THE OCCURRENCE OF THE PERIODIC ACID-SCHIFF REACTION IN VARIOUS NORMAL CELLS OF BLOOD AND CONNECTIVE TISSUE

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The periodic acid-Schiff method stains glycogen, mucus, reticulum and basement membranes, some kinds of elastic tissue, fibrin, and various substances of quite unknown composition (McManus; Lillie et al.; Wislocki and Dempsey). Glycogen may be differentiated from these other substances by the fact that it is soluble in saliva.

In applying recent histochemical methods to hematology, we have observed the staining reactions of a variety of normal blood and connective tissue cells by the periodic acid-Schiff procedure. The present paper gives a detailed account of these observations with interpretations of the findings.

Material and Methods

The principal tissues used were obtained from man and rhesus monkey. The human material consisted of smears of peripheral blood and bone marrow of normal subjects, as well as pieces of uterine tube, uterine cervix, vermiform appendix and mammary gland obtained from operative specimens. A few smears of patients with chronic lymphatic leukemia were also examined. Peripheral blood was obtained by finger puncture and marrow by aspiration, usually from the sternum.

The material from young rhesus monkeys (Macaca mulatta) comprised bone marrow, spleen, lymph glands and pieces of connective tissue from the mediastinum, peritoneum and skin.

In addition to these, occasional tissues from rabbit (bone marrow of a young animal), sow (endometrial stroma) and rat (various areas of connective tissue) were utilized.

The blood and bone marrow smears as well as the blocks of tissue were fixed in Rossman's mixture (sat. sol. picric acid in abs. alc., 90 cm³; formaldehyde (added just before using) 10 cm³). The blocks were embedded in paraffin and sections were cut at 5 μ. Both the smears and the deparaffinized sections were stained by the periodic acid-Schiff technique. After this fixation and method of staining, glycogen, some acid mucopolysaccharides, fibrin and other substances are stained red or pink. Glycogen is distinguishable from mucus and other positively reacting substances by the use of control sections exposed to saliva. Control sections were placed in saliva at room temperature for one hour before staining them. The periodic acid-Schiff method was applied according to the directions of McManus in slightly modified form. The smears and deparaffinized sections were treated with a 1 per cent solution of periodic acid for five minutes, followed by Schiff's leukofuchsin reagent for fifteen minutes and subsequent rinsing in sulfurous acid. When a counterstain seemed desirable, light green or hematoxylin was used. The sections were then dehydrated in alcohols, cleared in xylol and mounted in balsam.

Besides the regular use of saliva on control sections for the identification of glycogen, a few sections of rabbit's and monkey's bone marrow were exposed to male diastase (Fisher Scientific Co.—Eimer and

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Amend). The sections were incubated for one hour at 37 C. in a 1 per cent solution of malt diastase buffered with phosphate at pH 6.8. The results obtained with diastase differed in some respects from those following the use of saliva, as will be discussed in a subsequent passage.

The connective tissues enumerated above were drawn into the investigation for the purpose of studying mast cells and tissue eosinophils. To identify these two cell types with certainty, sections were prepared from the same regions after the use of other fixatives and stains. For the cross-checking of mast cells, blocks of tissue were fixed for twelve hours in a 4 per cent solution of basic lead acetate and the sections were stained for thirty minutes in a 4 per cent aqueous solution of toluidin blue according to the method of Holmgren and Wilander4 and Holmgren.5 By this procedure the granules of the mast cells become brilliantly metachromatic. For the identification of eosinophils in connective tissue or bone marrow, blocks fixed in Zenker’s fluid were sectioned and stained in eosin and methylene blue. Following this procedure the eosinophils are readily distinguishable by their red-stained granules. Blood platelets, megakaryocytes and polymorphonuclear neutrophils could be readily identified in the periodic acid-Schiff preparations without resorting to other means for checking them. Basophilic leukocytes were uncommon but readily recognizable in peripheral blood. Lymphocytes and monocytes were investigated in human blood smears and in sections of spleen and lymph glands of the monkey.

