The Shortest Peroxidase Stain Time and the Mean Cellular Peroxidase Content of Blood Leukocytes*

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A new modification of the shortest peroxidase stain time (SPST) method for blood leukocytes, originally studied by Suzuki, based on the Sato-Sekiya's stain, was described. This method can be used for a quantitative determination of the peroxidase activity of leukocytes. The rationale of the method was demonstrated.

The mean cellular peroxidase content (MCP) of myelogenous leukocytes of ten hospitalized patients estimated by the present SPST method, was equivalent, in average, to $(15.29 \pm 3.71) \times 10^{-8}\gamma$ of horseradish peroxidase (HRP) in eosinophils, $(8.18 \pm 4.08) \times 10^{-8}\gamma$ of HRP in neutrophils and $(4.97 \pm 2.72) \times 10^{-8}\gamma$ of HRP in monocytes, respectively.

The copper peroxidase reaction method was originally devised by Sato and Sekiya for the purpose of differentiating between myelogenous and lymphatic cells.^{10,21,24,38} It became soon apparent that the peroxidase activity was likely to be essential for a normal life process as suggested by the observation of the loss of this enzyme activity in cases of encephalitis lethargica (Economo type).^{22,23} Since then, some quantitative alterations of peroxidase activity of leukocytes, estimated by the use of modifications of Sato-Sekiya's original method, i.e., the shortest peroxidase stain time (SPST) method^{6,7,32-34} and the counting chamber method,^{11,25} have been studied in their laboratory and others.²⁸

The purpose of the present paper is to describe a new method for the SPST test, by which the beginning reactivity of peroxidase of individual blood cell under carefully standardized conditions of Sato-Sekiya's peroxidase stain, or the shortest time (in seconds) during which a neutrophil, a monocyte or an eosinophil has begun to take peroxidase stain, can be estimated more accurately than by the original method. The rationale of the method was demonstrated by experiments. And it was shown that the cellular peroxidase content in gravimetric units of a peroxidase preparation could be estimated on the basis of SPST of these leukocytes.

Received for publication, February 14, 1966.

* Read at the 67th Meeting of Japan Pediatric Society, held in Sapporo, June 19, 1964 and also at the 19th Meeting of the Tohoku Hematological Society, held in Sendai, February 21, 1965.

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MATERIALS AND METHODS

Capillary blood was taken either from ear lobe, finger, toe or sole. Blood smears were prepared by the cover glass technic and were air-dried well. The cover glass of 18×18 mm,² in size was used. A large number, at least thirty, of blood smears were prepared from each patient, so that a good homogeneous smear, neither too thick nor too thin, might be chosen out of them. Blood smears were tested within a definite time, always within four hours after blood taking. The reaction was tested always at the room temperature.

Reagents The reagents of Sato-Sekiya's copper peroxidase stain were used with a slight modification.

Solution A: Copper sulfate solution. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.1g., NaCl 0.75 g., distilled water 100 ml.

The copper sulfate solution in the present method is more diluted than in the original method to slow down the reaction process. Sodium chloride is added in order to adjust the ionic strength of the solution.

Solution B: Benzidine peroxide solution. Rub 0.5 g of benzidine base in a mortar with a few drops of water. Add 500 ml of water and filter. Then add 0.5 ml of 3% hydrogen peroxide to the filtrate. Keep in brown bottle. It is advisable to prepare solution B fresh once a week for the purpose of the present test.

Solution C: 1% aqueous safranin-O solution for counter stain.

Technic Apply 0.3 ml of solution A on to a fresh air-dried blood smear for one minute. (Do not pour it off.) Then add 0.1 ml of solution C and wait for five minutes. (Do not pour it off.) Then add 0.5 ml of solution B to the mixture above described, timed by use of a metronome. When a definite time (seconds) has elapsed, wash the staining solutions off thoroughly with running water. The smears, thus stained, are air-dried and examined microscopically with oil-immersion. A magnification 7×100 is used.

How to record the results of SPST tests? There is a range of the peroxidase reacting time, usually from one to ten seconds, during which the peroxidase reaction is allowed to proceed. One hundred consecutive blood neutrophils, for example, on each smear were examined under microscope with oil immersion to determine the percentage of peroxidase-positive cells. Here the peroxidase-positive cell was such a one as had first taken peroxidase stain, regardless of the size of peroxidase granule, either solitary or in group (cf. Fig. 1). The SPST curve was constructed by plotting on graph paper the percentage of peroxidase-positive cells on each smear against the corresponding peroxidase reacting time (cf. Fig. 2). In the early stage of the present investigation, the results of SPST tests were recorded as the SPST curves. But, as the study progressed, it became clear that the SPST could be converted into gravimetric units of the enzyme.

