion with saliva. A significant difference became apparent
when films were only air-dried or had been fixed with absolute alcohol. After fixation the stainable substance, in leukocytes as well as in platelets, disappeared fifteen to thirty minutes following salivary digestion at room temperature. In unfixed films, the diastatic effect of saliva was considerably less pronounced, although varying in intensity in different slides. Salivary digestion occurred to a varying degree in alcohol-fixed films from bone marrows as well as in the peripheral blood of patients with myeloid leukemia. In occasional films, the cells proved resistant to the diastatic enzyme.

The results of these experiments make it seem very probable that the substance giving the aldehyde reaction after treatment with periodic acid in blood cells is glycogen. The glycogen in hematic elements, however, is relatively resistant to salivary digestion, unless first treated with alcohol. This may be due to the fact that the cells contain the glycogen in some chemical combination, possibly with protein. Such an assumption was made many years ago by Best. Willstaetter and Rhodewald discuss the peculiar fact that the glycogen becomes more demonstrable in leukocytes of exudates than in the blood. Ehrlich thought that the leukocytes which migrate from the blood stream are being changed in such a way that after some time free glycogen occurs. According to Willstaetter and Rhodewald, it can be assumed that the glycogen is not present in its usual form but possibly in some absorption, or more probably, in chemical connection with the cell protein.

As has been previously found, employing iodine as well as Best's carmine, the platelets give a strong staining reaction. Moreover, in the bone marrow films most of the megakaryocytes are stained. This is obviously due to glycogen, since the staining reaction is prevented by digestion with saliva. In contrast, Wagner found that the reducing substance which is formed after acid digestion of platelets was not digestible by yeast. He therefore concluded that it originated from ribonucleic acid rather than glycogen. By histochemical methods Wislocki, Bunting and Dempsey, found some ribonucleoprotein in the cytoplasm of megakaryocytes.

The behavior of histochemically demonstrable glycogen resembles that of stainable oxidase. This, however, should not be expected to be of practical value for the differentiation of myeloid from lymphatic cells, since some myeloblasts as well as occasional lymphocytes reveal glycogen granules.

A certain parallel of the glycogen reaction in the leukocytes and the histochemically demonstrable phosphatase is quite obvious. Phosphatase activity becomes apparent in myelocytes. The intensity of the phosphatase reaction increases with the maturation of the myeloid cell and is particularly prominent in films of patients with infectious diseases and in exudates. It was previously assumed that this increase in phosphatase may indicate an intensification of metabolic processes. Glycogen is probably the main source of energy for the leukocytes.

The importance of phosphate-splitting enzymes in the intermediary carbohydrate metabolism is well recognized. The histochemically demonstrable relationship between glycogen and phosphatase activity has recently been discussed by Dempsey and Wislocki.

**SUMMARY**

By applying Schiff’s reagent after periodic acid treatment to blood and bone marrow films, a cytoplasmic staining reaction is seen in some cells of the myeloid
series, as well as in megakaryocytes and platelets. The intensity of the staining reaction in the myeloid cells increases with their maturation. The staining reaction can be prevented altogether in alcohol-fixed films by salivary digestion, but only incompletely in air-dried films. The staining reaction is due to the presence of glycogen in some chemical association, possibly with protein.

REFERENCES


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MAX WACHSTEIN

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