**Observations on Mast Cells and Tissue Eosinophils**

**Mast cells.** In the connective tissues of man and rhesus monkey mast cells stained quite intensely following the periodic acid-Schiff procedure (fig. 1a). Stained mast cells were encountered in the stroma of the human mammary gland, uterine tube and cervix and, in the monkey, in the stroma of the skin, mediastinum and retroperitoneal tissue. The granules were quite heavily stained but the cytoplasm was also involved to some degree, giving a certain haziness to the granules. The reaction was not abolished by previous treatment with saliva, a result which indicated that the staining was not due to glycogen.

Mast cells encountered in the mucosa of sows’ uteri also stained deeply by the periodic acid-Schiff procedure, but the granules were less distinctly differentiated than in the mast cells of monkey and man. This staining was not prevented by previous treatment with saliva. On the other hand, the mast cells in the connective tissues of the rat were rarely and, at best, faintly stained.

In contrast to these species differences, the mast cells of all of these animals exhibited uniform and intense metachromasia of their granules following staining with toluidin blue. Instead of being hazy, the metachromatic reaction was sharply confined to the granules.

**Tissue eosinophils.** These cells were found by chance in great abundance in the mucosa of a human vermiform appendix. The eosinophils were easily identified by virtue of their brilliant red granules in sections stained with eosin and methylene blue. By the periodic acid-Schiff technic these same cells exhibited a diffuse reddish staining involving both granules and cytoplasm. This staining was not influenced by treatment with saliva and consequently could not be attributed to glycogen.

**Observations on Cells of Bone Marrow and Peripheral Blood**

**Basophilic leukocytes.** Basophilic leukocytes were occasionally picked up in human blood smears. Following the periodic acid procedure, they exhibited a number of brilliantly stained, sharply outlined, small red dots located in a pale pink cytoplasm (fig. 1c). In several control smears exposed to saliva, we were unable to identify any basophils, so that the red-stained material may have been glycogen. This appar-
PERIODIC ACID-SCHIFF REACTION IN BLOOD CELLS

Ent finding needs further verification. In the event that basophilic leukocytes contain glycogen, they would appear to differ from mast cells which contain periodic acid-Schiff positive material which is insoluble in saliva.

Eosinophilic leukocytes. Eosinophilic leukocytes encountered in human blood smears showed a pink to reddish cytoplasm with clear granules. This staining diminished some, but did not disappear entirely after preliminary exposure of the sections to saliva.

Eosinophilic leukocytes in monkey's bone marrow were quite deeply stained, the granules appearing dark red against a paler background. This staining was not prevented by treatment with saliva (fig 1c). These cells stood out most conspicuously in preparations of marrow which had been treated with saliva which removed the similarly stained glycogen from the neutrophilic leukocytes.

The eosinophilic leukocytes of rabbit's bone marrow contained exceptionally large granules which stained a pale red by the periodic acid technique (fig. 1d). The staining of these granules was not influenced by previous treatment with saliva.

Neutrophilic leukocytes and myelocytes. The neutrophilic leukocytes, in smears and sections of blood and bone marrow of all species investigated, reacted strongly with the periodic acid-Schiff reagents (fig. 1b). The antecedent neutrophilic meta-myelocytes and myelocytes also reacted positively, the amount of reactive substance being minimal in the myelocytes and increasing as the cells mature into leukocytes. The reaction in the neutrophilic series was completely absent after preliminary use of saliva, indicating that glycogen was responsible for it. Although the glycogen seemed to occur in the cytoplasm in granular or punctate form, it did not appear to be actually localized in the neutrophilic granules, for, as in other

Fig. 1

All of the cells illustrated in this plate were fixed in Rossman's mixture (abs. alc., formaldehyde and picric acid) and were stained by the periodic acid-Schiff method. Figures e, h, i and j were counterstained with hematoxylin. Figures a to d inclusive and figure j were drawn with a × 90 objective and a × 15 ocular, whereas figures e to i inclusive were drawn with a × 90 objective and a × 10 eyepiece.

