Arakawa's Reaction and Cell Content of Human Milk.

89th Report of the Peroxidase Reaction.

By

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Introduction.

Since the publication of Arakawa's¹⁾ reaction from this Laboratory, a number of papers have appeared concerning the difference between the Arakawa-positive* and the Arakawa-negative milk. At the time of the publication of Arakawa's reaction we thought that the difference between these two kinds of human milk was that of peroxidase content, and that the other possible difference might be the presence of a poison or poisons in the Arakawa-negative milk.

When Nozaki²⁾ started to investigate chlorin content of human milk, it was our expectation that, in spite of the biological difference between those two kinds of human milk, such a chemical constituent as chlorin would remain of the same concentration in both kinds of milk. The result of his investigation was quite different as was described in his paper. Now that a number of papers concerning Arakawa's reaction from this Laboratory have been published, we have come to think that a quantitative difference of chemical constituents

1) T. Arakawa, Tohoku J. Exp. Med., 1930, **16**, 118.

* "Arakawa-positive" may be used in two different senses. One of these is: Arakawa-positive in a biochemical sense. A sample of human milk is said to have become Arakawa-positive, when it became blue on the addition of Arakawa's reagent. Here it means that the sample was not negative to Arakawa's reaction. The other of these two senses is: Arakawa-positive in a clinical sense. A sample of human milk is clinically Arakawa-positive only when it shows such a reaction as ++ or +++ in one minute of the addition of Arakawa's reagent. Another sample of human milk may be Arakawa-positive in the first described sense, but yet clinically negative.

2) J. Nozaki, Tohoku J. Exp. Med., 1934, **23**, 60.

between the Arakawa-positive and the Arakawa-negative milk is even rather natural.

We have started the present work assuming that a cytological difference will be found between these two kinds of human milk, especially because peroxidase-positive cells of milk interested A. Sato, one of us, long ago. It will here be remarked how A. Sato and K. Shoji³⁾ succeeded in evolving a device of the counting chamber method of peroxidase reaction—perhaps the only humid method of blood counting utilizing peroxidase reaction. The following is a short history of the device:—

Since the device of the copper peroxidase reaction by Sato (one of us) and Sekiya⁴⁾ in 1919, the former and K. Shoji had been contriving to apply the copper peroxidase reaction (of blood films) to the counting chamber method, so that simultaneously with the total white cell count, myeloid and lymphatic cells might be easily differentiated. They tried and tried, but in vain. At last they tried it on milk and, after several trials, found one blue stained cell in a specimen, which convinced them of a possibility of the counting chamber peroxidase method of blood. And they succeeded at last. It was about two years after the device of the copper peroxidase reaction on blood films.

Method of Investigation.

1. Materials. Milk was tested with Arakawa's reagents and the result was noted.

2. Technique of the examination of peroxidase-positive cells. After centrifugalization of a milk sample (2 ccm.) for about fifteen minutes at ca. 3,000 turns per minute, the plasma—the second layer—was tested with Arakawa's reagents and the result was noted too, and the third layer—the bottom layer—smears were made. These films were stained by Sato-Sekiya's⁴⁾ original copper peroxidase method and peroxidase-positive cells were estimated at the microscopic examination (Cf. Fig.).

Result of Investigation.

1. Relation between Arakawa's reaction and content of peroxidase-positive cells.

3) A. Sato and K. Shoji, *Journ. of Labor. and Clin. Med.*, 1928, **13**, 1058.

4) A. Sato and S. Sekiya, *Tohoku Igaku-Zasshi*, 1920, **6**, 534; *Tohoku J. Exp. Med.*, 1926, **7**, 111.

A glance at Table 1 will give one the impression that Arakawa's reaction goes hand in hand with cell content. A milk sample of normal Arakawa's reaction is of a high cell content, while one of weak reaction is a low cell content (Cf. Table 1).

From this impression, we may arbitrarily assume that a milk sample of strong or normal Arakawa's reaction corresponds to the cell content of the sign # (Cf. Footnote to Table 1), one of intermediate reactions to that of the sign + and one of the completely or almost negative reaction to that of the sign +.

