Three Unexpected Products of Peroxidase Reaction*;  
Brain Site of Lesion, Beriberi Poison  
and New Disease

By

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In 1922 I published with Sekiya a new peroxidase reaction (often quoted in the literature as Sato and Sekiya's peroxidase stain or as Sato's peroxidase reaction). As my proper object of research was infantile nutrition, I made light of our own peroxidase reaction— which was then used only for the differentiation of myelocytic and lymphatic leucocytes in leukemic cases (then very rare). But in course of time it gave me a hint to finding 1) the localisation of a brain lesion, 2) the poison of infantile beriberi (methyl glyoxal 1934; strictly chemical identification 1950) and 3) a new disease (Chediak-Higashi's Disease).

In 1922 I devised in collaboration with Sekiya a new peroxidase reaction. The only reason for this new device was the high price of one (—dimethyl-paraphenylene diamine) of the two reagents of the oxidase reaction (Schultze) — to say nothing of its speedy deterioration when dissolved.

At that time I did not intend to make a point of studying blood in spite of our device of the new peroxidase reaction, because then almost all infants of summer diarrhea admitted into our Hospital and about half the number of infantile cases of pneumonia died. Morphological hematology would not contribute a bit towards curing these diseases. My research ought of course to be infantile nutrition — a chemical problem.

I did not, however, discard our own peroxidase reaction utterly, because it differentiated lymphocytes (red without blue granules) and monocytes (red with blue granules) quite easily, so easily that beginners felt no difficulty in the differentiation even in bad smears. So the reaction was daily used in our Laboratory in the routine hematological work.

In the meantime we had in our Laboratory been preparing some micro methods for the coming study of infantile nutrition. (The device of micro methods may seem to be an absurd work for the clinician, but many micro methods...
were at that time half-micro methods, so that they were not "micro" enough for infant study). And I had been groping about in the dark, thinking where I should attack the problem chemically.

Now, while I was continuing to make light of our own peroxidase reaction, this produced in the course of time three unexpected results, which will be related in the following:

I. The First Product of the Peroxidase Reaction: the "Striatal Blood Syndrome\(^2,3\)" (or Picture)"

Though I did not, as stated above, make much of our own peroxidase reaction, I did not neglect to stain blood smears from every case admitted into our Department with our peroxidase stain, because I wanted conscientiously to know whether our new reaction should always prove a good substitute for the original oxidase reaction.

One day in 1922, I came to examine blood smears of an acute case of lethargic encephalitis Economo\(^3\). To my great surprise, the peroxidase reaction was negative, that is, not a single myeloic leucocyte was peroxidase-positive. All in the Laboratory who had become skilful in the stain tried and tried, but in vain. Then I tried the original oxidase reaction, wondering whether it should turn out positive or negative. The oxidase reaction was positive, and normally positive. I doubted the evidence of my eyes. How badly I wished at that time that our substitute reaction could have stained the smears from the Economo case peroxidase-positive!

Then I tried our reaction on the other acute case of the Economo disease — there were then two children of acute encephalitis in our Department. This second case was peroxidase-negative too, the oxidase reaction being normally positive.

Later examinations showed the following relation between the two reactions (cf. Table I).

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Oxidase</th>
<th>Peroxidase</th>
<th>Case 2</th>
<th>Oxidase</th>
<th>Peroxidase</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Course of disease</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Recovery →</td>
<td>Positive</td>
<td>Positive</td>
<td>Death →</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Death itself does not make the peroxidase reaction negative; leucocytes of the corpse are always peroxidase-positive. It was evident that the negative peroxidase reaction had something to do with Encephalitis of Economo type. (Patients of Japanese Encephalitis are peroxidase-positive).

After some long time I succeeded in producing the same picture — later I suggested the name; “the Striatal Blood Syndrome” (Syndrome, because of positive oxidase and negative peroxidase reactions) — in the rabbit. I had to use many, many rabbits for this purpose because of the two conditions necessary for a successful production of blood picture: 1. The animal operated on must outlive the operation at least a few days; and 2. the operation must destroy the bilateral parts of the “peroxidase centre” completely. Either condition was difficult to fulfill. Most of the rabbits operated on, which had outlived the operation, failed to develop the “Striatal Blood Picture” due to a much too sparing operation. And the animals with a too aggressive operation died immediately without living long enough to develop the blood picture, though it was of course utterly unknown, whether or not the operation was successful.

