A Peroxidase Reaction of the Leucocytes with Pharmacopeial Tinctures.

(7th Report of the Peroxidase Reaction.)

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Since the device of Arakawa's reaction¹⁾ of milk, we have tried to apply it on blood smears and to compare the result with that of the copper method devised by Sato and Sekiya²⁾ as the standard peroxidase reaction.

Method used.

A. Peroxidase reaction (Copper Method).

Reagents used.

1. Solution A. Copper sulphate solution, 0.5 per cent.
2. Solution B. To 100 c.cm. of saturated aqueous solution of benzin-dine, 2 drops of hydrogen peroxide (3 per cent) are added.

Method used.

To a fresh, dry blood smear apply solution A. Pour off most of it and apply solution B for two minutes. Then wash thoroughly with running water.

B. Arakawa's reaction.

Reagents used.

1. Benzin-dine (Benzidin. pur. Merck).
2. One of pharmacopeial tinctures, as old as possible, because most freshly prepared tinctures give no reaction.

Method used.

To one of the pharmacopeial tinctures, sufficient benzin-dine to saturate

1) Arakawa's reaction, to be reported in details in the near future. Compare also the paper of Arakawa and Sato, Tohoku J. Exp. Med., 1927, 9, 646.

2) A. Sato and S. Sekiya, Tohoku J. Exp. Med. 1926, 7, 111.

the tinctures is added before using and the contents are vigorously shaken. Now to a fresh, dry blood smear apply the respective tincture with benzidine and wait two minutes. Then wash thoroughly, at first with alcohol and next with water, and then dry.

Result of the reaction: the peroxidase granules of the leucocytes are stained deep blue or indigo blue.

C. Combined reaction (Copper-Tincture-Benzidine Reaction).

Reagents used.

- (1. 0.5 per cent copper sulphate solution.
- (2. Tincture with benzidine, as described under B.

Method used.

To an air dried blood smear apply the copper solution and pour off most of it. Without washing, pour on the second solution immediately and wait 2 minutes. Wash thoroughly with alcohol at first and then with water.

Result: the peroxidase granules of the leucocytes are stained especially deep bluish green.

The results of the experimentation are tabulated below. The reaction of each tincture is shown by the number of the marks (+), as +, ++, + + +, according to the intensity, whereas the intensity of the copper method of Sato and Sekiya as the standard, is expressed by + + +.

TABLE I.

Kinds of tinctures	Time past since preparation	A r a k a w a's reaction		Combined method	
		intensity	color	intensity	color
T. ipecacuanhae	unknown	##	indigo	+++	deep bluish green
T. cantharidum	4y. 7m.*	##	"	+++	"
T. colombo	2y. 11m.	++	"	##	"
T. aurantii	about 3y.	++ or +	greenish indigo	##	"
T. cinnamomi	unknown	++ or +	light indigo	##	"
T. aromatica	"	—		##	"
T. digitalis	1y. 8m.	+	gray	##	deep blue
T. colchici	over 2y.	+	light indigo	##	deep bluish green
T. croci	"	+	"	## or ++	"
T. cascarillae	unknown	##	indigo	##	"

Kinds of tinctures	Time past since preparation	Arakawa's reaction		Combined method	
		intensity	color	intensity	color
<i>T. valerianae aetherae</i>	unknown	+	light indigo	## or ++	deep bluish green
<i>T. alōes compositae</i>	„	+	„	##	„
<i>T. colocynthidis</i>	„	++	„	++	bluish green
<i>T. aconiti</i>	over 2y.	—		++	„
<i>T. chinae compositae</i>	unknown	—		++ or +	„
<i>T. valerianae</i>	„	(+)	light gray	++	„
<i>T. lobeliae</i>	„	—		+ or (+)	light blue
<i>T. aromatica acida</i>	„	—		+	yellow
<i>T. opii</i>	„	(+)	light gray	+	light blue
<i>T. gentianae compositae</i>	„	—		(+)	„
<i>T. catechu</i>	„	—		+	light sepia
<i>T. quassiae</i>	„	—		+	light blue
<i>T. cannabis indiae</i>	„	—		(+)	„
<i>T. chinae</i>	„	(+)	light blue	(+)	„
<i>T. amara</i>	„	—		(+)	„
<i>T. asae foetidae</i>	„	—		—	
<i>T. strophanthi</i>	„	—		—	
<i>T. scopoliae</i>	„	(+)	light yellow	—	
<i>T. zingiberis</i>	„	—		—	
<i>T. strychni</i>	„	—		—	
<i>T. opii benzoica</i>	over 2y.	—		—	
<i>T. capsici</i>	„	—		—	
<i>T. gallarum</i>	unknown	—		—	
<i>T. ferri pomati</i>	„	—		—	

* 4y. 7m. = 4 years and 7 months. (+) = weaker than +.

As already pointed out by Arakawa and Sato,³⁾ even those tinctures

3) T. Arakawa and A. Sato, Tohoku J. Exp. Med., 1927, 9, 646.

with + + + in the above table will not give the slightest reaction, if these have been only freshly prepared.

Now the same experiment was tried under the following conditions ; (1) cases in which some fresh tinctures were used in combination with hydrogen peroxide, and (2) cases in which both some fresh tinctures in combination with 0.5 per cent copper sulphate solution and with hydrogen peroxide were used. These experiments are shown in Table II and III. Table II will show the difference between fresh and old tinctures very distinctly, while from Table III will be seen how fresh tinctures may be made exceedingly " active " like old ones.

TABLE II.

Kinds of tinctures	Fresh tinctures + Benzidine	Old tincture + Benzidine		
		Time past since preparation	Intensity	Color
T. cantharidum	—	4y. 7m.*	##	deep blue
T. ipecacuanhae	—	about 3y.	##	deep bluish green
T. colombo	—	2y. 11m.	++	deep blue
T. digitalis	—	over 2y.	## or ++	"
T. auranti	—	about 3y.	++ or +	indigo
T. colchici	—	over 2y.	+	blue

* 4y. 7m. = 4 years and 7 months.

TABLE III.

Kinds of tinctures	Cu SO_4 + Fresh } + Benzid. tinct. }	Fresh tincture + H_2O_2 + Benzidine		Cu SO_4 + Fresh } + H_2O_2 + Benzidine tinct. }	
	Reaction	Intensity	Color	Intensity	Color
T. cantharidis	—	+++	deep blue	+++	deep bluish green
T. ipecacuanhae	—	##	deep bluish green	+++	„
T. colombo	—	##	deep blue	+++	„
T. digitalis	—	++	„	+++ or ##	„

Summary.

In an attempt to apply Arakawa's reaction (which is originally designed for milk) to blood leucocytes, the authors have found the following facts :

(1) Different pharmacopeial tinctures with benzidine give different results as to the intensity and color of the reaction, and the reaction with some of them is almost as strong as the copper peroxidase method of Sato and Sekiya.

(2) Even those tinctures which give a very strong reaction will not give even the slightest reaction, if these have been only freshly prepared.

(3) If to these fresh tinctures hydrogen peroxide is added, they react instantly like the old tinctures, so that Arakawa's reaction, as tested on blood leucocytes, is a peroxidase reaction.

(4) The reaction with these old pharmacopeial tinctures may be made still stronger, when copper sulphate is used at first, as in the case of the copper peroxidase reaction of Sato and Sekiya.