Then the milk samples tabulated in Table 1 will be distributed as will be shown in Table 2 (Cf. Table 2).

As has been shown in Table 2, 9 milk samples out of the 20 belonging to strong or normal Arakawa's reaction were of the cell content #, and 11 samples of the cell content +. But there was no sample of the cell content +. And of 10 samples belonging to the intermediate reaction, 9 samples were of the cell content +, and only one of the cell content #. There was no sample of the cell content +. As to the completely or almost completely negative reaction, 20 samples were of the cell content + and 7 of the cell content +. There was none of the cell content # (Cf. Table 2).

From this Table (Cf. Table 2), it will be seen that there is an approximate proportion between the strength of Arakawa's reaction and the cell content. Milk samples of strong Arakawa's reaction are of a high cell content, and ones of negative reaction of a low cell content.

Of all the 58 samples examined, there was an exceptional case. That was milk sample from the left side of Case 27. It was of an intermediate Arakawa's reaction and that a weak reaction. Its cell

Fig. Smear of centrifuged human milk stained with Sato-Sekiya's copper peroxidase reaction (original method). Blue cells are peroxidasepositive.

TABLE 1.

Arakawa's reaction and peroxidase-positive cells of human milk.

No.	Name	Side	A r a k a w a's reaction of mother's milk												Peroxidase-positive cells*		
			Whole milk						Milk plasma								
			(0')	(1')	(2')	(3')	(4')	(5')	(0')	(1')	(2')	(3')	(4')	(5')			
1	Ohmi	right	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(±)	(±)	(+)	
		4 days after daily administration of vitamin B															
		right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(±)	(±)	(+)	
2	Daikoku	right	(-)	(-)	(-)	(±)	(±)	(±)	(-)	(-)	(±)	(+)	(+)	(+)	(+)		
		6 days after daily administration of vitamin B															
		right	(-)	(-)	(-)	(±)	(±)	(±)	(-)	(-)	(±)	(+)	(+)	(+)	(+)	(+)	
		7 days after daily administration of vitamin B															
		left	(+)	(#)	(#)	(#)	(#)	(#)	(+)	(#)	(#)	(#)	(#)	(#)	(#)	(#)	
3	Hanasaka	left	(-)	(-)	(±)	(±)	(±)	(±)	(-)	(±)	(+)	(+)	(+)	(+)	(+)		
		4	Ohto	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	
				right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	
				2 days after daily administration of vitamin B													
				right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
3 days after daily administration of vitamin B																	
5	Chiba	right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
6	Kohriyama	left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
7	Takahashi	left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
8	Fukushima	on the next day															
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		2 days after															
		right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(±)	(±)	(±)	(±)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)		
9	Furusawa	left	(-)	(-)	(±)	(±)	(±)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		3 days after daily administration of vitamin B															
		right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(±)	(±)	(±)	(±)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(-)	(-)	(±)	(±)	(±)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
10	Tsuji	left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)		
		13 days after															
		right	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
11	Uchimi	left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
		right	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
12	Suzuki	right	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
		right	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
13	Kurosawa	right	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
		left	(±)	(+)	(+)	(+)	(+)	(+)	(+)	(#)	(#)	(#)	(#)	(#)	(#)		
		right	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
14	Shoji	right	(±)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
15	Suzuki	right	(±)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		right	(±)	(+)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		
		left	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(±)	(±)	(+)		