Fig. 1. A: Blood neutrophils stained with the original method of Sato-Sekiya's peroxidase stain. All the cells are loaded with a great number of peroxidase granules.

B: Blood neutrophils stained with the present SPST method, showing the beginning reactivity of peroxidase of the cells. The cells a and b, peroxidase-positive forms, have few peroxidase granules, while the cell c, a peroxidase-negative form, has no peroxidase granule at all.

Fig. 2. The SPST curves of blood eosinophils (Eos.), neutrophils (Neut.) and monocytes (Monoc.). Note the difference of SPST curves among various kinds of leukocytes. The blood was drawn from a 15-year-old boy, case No. 102 in Table 4 (cf. Table 4), who was under thyroid medication for his hypothyroidism, in a heparinized syringe. Smears were prepared from the buffy coat after centrifugation.

And thence, the results of SPST tests were recorded as the mean cellular peroxidase content (MCP) of each type of myelogenous leukocytes, in the way which will be described in the present paper.

N.B. Any student can try the original Sato-Sekiya's peroxidase stain with success within a few hours of his trial, but the present SPST method is technically much more difficult and it may take one a month or so to master it.

Not infrequently, the cells near the edges of a blood smear may, for some unknown reason, show a more or less prolonged SPST than the cells in the central parts. So it

is better to examine the cells in the central parts of a smear.

It is preferable to complete the microscopic examination of stained films within a few days after staining, though the peroxidase stain usually remains unchanged for a week or longer, if stained smears were kept at room temperature in a covered box.

The SPSTs of peroxidase solution in known concentrations

1. The effect of the concentration of horseradish peroxidase on the reaction between solutions A (copper sulfate) and B (benzidine and hydrogen peroxide): The following experiment was performed to see the effect of peroxidase on the reaction between solutions A and B.

About 0.004 ml of (water or enzyme added) solution A (mixture of three parts of 0.1% copper sulfate, i.e., solution A, and one part of water or aqueous solution of peroxidase,* 500 to 10 γ /ml) and about 0.005 ml of solution B were placed apart on the glass surface of a counting chamber or a tissue culture slide.†

TABLE 1. *The determination of SPSTs of horseradish peroxidase (HRP) solutions in known concentrations*

Solution A				SPST*
Enzyme (H.R.P.) solution		Water	0.1% Copper sulfate solution	
Concentration	Amount			
γ /ml	ml	ml	ml	
500	0.1	0	0.3	1.0± 0.1
200	0.1	0	0.3	2.3± 0.2
100	0.1	0	0.3	3.4± 0.3
50	0.1	0	0.3	5.3± 0.5
30	0.1	0	0.3	8.5± 0.7
20	0.1	0	0.3	10.8± 0.8
10	0.1	0	0.3	15.9± 0.9
	0	0.1	0.3	140.0±10.0

* At least three determinations of the SPST were made for each concentration of the enzyme solution.

The reaction mixture contained approximately 0.004 ml of solution A and 0.005 ml of solution B (benzidine and hydrogen peroxide).

The cover glass was next placed on the chamber. The slide was examined under a microscope (not with the oil immersion in this case) with a magnification of 7 $\times$ 10 or 7 $\times$ 20. When two drops of solutions A and B came into contact and both solutions were partly mixed, a stop watch was started and the time required by the mixture to develop any blue precipitate, recognizable under the microscope, was measured. The results were summarized in Table 1 and Fig. 3.

* Horseradish peroxidase (Nutritional Biochemical Co., Cleveland, Ohio, USA) was used.

† Hiraki's tissue culture slide, No. 1, depth 0.2 mm, Nihonrinshokikai Co., Tokyo, was used.

Fig. 3. The relationship between the concentration of enzyme (HRP) solution and the SPST.

With this technic, the time required by the mixture, at the site of contact of solution A (containing no peroxidase) and solution B, to develop any blue precipitate was prolonged to even 140 seconds or more. But, in the presence of peroxidase in solution A, the time was greatly shortened, i.e., up to 1 second or less (cf. Fig. 3). And the time required by the reaction mixture of solutions A and B to begin to develop any blue precipitate (at the site of contact of both solutions) could be correlated with the concentration of peroxidase in solution A (cf. Fig. 3).