At first I did not expect at all that a mere brain puncture might produce the “Striatal Blood Picture”, because the “poison” produced in lethargic encephalitis Economo would be the cause for that peculiar blood picture. I was very much surprised at the result that the bilateral “peroxidase puncture” did produce the blood picture.

That the “Striatal Blood Syndrome” was not due to encephalitis Economo (or the poison produced), but to the brain lesion itself was indubitable on the basis of the above mentioned animal experiment. Later it was clinically proved; the “Striatal Blood Syndrome” was reported by Simmel in an adult case of severe chorea, not affected by Economo encephalitis. The following cases presenting the peculiar blood picture, later reported also in Europe, were not cases of the encephalitis either; they were cases of “Blitzkrampf” and syphilis. Animal experiments concerning the blood picture were also reported by Italian authors.

I shall not go further into the details of this blood picture, because the paper of Mascher was an almost exhaustive treatment of the literature of the picture up to 1933.

“Contra-striatal Blood Picture” (1939) or “Anencephalic Blood Picture” — I suggested the name for the blood picture somewhat contrary to the “Striatal Blood Syndrome”. These blood pictures are compared with each other in the following table (cf. Table II).

The “Contra-striatal Blood Picture” was also accidentally found. When Kinugawa in the Pharmacological Department (of the late Prof. Yagi at that time) was performing the decerebration (in the meaning of removal of bilateral hemispheres) in rabbits, I obtained some blood specimens immediately after the operation, because the removal of the hemispheres — the cranial parts of the
TABLE II. Comparison between the "Striatal" and "Contra-striatal" Blood Pictures

<table>
<thead>
<tr>
<th>Oxidase</th>
<th>Normal</th>
<th>&quot;Striatal blood picture&quot;</th>
<th>&quot;Contra-striatal blood picture&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>+</td>
<td>-</td>
<td>#</td>
</tr>
</tbody>
</table>

"peroxidase centre" — would, I believed then, never affected the normal peroxidase picture. The actual result of the reaction presented quite a normal picture; the peroxidase of leucocytes was normal.

Much later, I asked Kuribayashi in our Pediatric Department to do the same operation on rabbits as Kinugawa did in order to see how leucocytes morphologically — by use of the Giemsa stain — would change, while the peroxidase reaction remained the same (— this very result did I see in the above mentioned instance). Kuribayashi's result was quite astonishing. The Giemsa picture remained the same until death of the animals, while peroxidase reaction became more and more intense as time went on (cf. Picture in Kuribayashi's11 paper).

I became for the first time convinced that it will take time a few days at least after the respective operation — for the "Striatal Blood Picture" as well as for the "Contra-striatal Blood Picture" to be fully developed as will be schematically seen from Table III.

As to the mechanism how such a rather strange peroxidase picture should occur, it was described in Sato and Kuribayashi's10 paper.

Remarks: 1. At first I thought that the brain lesion causing the "Striatal Blood Picture" — the site of the "peroxidase centres" — ought to lie in the striatal system, but it must be in the middle brain (in the bottom of ventriculus III) caudal of Nuclei oculomotoria. But wishing that many a hematologist would test the peroxidase reaction in every case with clinically striatal symptoms, I retained the name: "striatal"*. — So saw Simmel5 our "Striatal Blood Syndrome", as above cited, in a grave case of chorea.

2. It was a great regret that the "Striatal Blood Syndrome" was not discussed in Japan at that time, probably because, when I reported the blood syndrome, there was no more acute case of the disease in Japan. Those who were interested in the strange blood syndrome and tested the peroxidase stain failed of course to see the syndrome, because the patients tested were highly probably only convalescents of the disease (cf. Table I).

Conclusion to Paragraph I. If had used the original oxidase reaction alone — without using my own new substitute reaction (which I thought as of the exactly same biological significance) — , I could never have found the "Striatal Blood

* I am grateful to Prof. Schulten so much more for mentioning "Striäres Blut-syndrom" in two places in his text-book21, so that a hematologist may test the peroxidase reaction in a case with acute striatal symptoms.
Syndrome” and the “Contra-striatal Blood Syndrome”.

II. The Second Product of the Peroxidase Reaction: Poison of Infantile Beriberi Found

From our Laboratory it was published in 1934\textsuperscript{12}) that the poison of infantile beriberi was methyl glyoxal (or pyruvaldehyde like substance), though the final strictly chemical identification was published in 1951\textsuperscript{13}).