No.	Name	Side	Arakawa's reaction of mother's milk												Peroxidase-positive cells*
			Whole milk						Milk plasma						
			(0')	(1')	(2')	(3')	(4')	(5')	(0')	(1')	(2')	(3')	(4')	(5')	
16	Sasaki	right	(-)	(±)	(±)	(±)	(±)	(+)	(-)	(±)	(±)	(±)	(±)	(+)	(+)
		right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		6 days after													
17	Igari	right	(-)	(-)	(-)	(-)	(±)	(±)	(-)	(-)	(±)	(±)	(+)	(+)	(+)
		left	(-)	(-)	(-)	(-)	(±)	(+)	(-)	(-)	(±)	(+)	(+)	(+)	(+)
18	Watanabe	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(±)	(+)	(+)	(+)	(+)
19	Masu	right	(-)	(±)	(+)	(+)	(#)	(#)	(-)	(-)	(±)	(±)	(±)	(±)	(+)
		left	(-)	(±)	(+)	(+)	(+)	(#)	(±)	(+)	(+)	(+)	(+)	(+)	(+)
20	Tsukashima	right	(-)	(+)	(+)	(+)	(+)	(#)	(±)	(#)	(#)	(#)	(#)	(#)	(+)
		left	(±)	(+)	(+)	(+)	(+)	(#)	(±)	(#)	(#)	(#)	(#)	(#)	(#)
21	Sumitani	right	(-)	(±)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)
		left	(-)	(+)	(+)	(+)	(+)	(+)	(±)	(+)	(+)	(+)	(+)	(+)	(+)
22	Sato	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
23	Ito	right	(+)	(+)	(#)	(#)	(#)	(#)	(+)	(+)	(#)	(#)	(#)	(#)	(#)
		left	(+)	(+)	(#)	(#)	(#)	(#)	(+)	(+)	(#)	(#)	(#)	(#)	(#)
24	Iwai	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(±)	(+)	(+)	(-)	(-)	(±)	(±)	(±)	(±)	(+)
		right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		one week after daily administration of vitamin B													
25	Kurihara	right	(-)	(±)	(±)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(#)	(#)	(#)
26	Yoshida	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		right	(-)	(-)	(-)	(-)	(±)	(±)	(-)	(-)	(-)	(±)	(±)	(±)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(±)	(+)
		on the next day													
		right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		3 days after daily administration of vitamin B													
		right	(-)	(-)	(-)	(-)	(-)	(±)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		5 days after daily administration of vitamin B													
27	Yuki	right	(-)	(±)	(±)	(±)	(±)	(±)	(-)	(±)	(±)	(+)	(+)	(+)	(+)
		left	(-)	(±)	(+)	(+)	(+)	(+)	(-)	(±)	(±)	(+)	(+)	(+)	many
		Mastitis acuta sinistra													
28	Sato	right	(-)	(+)	(+)	(+)	(+)	(#)	(+)	(+)	(#)	(#)	(#)	(#)	(#)
		left	(-)	(+)	(+)	(+)	(+)	(#)	(±)	(+)	(+)	(+)	(+)	(#)	(#)
29	Ito	right	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
		left	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
30	Kadowaki	right	(+)	(+)	(#)	(#)	(#)	(#)	(+)	(+)	(#)	(#)	(#)	(#)	(#)
		left	(+)	(+)	(#)	(#)	(#)	(#)	(+)	(+)	(#)	(#)	(#)	(#)	(+)

* Note to the Table:—

(#) The number of cells in one field of vision averages above 3.
 (+) " " 2 or 1.
 (-) " " below 1.

content was very high, so that one field of vision presented many peroxidase-positive cells. The mother had mastitis acuta on her left breast.

2. Effect of vitamin B** on cell content.

** Vitamin B₁ is meant throughout the paper.

TABLE 2.

Relation between Arakawa's reaction and cell content.

Arakawa's reaction		Strong or normal	Intermediate	Completely or almost completely negative
Total number of samples		20 samples	10 samples	27 samples
Approximate content of peroxidase-positive cells*	##	9 samples (45 %)	1 sample (10 %)	0
	++	11 samples (55 %)	9 samples (90 %)	7 samples (26 %)
	+	0	0	20 samples (74 %)
	Average	##	++	+

* Cf. Foot-note to Table 1.

Table 3 shows a collection of only a few cases of lactating women treated with vitamin B.

TABLE 3.

Effect of vitamin B on content of peroxidase-positive cells.