In a parallel study with the same technic as described above, it was shown that, in the absence of copper sulfate, peroxidase reacted with solution B to develop brown precipitates, but no blue precipitates were produced, unless much higher concentrations of peroxidase were employed.

2. The effect of the concentration of myeloperoxidase on the reaction between solutions A and B: Myeloperoxidase could substitute horseradish peroxidase in the oxidation of benzidine under the conditions of the method employed in Fig. 3. The myeloperoxidase solution used in the present experiments was prepared from canine leukocytes by the method of Agner^{1,2} and had ratios absorbancy 430/absorbancy 280 of 0.62 and absorbancy 430/absorbancy 390 of 1.62 and its optical density at 430 $m\mu$ was 0.22. The effect of the concentration of myeloperoxidase (MPO) on the reaction between solutions A and B is shown in terms of SPST in Table 2 and Fig. 4. (cf. Table 2 and Fig. 4).

TABLE 2. *The determination of SPSTs of myeloperoxidase (MPO) solutions at various concentrations**

Solution A				SPST†
MPO solution		Water ml	0.1% Copper sulfate solution ml	
Concentration, % of original solution	Amount ml			
100 %	0.1	0	0.3	7.0± 0.5
75 %	0.1	0	0.3	9.5± 0.7
50 %	0.1	0	0.3	12.6± 2.6
30 %	0.1	0	0.3	21.4± 2.1
20 %	0.1	0	0.3	24.0± 3.0
10 %	0.1	0	0.3	45.0± 5.0
5 %	0.1	0	0.3	86.5± 6.5
	0	0.1	0.3	145.0±10.0
100 %	0.1	0.3	0	17.0± 3.0‡

* The canine leukocyte myeloperoxidase (MPO), prepared by the method of Agner,^{1,2} was used. The preparation had A430/A280 ratio of 0.62 and A430/A390 ratio of 1.62, and its optical density at 430 m μ was 0.22. This preparation was dissolved in water and used as a 100% enzyme solution. Dilution of the enzyme solution was made with distilled water.

† At least three determinations of the SPST were made for each concentration of the enzyme solution. The reaction mixture contained approximately 0.004 ml of solution A and 0.005 ml of solution B (benzidine and hydrogen peroxide).

‡ Brown precipitates, instead of blue precipitates, developed at the time shown in the table.

Fig. 4. The relationship between the concentration of enzyme (MPO) solution and the SPST.

The Shortest Peroxidase Stain Time of Leukocytes

The results of above described observations support the view that the present SPST method is rational and the SPST of blood leukocytes can reflect, to some extent, the concentration of peroxidase in the cells. It is needless to add that blood leukocytes are not homogeneous in their structure. They contain, in addition to peroxidase itself, other substances, which may influence the peroxidase activity in some way or other, as will be discussed later.

Conversion of SPST to gravimetric units of horseradish peroxidase (cf. Table 3):

When the SPSTs, determined as shown in Table 1, were plotted against the concentration of horseradish peroxidase (HRP) on a log/log scale, a linear relation was obtained (cf. Fig. 3). Under standard conditions, this relation can be used to convert SPSTs into gravimetric units of peroxidase (HRP) as equivalents of myeloperoxidase activity in the cell, assuming that the mean cellular volumes of neutrophils and monocytes are 450 $\text{cu}\mu$ and 470 $\text{cu}\mu$ respectively on the basis of the data reported³⁶ (cf. Table 3).

TABLE 3. *The table for conversion of SPST to gravimetric units of HRP*

SPST	HRP conc.	For neutrophils and eosinophils, HRP	For monocytes, HRP
sec	γ/ml	$\gamma/450\text{cu}\mu$	$\gamma/470\text{cu}\mu$
1"	500	22.50×10^{-8}	23.50×10^{-8}
2"	230	10.35 "	10.81 "
3"	130	5.85 "	6.11 "
4"	70	3.15 "	3.29 "
5"	58	2.61 "	2.73 "
6"	45	2.03 "	2.12 "
7"	35	1.58 "	1.65 "
8"	28	1.26 "	1.32 "
9"	24	1.08 "	1.13 "
10"	20	0.90 "	0.94 "
11"	17	0.77 "	0.80 "
12"	15	0.68 "	0.71 "
13"	13	0.59 "	0.61 "
14"	12	0.54 "	0.56 "
15"	11	0.50 "	0.52 "
16"	10	0.45 "	0.47 "
17"	9	0.41 "	0.42 "
18"	8	0.36 "	0.38 "
19"	7.5	0.34 "	0.35 "
20"	7	0.32 "	0.33 "