a. Mast cells from stroma of human uterine tube, stained after exposure of the section to saliva.

b. Neutrophilic leukocytes from the bone marrow of a young rhesus monkey.

c. Eosinophilic leukocyte from the bone marrow of a young rhesus monkey, stained after exposure of the section to saliva.

d. Eosinophilic leukocyte from the bone marrow of a young rabbit, stained after exposure of the section to saliva.

e. Basophilic leukocyte from smear of human peripheral blood.

f. Megakaryocyte from the bone marrow of a young rabbit.

g. Megakaryocytes from the bone marrow of a young rhesus monkey. The cell on the right was untreated, whereas the one on the left was drawn from a section which had been exposed to saliva before staining it.

h. Megakaryocyte and blood platelets (lower left) from smears of human bone marrow and peripheral blood.

i. Megakaryocyte and blood platelets (lower right) from smears of human bone marrow and peripheral blood, stained after exposure to saliva.

j. A typical lymphocyte from a case of chronic lymphatic leukemia showing the maximal number of stained, cytoplasmic bodies.
glycogen-bearing cells, it frequently shifted with fixation to one side of the cell (fig. 1b).

**Lymphocytes.** The lymphocytes of human peripheral blood were for the most part negative, but about 1 in 10 showed a few deep red Schiff-positive cytoplasmic granules which did not seem to be soluble in saliva. In smears from several patients with chronic lymphatic leukemia, the number of lymphocytes showing these granules was both relatively and absolutely increased. In one case, practically all of the lymphocytes contained from 6 to 12 bright red dots (fig. 1j).

The cytoplasm of the lymphocytes observed in stained sections of the spleen and lymph glands of the rhesus monkey was negative.

**Monocytes.** In smears of human peripheral blood these cells exhibited pale pink, diffuse cytoplasmic staining which did not seem to be influenced by saliva. It was our impression from comparing the staining observed in the various cells of blood and bone marrow that this faint staining of the monocytes was a nonspecific reaction.

**Megakaryocytes.** In sections of monkey bone marrow these cells exhibited a multitude of indistinct, dustlike, reddish particles located in more faintly stained cytoplasm. This staining was not affected by preliminary treatment with saliva (fig. 1g).

In sections of human bone marrow the megakaryocytes exhibited somewhat more intense staining. The diffusely pink cytoplasm contained uneven sized, irregularly scattered, red particles. After treatment with saliva, the red material was no longer visible although the pink background tone survived (figs. 1h and i).

In the bone marrow of the rabbit the megakaryocytes stained more intensely than in either monkey or man. Larger red particles filled a good portion of the cells, appearing against a finely punctate reddish background (fig. 1f). Treatment with saliva diminished this staining but by no means abolished it.

**Platelets.** These were only examined in human peripheral blood. The platelets showed a fine red stippling similar to that seen in the megakaryocytes (fig. 1h). This staining failed to occur after exposure to saliva (fig. 1i).

**DISCUSSION**

**Comparison of saliva and malt diastase.** Malt diastase was briefly compared with saliva in reference to its effect on the periodic acid-Schiff reaction and the identification of glycogen. Diastase was tested on several sections of monkey's and rabbit's bone marrow in which neutrophilic leukocytes, neutrophilic myelocytes, eosinophils and megakaryocytes were readily identifiable. Similar to saliva, the use of diastase prevented completely the staining of neutrophilic leukocytes and their myelocytic precursors; but, unlike saliva, it reduced very markedly the staining of both eosinophils and megakaryocytes. These results indicated that saliva and the preparation of malt diastase employed were not completely identical in their action. The latter attacked a wider range of substances than saliva. In connection with other studies we have observed that malt diastase is capable of preventing the staining of basement membranes and reticulum by the periodic acid-Schiff method.

