
24th Hematological Paper.

By

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A close morphological relation between hemoglobin and peroxidase has been demonstrated by Minagawa1) in a couple of papers published from this Laboratory. He was able to show the close relation in the case of frog's blood much better in the counting chamber peroxidase method of Sato-Shoji2) than in the original copper peroxidase reaction of Sato-Sekiya3). And in the case of human blood, he demonstrated it only by use of the counting chamber peroxidase method; besides, the blood was pathological, while frogs used were all normal, at least not pretreated ones. In the present paper I desire to report further on the relation.

Own Work.

In the case of human blood Minagawa did not see distinctly peroxidase-positive red cells or normoblasts on blood smears, even in pa-

Peroxidase-positive Erythrocytes and Normoblasts

As the peroxidase stain of blood films he used only the original Sato-Sekiya copper peroxidase reaction. I used besides this original method the Tohoku Pediatric Method, a modification of the former. Though I do not go into details here concerning the difference between both methods, one of the most striking differences between them is that red cells are retained on the film in the original method, while in the modification they are completely out of sight on principle. But even in the latter method, normoblasts and young erythrocytes are retained on the film, however fresh the blood smear may be. From this fact it is not difficult to suppose that red cells retained on a fresh smear in the method must be some pathological or abnormal erythrocytes, even if they are not nucleated.

Fig. 1 shows besides two neutrophiles and one lymphocyte one peroxidase-positive red cell. The cell is not nucleated, but evidently pathological, first, because all the other red cells are not seen due to the application of Solution I of the Tohoku Pediatric Method and second, because red cells are not peroxidase-positive under the method. This fact will be shown in the case of an old smear. Even normal red cells on an old air-dried blood smear are not taken away by Solution I of the Method, but kept retained on the film. But they do not take peroxidase stain at all in the Tohoku Pediatric Method. Therefore, the cell in Fig. 1 above referred to is an abnormal red cell. This may indeed have thrown away its nucleus but remains still in a stage of youth; it must be a young erythrocyte, though the cell body is almost unstained with safranin, because a young erythrocyte has, as above stated, a strong resistance to the copper solution. With this result in view, it will be easily presumed that in blood films stained with the original Sato-Sekiya’s peroxidase method, blue erythrocytes are seen among non-stained erythrocytes, then blue cells are pathological or abnormal.

Fig. 2 shows a number of such cells on different blood films as has been illustrated in Fig. 1. They are all non-nucleated, but peroxidase-positive, though the grade of positivity is very different according to cells, probably due to the difference of youth. Some of them are only faintly stained with safranin. With the use of the original Sato-Sekiya’s peroxidase method those red cells are stained blue (peroxidase-positive) too.


* Unripe non-nucleated erythrocytes usually become red stained with safranin (the counter stain of the Tohoku Pediatric Method or of Sato-Sekiya’s original peroxidase method) probably due to the yet basophile property of the cytoplasm.
Fig. 1. Proxidase-positive erythrocyte on a blood film stained with the Tohoku pediatric Method. 2 neutrophiles, 1 lymphocyte (red) and 1 Peroxidase-positive erythrocyte (non-nucleated).

Fig. 2. Different peroxidase-positive erythrocytes seen in blood films from various cases stained with the Tohoku Pediatric Method.

Fig. 3. Different peroxidase-positive normoblasts in the smear of bone marrow juice from a case of myelosis under the copper peroxidase reaction by the use of Tohoku Pediatric Method. The juice was obtained by sternal puncture.

Fig. 4. Different peroxidase-positive normoblasts in the smear of bone marrow juice from a case of sepsis under the copper peroxidase reaction by the use of Sato-Sekiya's original method. The juice was obtained postmortal.
The above described fact shows that even on blood smears peroxidase-positive red cells in human blood can be shown by the Tohoku Pediatric Method or Satô-Sekiya's original method. It is to be noted here that, if I have stated that peroxidase-positive red cells shown in Figg. 1 and 2 must have thrown away their nucleus, it does not follow by any means that normoblasts should be peroxidase-positive. As our experience with the Tohoku Pediatric Method hitherto has shown, normoblasts on blood films are always peroxidase-negative (cf. Fig. 1 in the paper of Shiraishi and I\textsuperscript{5}). But when I came across a case of myelosis, I examined the bone marrow juice of the case. There were a number of peroxidase-positive normoblasts and erythrocytes as well as peroxidase-negative ones. The peroxidase-positive normoblasts are shown in Fig. 3. The cell body of normoblast is very faintly red with safranin.

Fig. 4 shows again normoblasts on a smear of bone marrow juice, but the case was sepsis and the stain was the original Satô-Sekiya copper peroxidase reaction, not the Tohoku Pediatric Method. They appear more strongly peroxidase-positive than those in Fig. 3, but this is due to the difference of the methods. The cell body is stained peculiarly brownish-yellow.

\textsuperscript{5} T. Suzuki and Sh. Shiraishi, Tohoku J. Exp. Med., 1936, 29, 527.
Fig. 5 shows one lymphocyte and one peroxidase-positive normoblast on a blood smear. The case was agranulocytosis. This was my first opportunity of meeting with a peroxidase-positive normoblast, as far as blood films are concerned.

Now what is the difference of peroxidase-positive and -negative normoblasts as to the age? It is difficult to say anything definite. Peroxidase is closely related to hemoglobin. A normoblast which has reached the stage about to throw away its nucleus may have been furnished with almost normal cell body, then the body may be too ripe to show any distinct peroxidase reaction, while in a very immature normoblast, peroxidase reaction will be yet very weak or negative. As to the relation between hemoglobin and peroxidase I shall relate further by way of appendix.

Appendix.

Normal Red Cells with Blue Peroxidase Reaction.

Those who look at Fig. 6 will think that it is oxidase reaction or copper peroxidase reaction of blood leucocytes without the counterstain. But these cells are only normal human red cells (normal rabbit's red cells also can show a similar picture).

I modified Cooke's method with an addition of acetic acid, though the recipe is different in other respects too. The time is very different too, because in Cooke's method it is as long as 20 minutes, while in my own it is only 5 seconds. The picture reminds one, as above mentioned, of copper peroxidase reaction of leucocytes. This may, I think, contribute to show a close relation between hemoglobin and peroxidase.

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6) My own modification of Cooke's method is described here:

The Reagent used:—
- Benzidine 0.1 grm.
- $H_2O_2$ (3%) 30.0 c.c.
- Acetic acid (30%) 14 gtt.
- Absolute alcohol 70.0 c.c.

Technique used:— Apply the reagent on an air-dried blood smear for about five seconds, and then wash thoroughly with water.


Cooke's method is quoted here:—

The reagents are benzidine 0.01 per cent in 80 per cent alcohol and hydrogen peroxide, and the reagents are applied on air-dried blood films and the reaction is allowed to continue for twenty minutes.
However, a word is necessary before a definite conclusion is drawn. The peroxidase reaction which happens to occur in the system of red cells in the case of the copper peroxidase reaction will contribute very much to the relation between hemoglobine and peroxidase. But in Cooke's experiment and in my part of the experiment related in this appendix, a further experiment on the reagents may from the chemical point of view be necessary, so that this will be made a problem for future investigation.

Conclusions.

1. Peroxidase-positive red cells of human blood may be shown on blood films by use of the Tohoku Pediatric Method, a modification of Sato-Sekiya's original copper peroxidase reaction, though they can also be shown by the original method.

2. Normoblasts and young non-nucleated red cells of human blood can be peroxidase-positive in the copper peroxidase reaction.

3. A method is described, by which normal human or rabbit's red cells on a blood smear will be made bluely peroxidase-positive.