I did not have even the slightest idea that the peroxidase reaction of leucocytes could lead to the clue to infantile beriberi poison. Those who have seen fatal cases of acute infantile beriberi will believe that the death is not due to avitaminosis Bi, but that it must be the result of acute poisoning. Hirota\textsuperscript{14}), who described “infantile beriberi” as a clinical entity for the first time 1891 in Japanese (1898 in German), stated in his paper:—

“The disease in question of infants will be caused through the milk of the beriberi-diseased mother and is nothing else than an intoxication through same” (literally translated). I think that a doctor who saw for the first time a breast-fed (and well fed) baby die of acute infantile beriberi would insist that somebody must have given him some deadly poison.

An apparently well fed baby (of three months, e.g) will die almost suddenly in a day or two. Even in a relatively recent publication, Raid\textsuperscript{15}) experienced cases of fatal infantile beriberi in Hong Kong and stated: “Sato regards sudden death as the most typical symptom of infantile beriberi”. While nowadays thiamin will rescue severe cases of the disease, “not every case of acute fulminating infantile beriberi may respond to even massive doses of thiamin”\textsuperscript{15})

In 1934 we published for the first time that the poison was methyl glyoxal\textsuperscript{12)} (or methyl glyoxal like substance). This was the time that methyl glyoxal had long been discarded or considered as an insignificant side product of carbohydrate fermentation. So only few authors paid attention to our opinion. But we repeated our opinion frequently, until the poison was identified by Wako\textsuperscript{13}) under the guidance of Prof. Tatsuta (of the Department of Science) who had made the investigation of carbonyl compounds his life work.

How did we come to think methyl glyoxal as the poison causing infantile beriberi? The first hint to it was given by the “Striatal Blood Picture” (cf. the First Product of the Peroxidase Reaction).

The “Striatal Blood Picture” has nothing to do with vitamins, it was a matter of course, but at that time the vitaminology was still in early infancy. And, long before we had come to perform the “peroxidase puncture”, we thought that vitamin B deficiency might possibly have something to do with the picture. (In this treatise I used the word $\text{Vitamin B}_1$ frequently on purpose instead of the simpler word thiamin, because, when we started the work on infantile beriberi, the word vitamin $\text{B}_1$ itself did not exist in the medical literature. But when we, for
instance, wanted to make food free of vitamin $B_1$, we autoclaved it with due
technique.) So, Suzuki\(^{16}\) attempted the experiment with pigeons as experimental
animals and with autoclaved tofukara as food. Blood did not present the "Stria-
tal Blood Picture" at all, even when the pigeons died.

We of course did not intend to publish the result (we published it only
several years later\(^{16}\), because we had only then begun to know its biological
importance). Fortunately, however, we remembered that in that pigeon ex-
periment peroxidase reaction was getting weaker and weaker as $B_1$-avitaminosis
advanced, though peroxidase was still distinctly stained even at the time of death.

This finding — weaker peroxidase reaction in $B_1$-avitaminosis, which had
appeared to show only an insignificant importance, began to play a great part in
making a later devised test useful. This new test, named Arakawa's reaction by
myself — because it was devised by T. Arakawa\(^{16}\) in our Laboratory who had
been much interested in our peroxidase reaction. It is an exceedingly sensitive
peroxidase reaction of human milk (usual peroxidase regagents will show an
intensely blue color in case of raw cow's milk, but no or almost no color in case of
human milk. Arakawa's test will show a very intensely blue color in case of normal
human milk.)

Human milk shows a positive (intensely blue) and a negative (strawyel low)
reactions with intermediate reactions. For the sake of simplicity only positive
and negative reactions may be related here. Now on the basis of the pigeon
experiment just mentioned, I presumed that the human milk negative to
Arakawa's reaction might possibly be milk from a $B_1$-avitaminotic body. And
animal experimentation\(^{16}\) was performed. Pregnant rabbits fed on $B_1$-avitami-
notic food secreted Arakawa-negative milk, but, when vitamin $B_1$ was administered,
they began to secrete Arakawa-positive milk.

Human cases with negative Arakawa's reaction began to secrete Arakawa-
positive milk on vitamin-$B_1$ administration. Papers on such a relation — more
than 200 in number — had been published since 1930 from our Laboratory.

When we published Arakawa's reaction in 1930, — when only subclinical
cases of infantile beriberi, apparently healthy, were seen — , I wanted very much
to test the reaction in cases of fulminant infantile beriberi, such as were prevalent
in the era of Meiji (1874–1910). I thought — and it was a great mistake — at
that time there was no such fatal case of the disease in the world. How I regretted
that Arakawa's reaction was devised only much too late — at least 30–40 years
too late.