The nature of the periodic acid-Schiff reaction in blood cells. The action of periodic
acid depends on the oxidation of carbohydrate compounds. As a result, aldehydes are formed and these are revealed by their colored reaction with the leukofuchsin of Schiff's reagent. The reaction produced in some types of blood cells by this technic appears to be due to glycogen, but in other blood cells the saliva-resistant substances which stain must contain other kinds of carbohydrates. In the case of the neutrophilic leukocytes and their myelocytic precursors, the stained substance is undoubtedly glycogen in all species examined. In man the megakaryocytes and platelets also appear to contain glycogen. In the monkey, on the contrary, the megakaryocytes are stained but the substance involved does not seem to be soluble in saliva.

It is well established that the periodic acid-Schiff reaction occurs with a variety of acid mucopolysaccharides (particularly epithelial mucus), and it is probable that the reaction is associated with the carbohydrate fraction of these substances. In mast cells, which possess granules containing an acid mucopolysaccharide, the positive reaction may well be explained in such a way. Species differences exist in the staining of mast cells by the periodic acid-Schiff reagents; in man and monkey their granules stain quite intensely, whereas in the rat they are at best very faintly differentiated. This variability suggests species differences in the availability of the carbohydrate radicals. In this connection it is of interest to note that the intense metachromatic staining of the mast cell granules with toluidin blue shows no such species variability. However, metachromatic staining depends upon the presence of sulphate groups rather than upon the carbohydrate moieties of mucopolysaccharides.

Concerning basophilic leukocytes, there is little that we can say at present. The occasional basophils, encountered in normal blood smears of human blood, contain numerous small red dots in their cytoplasm. The fact that we have not identified any similarly stained cells in several smears exposed to saliva suggests that these droplets consist of glycogen. Yet, these findings seem too few to establish this point definitely. If the above result proves to be consistent, it would indicate a difference in the histochemical composition of mast cells and basophilic leukocytes.

In a previous investigation of the blood cells of the rhesus monkey by the Bauer-Feulgen method, Wislocki and Dempsey observed that only the polymorphonuclear neutrophils and their metamyelocyte precursors gave a positive reaction, and this staining was shown to be due to glycogen. Subsequently, Rheingold and Wislocki described the megakaryocytes of human marrow as giving a faint Bauer-Feulgen reaction in contrast to the negative megakaryocytes of the rhesus monkey. Comparison of these previous findings with the present ones indicates that the Bauer-Feulgen technic, as we have carried it out, is not as sensitive as the periodic acid-Schiff reaction. Regardless, however, of the fact that the two methods have not been quantitatively alike, as we have used them, they have corroborated one another in indicating that there are histochemical differences between the megakaryocytes of man and rhesus monkey.

The reaction in the several types of eosinophils does not appear to be due to glycogen. Nor can it be possibly ascribed to an acid mucopolysaccharide when one
considers the fact that the cytoplasm of these cells is alkaline in nature. It is conceivable that it might be attributable to the presence of a neutral mucopolysaccharide. Noteworthy also is the observation that, whereas in the eosinophils of the bone marrow of rabbit and monkey it is principally the granules which are stained, in the eosinophils of human peripheral blood it is the cytoplasmic ground substance which is chiefly colored.

**Summary**

An account is given of the periodic acid-Schiff reaction in the cytoplasm of various normal cells of blood and connective tissue of man, rhesus monkey and rabbit. Saliva treated control sections were used to distinguish glycogen from other reactive substances. The effects of malt diastase were compared briefly with those of saliva. The results of the present study may be summarized as follows (table 1):

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<thead>
<tr>
<th>Cell</th>
<th>Man</th>
<th>Monkey</th>
<th>Rabbit</th>
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<tbody>
<tr>
<td>Basoph. leuk.</td>
<td>*Pos.</td>
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<tr>
<td>Lymphocytes</td>
<td>†Pos.</td>
<td></td>
<td>Neg.</td>
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<tr>
<td>Monocytes</td>
<td>Ft.</td>
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<td>Megakaryocytes</td>
<td>Glyc.</td>
<td>Pos.</td>
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<td>Tissue eosinos.</td>
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<td>Mast cells</td>
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**REFERENCES**

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