For example, if the SPST of a neutrophil is three seconds, the peroxidase activity of the cell, determined from the standard curve in Fig. 3, would be equivalent to that of enzyme solution containing 130 γ HRP per ml, (or $5.85 \times 10^{-8}\gamma$ of HRP per 450 $\text{cu}\mu$). The peroxidase content of the neutrophil may thus be calculated as equivalent to $5.85 \times 10^{-8}\gamma$ of HRP.

The mean cellular peroxidase content

Multiply each amount of the enzyme (in γ of HRP) by the number (in percentage) of cells found with that amount of enzyme, add all the products, and divide by the total number (in per cent) of cells counted.

Sample calculations:

1. If the totals of peroxidase-positive forms of neutrophils at each reaction time are 5% at 1 sec, 25% at 2 sec, 71% at 3 sec and 100% at 4 sec, respectively under the present standard method, then the mean cellular peroxidase activity (MCP) can be calculated as follows.

$$\text{MCP of the neutrophils, in } 10^{-8} \times \gamma \text{ of HRP} = [(22.50 \times 5) + \{10.35 \times (25 - 5)\} + \{5.85 \times (71 - 25)\} + \{3.15 \times (100 - 71)\}] \div 100 = 6.80$$

2. If the totals of peroxidase-positive forms of monocytes at each reaction time are 0% at 1 sec, 9% at 2 sec, 25% at 3 sec, 41% at 4 sec, 51% at 5 sec, 71% at 6 sec, 84% at 7 sec, 89% at 8 sec, and 91% at 9 sec respectively under the present standard method, then the mean cellular peroxidase activity (MCP) can be calculated as follows.

$$\text{MCP of monocytes, which became peroxidase-positive within 9 sec of reaction time, in } 10^{-8} \times \gamma \text{ of HRP} = [(10.81 \times 9) + \{6.11 \times (25 - 9)\} + \{3.29 \times (41 - 25)\} + \{2.73 \times (51 - 41)\} + \{2.12 \times (71 - 51)\} + \{1.65 \times (84 - 71)\} + \{1.32 \times (89 - 84)\} + \{1.13 \times (91 - 89)\}] \div 91 = 347.70 \div 91 = 3.82$$

MCP of monocytes may thus be expressed as equivalent to $3.82 \times 10^{-8} \gamma$ of HRP or less. If the maximum peroxidase-positivity* is known, then the MCP of monocytes as a whole may be determined more accurately. MCP of monocytes as a whole, namely, including both peroxidase-positive and peroxidase-negative forms, may be calculated as follows, assuming that the percentage of peroxidase-positive forms of monocytes reached the maximum value,* for example 97% at 10 sec of the reaction time.

$$\text{MCP of monocytes as a whole, in } \times 10^{-8} \gamma \text{ of HRP} = [347.70 + \{0.94 \times (97 - 91)\}] \div 100 = 3.53$$

As described already, the shortest time required to develop any blue granules (SPST) in the reaction mixture containing enzyme preparation of known concentration was measured by the microscopic observation, in which lower magnification was used for the sake of convenience than in the case of determination of SPST of leukocytes in blood smear. Therefore, the actual peroxidase content of leukocytes would be less than the value obtained from the above table (Table 3), although there will be a parallel relation between these two values.

Notwithstanding such drawback or uncertainty, the present SPST method

* The maximum peroxidase-positivity of monocyte is defined as the maximum percentage of peroxidase-positive forms of monocytes determined under the Sato-Sekiya's original method.

is still the most useful method at present for the determination of peroxidase activity of leukocytes, because this method has the advantage of evaluating the enzyme activity of leukocytes 'individually' under the microscope.

RESULTS

By the use of the present method, it is possible to determine the SPSTs of neutrophils, eosinophils and monocytes, respectively. An example is illustrated in Fig. 2. In general, the SPST of eosinophils is much shorter and that of monocytes is much longer than that of neutrophils, as might be expected.