One day a reprint came to us from Dr. Fehily, in Hong Kong, who had
been reading our papers on the peroxidase reaction in the Tohoku Journal of
Experimental Medicine and testing Arakawa's reaction. To my great surprise,
acute toxic cases of infantile beriberi — and it was Fehily herself who told the
Hong Kong medical world for the first time that those cases were suffering from
"infantile beriberi" of Prof. Hirota\textsuperscript{14}) were still prevalent among Chinese people. My impression on reading the paper was that Hong Kong was at that time, as far as acute and fatal beriberi is concerned, a veritable picture of Japan, about one quarter century prior to that time.

As Fehily\textsuperscript{18}) stated, mothers of cases of infantile beriberi secreted Arakawa-negative milk, but on vitamin B\textsubscript{1} administration, they began, as she further stated, to secrete Arakawa-positive milk. \textit{We could test Arakawa's reaction on "infantile beriberi cases" as of the Meiji era} (when such fulminating cases of the disease prevailed among Japanese people) with the expected result.

Remark 1. Is methyl glyoxal poisonous or non-poisonous? It is reported to be a non-poisonous substance\textsuperscript{19}). And I myself might not mind being daily injected with pyruvaldehyde (of course a good, painless technic being understood). Only then \textit{I will refuse that injection}, if I am forbidden to take any thiamin-containing food or drug. Methyl glyoxal is a fatal heart-enlarging poison\textsuperscript{19}) for B\textsubscript{1}-avitaminotic animals, and this poison is quite easily detoxicated by thiamin.

Remark 2. I thought that nowadays fatal cases of infantile beriberi would not be seen, but as will be presumed from Reid's\textsuperscript{15}) paper, many a fatal case may still be seen among rice-consuming people.

Remark 3. \textcolor{red}{Before I began to start human milk study,} I thought that the difference between normal and B\textsubscript{1}-avitaminotic human milks was the difference of thiamin content. The chemical difference of human milk was, I thought, subject to only individual difference, aside from the difference between immature and ripe milk.

It was only more than 10 years of study on Arakawa's reaction that I had begun to know that there was an essential difference\textsuperscript{20}) between normal and athiamic milks and that athiamic milk was that of poison (cf. Table IV).

| Table III. Oxidase and Peroxidase Reactions after the Bilateral “Peroxidase Puncture” and the Removal of Bilateral Hemispheres |
|--------------------------------------------------|--|
| | “Peroxidase puncture” | Hemisphere removal |
| | Immediate after | A few days after | Immediate after | A few days after |
| Oxidase | + | - | + | + |
| Peroxidase | + | + | + | + |
| “Striatal blood picture” | “Contra-striatal blood picture” |

III. The Third Product of the Peroxidase Reaction: Chédiak and Higashi's Disease

When I had have some experience concerning the peroxidase reaction, I began to believe firmly that all the people of the world — say, 3 billions in number — have the same (or almost the same) peroxidase picture, so that the

* We feel very grateful to Dr. Fehily for this work\textsuperscript{19}) of hers and for her subsequent visit to our Department to talk over the subject and to obtain some other papers of ours on the problem.
peroxidase picture of the neutrophil, e.g. of all the people of the world is like those shown in the pictures of different books. I had become firmer and firmer in the belief in the course of about 30 years of clinical peroxidase study. It was therefore a great surprise to me, when I saw the specimens shown me by Dr. Higashi (who had been studying peroxidase reaction in our Laboratory since his student time), — blood smears from his own patient. They presented an unexpected blood picture, — quite a monstrous peroxidase picture!

It was with a good reason that Higashi suggested the name of “Congenital Gigantism of Peroxidase Granules” for the new disease. In a word, with this anomaly lived a more or less normal life until an acute infection — with a clinically somewhat leukemic syndrome — ended their life in a very early life.

At first I thought that this inborn error of metabolism was a very, very rare one — I had never come across such a case during my 30 years' experience with the peroxidase reaction. But very soon I accidentally read Chédiak's case with a leucocytic anomaly in a journal, and I found that there was a close similarity between Chédiak's and Higashi's cases. So I published a treatise: “Chédiak-Higashi’s Disease; Probable Entity of Familial Anomaly of Leucocytes (Chédiak) and Congenital Gigantism of Peroxidase Granules (Higashi).