No.	Name	Side	Arakawa's reaction of milk plasma						Content of peroxidase-positive cells*	Days of administration of vitamin B
			(0')	(1')	(2')	(3')	(4')	(5')		
1	Ohmi	right	(-)	(-)	(-)	(-)	(±)	(±)	(+)	0
	"	"	(-)	(-)	(-)	(-)	(-)	(±)	(+)	4
	"	"	(-)	(-)	(±)	(+)	(+)	(+)	(+)	6
	"	"	(-)	(-)	(±)	(+)	(+)	(+)	(+)	7
4	Ohto	"	(-)	(-)	(-)	(-)	(-)	(-)	(+)	2
	"	"	(-)	(-)	(-)	(-)	(-)	(-)	(+)	3
	"	"	(±)	(+)	(+)	(+)	(+)	(+)	(+)	5
	"	"	(+)	(+)	(+)	(+)	(+)	(+)	(+)	7
	"	"	(±)	(+)	(+)	(+)	(+)	(+)	(+)	16
9	Furusawa	left	(-)	(+)	(+)	(+)	(+)	(+)	(+)	0
	"	"	(±)	(+)	(+)	(+)	(+)	(+)	(+)	3
24	Iwai	right	(-)	(-)	(-)	(-)	(-)	(±)	(+)	0
	"	"	(-)	(-)	(-)	(-)	(-)	(±)	(+)	7
	"	"	(-)	(-)	(-)	(-)	(-)	(±)	(+)	0
26	Yoshida	(right	(-)	(-)	(-)	(-)	(-)	(-)	(+)	0
		left	(-)	(-)	(-)	(-)	(-)	(-)	(+)	0
		(right	(-)	(-)	(-)	(-)	(-)	(-)	(+)	3
		left	(-)	(-)	(-)	(-)	(-)	(-)	(+)	3
		(right	(-)	(-)	(-)	(-)	(-)	(-)	(+)	5
		left	(-)	(-)	(-)	(-)	(-)	(-)	(+)	5

* Cf. Foot-note to Table 1.

As has been shown in a number⁵⁻¹⁰⁾ of papers published from this Laboratory, Arakawa's reaction will become stronger on an administration of vitamin B to a lactating woman, or at least Arakawa-negative milk will, if a lactating woman has been treated with vitamin B, become less poisonous¹¹⁾ even allowing that the reaction should remain negative all the same.

A certain effect of vitamin B will also be seen from the change of cell content of milk. When a lactating women with negative or weak Arakawa's reaction has been treated with vitamin B for some time, the cell content will become large, notwithstanding that the reaction has not improved much (Cf. Table 3).

3. Arakawa's reaction of milk plasma.

54 samples (75%) showed no difference of Arakawa's reaction between whole milk and milk plasma, and in 10 samples (14%) milk plasma became more intensively positive to Arakawa's reaction than the whole milk, but there were 8 samples (11%) that showed a weakening of the reaction of milk plasma. But in those samples that showed a stronger reaction or a weakening of the reaction, the grade of the change was not remarkable in most cases, so that in practice it may be said that Arakawa's reaction of whole milk generally represents that of milk plasma.

Remarks.

As to the significance of the cell-rich and the cell-poor milk, it will be related in the next paper on the same problem¹²⁾.

Conclusion.

Human milk positive to Arakawa's reaction is of a higher content in peroxidase-positive cells than Arakawa-negative is.

5) A. Sato, Nippon no Ikai, 1930, **20**, 11; Jikken Iho, 1931, **17**, 323; 1932, **18**, 1292; 1933, **19**, 1554.

6) M. Chiba, Tohoku J. Exp. Med., 1932, **19**, 486.

7) T. Suzuki, Ibid., 1934, **23**, 23.

8) Y. Uga, Ibid., 1935, **25**, 169.

9) T. Suzuki, Ibid., 1935, **25**, 186.

10) J. Kimura, Ibid., 1935, **27**, 360 and 374.

11) A. Takamatsu, Ibid. 1934, **23**, 46.

12) Cf. T. Suzuki, the 91st Report of Peroxidase Reaction, to be published in this Journal.