The individual cells, even among the same kind of granulocytes, differed in SPST slightly from one other. Normally, some neutrophils, for example, of a smear begin to take stain a few seconds earlier or later than the majority of population. During the first one to two seconds, neutrophils seem to take stain rather reluctantly, but then, in the following one to two seconds, they will take stain rapidly, so that now the majority of neutrophils have begun to take stain. A steep slope of SPST curve (cf. Fig. 2) was thus formed usually between the first two and four seconds of the stain time. A few cells per hundred neutrophils, however, may require one to two seconds more to take their first stain. So the above-mentioned slope may be followed by a gentle slope ending at five or six seconds of the reacting time.

As described already, these SPSTs can be converted into gravimetric units of HRP, since the SPST is inversely proportional to the enzyme concentration. The results of SPST test on ten hospitalized patients, in which cases all the three myelogenous leukocytes, i.e., neutrophils, monocytes and eosinophils were observed, are given in Table 4. The mean cellular peroxidase (MCP) of these cells were $(8.18 \pm 4.08) \times 10^{-8} \gamma$ of HRP in neutrophils, $(4.97 \pm 2.72) \times 10^{-8} \gamma$ of HRP in monocytes and $(15.29 \pm 3.71) \times 10^{-8} \gamma$ of HRP in eosinophils, respectively.

DISCUSSIONS

The clinical studies of Sato-Sekiya's peroxidase stain has been reviewed by Sato.²⁶ The present SPST method can be a new approach for further studies, by the use of Sato-Sekiya's stain, on the clinical problems concerning peroxidase of blood leukocytes. Hitherto, there is a paucity of literature dealing with the quantitative determination of peroxidase activity of blood leukocytes. Schultz and Kaminker³⁰ determined the peroxidase content in gravimetric units of myeloperoxidase of the normal human leukocytes. Arakawa and Katsushima^{14,15} have reported new methods for colorimetric estimation of peroxidase in serum or plasma and for that of blood leukocytes. By the latter, the peroxidase content per single myeloid cell in the peripheral blood can, they state, be estimated, but one must be careful in reading the data because peroxidase activity varies greatly

TABLE 4. *SPSTs and peroxidase content in*

No. of case	Name of patient	Age	Sex	Date of test	Diagnosis	SPST of neutrophils; Percentages of peroxi- dase-positive neutrophils and their reacting time (sec)				
						1"	2"	3"	4"	5"
101	M.T.	3y9m	F	30,I,63	Virilizing adreno- cortical tumor and hemorrhagic chicken- pox	0	43	100		
102	H.H.	15y	M	6,II,63	Goitrous hypothyroidism	5	25	71	100	
103	S.H.	8y	F	11,III,63	Goitrous hypothyroidism	2	78	100		
104	M.K.	7y	M	4,III,63	Congenital hypothyroidism	8	89	100		
105	M.S.	4y6m	F	15,III,63	Congenital hypothyroidism	4	82	100		
106	T.C.	13y2m	F	19,IV,63	Struma (under mercazol therapy)	0	20	93	100	
107	F.K.	4y	F	7,V,64	Infectious hepatitis	0	8	75	100	
108	T.S.	12y5m	M	24,VI,64	Anemia	0	8	100		
109	S.N.	0y8m	M	9,III,64	Hereditary spherocytosis	6	100			
110	C.T.	7y	F	3,VII,64	Fever and splenomegaly	2	27	100		
						Mean $\pm$ S.D. = 8.18 ± 4.08				

M: mean,

 δ : standard deviation

$$\delta = \sqrt{\frac{\sum fd^2}{n-1} - \omega^2}$$

according to the kind of myeloid leukocytes as was shown by the present author (cf. Fig. 2 and Table 4). A comparison of these different assay methods is shown in Table 5 (cf. Table 5).

In Table 4, the MCP of neutrophils, monocytes and eosinophils showed a considerable variations among cases. The reason why such variations of MCP occur remains to be studied.

Myeloperoxidase was isolated from leukocytes or chloroma tissue by Agner^{1,2} and Schultz.²⁹ The mechanism of enzyme action of peroxidase was studied in detail by Chance.⁵ Methods of peroxidase assays were reviewed by Maehly.²⁰ The present SPST method is useful in a clinical assay of the overall activity of