As it is, the disease is not so rare a one as I thought. Soon Donohue's paper and Miller's report followed. Now about 30 cases of the disease (or similar syndromes) have been reported. And the disease is not restricted to a part of the world, but the reports have come from almost all the parts of the world. Dr. Ts. Arakawa (Prof. of Pediatrics, Tohoku University, Sendai), for instance, has recently been examining a case of this congenital error of metabolism with all his staff. — This last sentence was written on Feb. 26, 1964.

<table>
<thead>
<tr>
<th>Table IV. Difference between Normal and Athiamic Human Milk</th>
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<tr>
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<tr>
<td>Vitamin B1</td>
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<tr>
<td>Chlorine</td>
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<td>Calcium</td>
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<td>Lactic acid</td>
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<tr>
<td>Fat</td>
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<tr>
<td>Vitamin C</td>
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<tr>
<td>Methylglyoxal</td>
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</table>
DISCUSSION

The present treatise will show that mere morphology of an enzyme may give a hint to the unexpected finding of a new thing or a new relation, which could not possibly be dreamed of by a given method of morphology.

Most of those who have compared the oxidase (= nadi) reaction (Schultze) and our peroxidase reaction on blood smears will come to the conclusion that, though these reactions are chemically different, these do show one and the same biological thing. So states Schelch (26) that, after describing the oxidase reaction first, Sato's peroxidase reaction has the same significance as the former.

If I had used the original oxidase reaction instead of my own peroxidase reaction — which, I myself thought at the time of its device, was of the same biological significance — I should not have been able to find the "Striatal Blood Syndrome" (the first unexpected product of the peroxidase reaction) and the poison of infantile beriberi or the poison produced in case of B1-avitaminosis (the second unexpected product of the peroxidase reaction). The third unexpected product — Chédiak-Higashi's Disease could have been found, if the oxidase reaction alone had been used instead of the peroxidase reaction, though the monstrous oxidase picture must have been an unexpected one to the oxidase examiner. Only our peroxidase reagents keep well so that they can be used in the daily routine work, while the oxidase reagents must be prepared freshly each time for use. So such an enzyme abnormarity as in Chédiak-Higashi's disease will be much more easily found through the peroxidase study than through the oxidase study.

It was especially interesting to me that the poison of infantile beriberi was hinted by the "Striatal Blood Picture" — or rather by the B1-avitaminotic pigeon experiment. As I stated above, the peroxidase reaction of leucocytes became weaker and weaker, as B1-avitaminosis advanced.

As we learned much later, this was not true. The actual result was: the peroxidase became less and less reactive, as B1-avitaminosis advanced; less and less reactive due to an ever increasing accumulation of methyl glyoxal on blood smears. Dip the blood slides into ether27) an instant, then the peroxidase reaction will be as strong as in control cases. Or, we can make normal blood smears peroxidase-weak, if we cover them with synthetic pyruvaldehyde.

CONCLUSIONS

In 1922 I devised a new peroxidase reaction (which has come to be called "Sato's Reaction or Sato and Sekiya's stain" in the literature) as a cheap substitute for the high priced reagents of the oxidase reaction (Schultze). I myself thought at the time of device that both reactions were of the same biological significance. But they were not altogether the same reaction.

1. Mere morphological — of an enzyme in this case — study may give a hint to the unexpected finding of a new thing or relation, which result could not
possibly be dreamed of by the study of a given method of morphology.

2. The daily routine hematological examination of our peroxidase reaction led to the quite unexpected finding of —
   a. the localisation of a brain lesion or the "Striatal Blood Syndrome",
   b. the poison of infantile beriberi or the poison produced in athiaminosis through the "Striatal Blood Syndrome" and
   c. Chédiak-Higashi's Disease.

Acknowledgment

I am very grateful to Prof. Yamagata (the then President of Japanese Clinical Hematology), Prof. Arakawa, Dr. T. Higashi, Dr. N. Katsushima, Mr. Sh. Sugai (Tohoku University) and Dr. S. Takai of the State Hospital of Sendai and Dr. O. Higashi (Iwate Medical College, Morioka) for their kind assistance, and to Prof. Kojima (of Pathology, Fukushima Medical College) for a free use of his slides of peroxidase reaction (of Hassall's bodies) of thymus.

References


   Those concerning the "Striatal Blood Syndrome":
   Those concerning "Infantile Beriberi Poison":
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17) Fehily, L., The Caduceus (University of Hong Kong), 1940, 19, 78.
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ibid., 1944, 47, 117.


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