neutrophils, eosinophils and monocytes

MCP of neutrophils, ($\times 10^{-8}$ γ of HRP)	SPST of monocytes; Percentages of peroxidase- positive monocytes and their reacting time (sec)										MCP of monocytes * ($\times 10^{-8}$ γ of HRP)	SPST of eosinophils (sec)			MCP of eosinophils ($\times 10^{-8}$ γ of HRP)
	1"	2"	3"	4"	5"	6"	7"	8"	9"	10"		1"	2"	3"	
7.79	0 15 83 90 92 95										6.45 or less	48	95	100	15.96
6.80	0 9 25 41 51 71 84 89 91										3.82 or less	91	96	100	21.23
9.60	0 2 38 64 76 76 90 94 94										4.19 or less	46	100		15.94
10.83	7 20 46 83 85 86 90 93 95 98										6.21 or less	74	98		19.52 or less
10.04	0 28 70 82 84 90 93(90) (91)										6.69 or less	58	100		17.40
6.56	0 2 17 72 78 84 88 94										3.51 or less	25	78	100	12.39
5.54	0 0 15 40 60 — 83 — — 85										2.95 or less	36	100		14.72
6.21	0 5 63 81 91 — 93 94 — 95										5.04 or less	5	43	100	8.39
11.08	4 8 16 34 46 56 56 64										4.84 or less	18	100		12.54
7.31	0 13 72 94 96										6.03 or less	37	100		14.85
Mean $\pm$ S.D. = 4.97 ± 2.72											Mean $\pm$ S.D. = 15.29 ± 3.71				

* The maximum positivity of monocytes was not determined in the present series of cases.

peroxidase contained in blood neutrophils, eosinophils and monocytes, respectively.

Chance⁵ stated that the peroxidase had an activity towards ferrocytochrome c and that inasmuch as peroxidase was specific for ferrocytochrome c and could participate in intracellular coupled oxidations, this enzyme could provide an auxilliary path for biological oxidations. According to Klebanoff,¹⁷⁻¹⁹ a number of substances of biological importance, i.e., reduced diphosphopyridine nucleotide, reduced triphosphopyridine nucleotide, ferrocytochrome c, epinephrine, norepinephrine, uric acid, thyroxine and ascorbic acid, can be oxidized by the peroxidase system. According to Sato and his group,^{26,34,35,37} methylglyoxal which may occur in cases of B₁ avitaminosis has an inhibitory effect on the peroxidase

TABLE 5. *The comparison of different peroxidase assay methods of blood leukocytes*

Authors	1963 Katsushima ^{15,16}	1962 Schultz ²⁰	1965 Present author		
Peroxidase Activity	5.53×10^{-8} γ of HRP/cell	7.1×10^{-8} γ of MPO/cell	$(15.29 \pm 3.71) \times 10^{-8}$ γ of HRP/cell	$(8.18 \pm 4.08) \times 10^{-8}$ γ of HRP/cell	$(4.97 \pm 2.72) \times 10^{-8}$ γ of HRP/cell
Kinds of leukocytes	Average of all myeloic leukocytes	Average of all leukocytes	Eosinophils	Neutrophils	Monocytes
Method of observation	Spectrophotometry	Spectrophotometry	Microscopic observation		
Materials	Leukocyte suspension	Leukocyte homogenate	Blood smear		
Substrate and H donors	H ₂ O ₂ Benzidine α -Naphthol	H ₂ O ₂ Guaiacol	H ₂ O ₂ Benzidine		
Reaction time	long (30 minutes)	short (seconds)	short (seconds)		

reaction of both leukocytes and milk. A prolongation of the SPST of blood neutrophils in cases of beriberi is corrected by the administration of vitamin B₁. Thus many factors may play roles in the utilization of peroxidase. Various iron enzymes in iron deficiency have been studied by Beutler,⁴ Gubler⁹ and Dallman.⁸ Peroxidase is a hemoprotein and so iron should be essential in biosynthesis of this enzyme. Therefore, it is reasonable to think that iron might play an important part in the occurrence of variations of SPST and MCP of neutrophils, monocytes and eosinophils, respectively. In this connection, further studies on SPST and MCP of these cells remain to be desired.

The problems of 'peroxidase-negative neutrophils' were discussed by Sato, Arakawa and others in a recent issue of this journal.^{3,13,27} The present author reported, in 1954, a qualitative abnormality of the peroxidase granules of myelogenous leukocytes, 'the congenital gigantism of peroxidase granules'.¹² It would be very interesting to use the present method to see if any quantitative, as well as qualitative, abnormality of peroxidase by way of SPST and MCP might be encountered in such a disease.

Acknowledgment

The author is grateful to Drs. H. Wako, and Ts. Arakawa for their kind advices and to the late Dr. A. Sato, Professor Emeritus of Tohoku University, for his suggestions.

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