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THE EFFECT OF IMMUNE BLOOD UPON THE OPSONO-CYTOPHAGIC POWER OF THE BLOOD IN PERTUSSIS¹

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In comparison with the general renewal of interest in vaccine prophylaxis in pertussis, little attention has been given to passive protection by the use of immune blood. There seems to be sufficient evidence to indicate that this method is effective in the prevention and modification of the disease in infants. It is probable that the very young infant does not respond well to antigenic stimulation. Moreover, in the case of the exposed infant, the time interval may not permit a satisfactory application of active immunization. Clinical results obtained by the use of adult immune serum and convalescent serum in the prevention of whooping cough, along with a review of the literature, have been described (1). In this report an attempt to measure the effect of these agents in terms of humoral antibodies is made.

Since recent clinical and experimental evidence strongly favors *H. pertussis* as the cause of whooping cough, and since definite proof of a circulating soluble toxin in the disease is lacking, it is justified to assume that recovery depends to a great extent upon phagocytosis. This process has long been appreciated as important in the general mechanism whereby the host rids itself of infecting bacteria.

In the past, investigation of humoral immunity in pertussis has chiefly concerned agglutinins and complement-fixing antibodies. These antibodies appear at the beginning of the third week of the disease and diminish significantly as convalescence reaches the fifth or sixth month. They are demonstrable in the blood for a similar period of

time following active immunization with *H. pertussis* vaccine.

Recent attention has turned to the application of the opsono-cytophagic test, suggested by Veitch (2), and used by Huddleson et al. (3) in undulant fever, for studying immune processes in pertussis. As the word implies, this is a test for the opsonizing antibody of the serum as well as for the phagocytosing power of the leukocytes. Whether phagocytosis, as demonstrated by this method, is simply another way of studying an antibody identical with agglutinins, precipitins and complement-fixation substances, depends upon one's interpretation of the unitarian theory of antibodies and is a question which is beyond the scope of this report.

The technique of the test and the materials used have been described in the preceding paper (4).

By the use of this test, which is probably not an entirely specific reaction, we have observed that the degree of phagocytosis of *H. pertussis* is increased in the blood during the course of whooping cough. Children who have had the disease have shown higher titers than those who have not and mothers have had, in general, higher titers than have their newborn infants. We have likewise collected evidence that suggests placental transfer of the opsonizing antibody (4).

RESULTS

In Table I, the results obtained by adding immune adult serum to the whole blood-heparin-organism mixture in each of 11 individuals are shown. In these tests 0.05 cc. of the patient's blood obtained from a puncture of the finger tip was mixed with 0.05 cc. of heparin (1 to 1000 dilution in physiological salt solution). To this was added 0.05 cc. of the standard antigen (10

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TABLE I

The effect of adult immune serum upon phagocytosis of *H. pertussis* in vitro

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Saline (control)			Serum		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Su.....	18 months	0	13	12	0	1	24
Ro.....	7 years	3	15	7	0	0	25
Ed. J.....	2 years	0	16	9	0	4	21
Ed. W.....	5 years	0	18	7	0	3	22
Ro.....	Newborn	0	14	11	0	9	16
Sm.....	Newborn	1	22	2	0	11	14
Ru.....	15 months	3	22	0	0	3	22
Cl.....	4 weeks	0	8	17	0	4	21
Jo.....	Newborn	7	9	9	5	10	10
Fa.....	Newborn	0	14	11	2	20	3
Ja.....	Newborn	1	22	2	0	15	10
Average number of cells.....		1.4	15.7	7.9	0.6	7.3	17.1

billion organisms per cc. in physiological salt solution preserved by a 1 to 10,000 concentration of merthiolate) and 0.05 cc. of the immune adult serum. This serum was that of adults who had previously had the disease in childhood. In the controls 0.05 cc. of physiological salt solution was substituted for the adult immune serum. It is obvious that in all except one instance the number of cells exhibiting marked phagocytosis is definitely increased as compared with the respective saline control.

Table II shows the results obtained when a similar comparison is made between the influence of the serum of an individual before, and 48 hours after, receiving an intravenous blood transfusion. In this experiment, this influence was determined upon the leukocytes of two newborn infants in each case. The blood of newborn infants was

TABLE II

The effect of transfusion upon phagocytosis of *H. pertussis*

Patient receiving transfusion	Age	Cells of newborn infants	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed											
			Saline control			Serum of patient before transfusion			Serum of patient after transfusion			Serum of donor		
			0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Cl.	4 weeks	a	0	14	11	2	20	3	0	10	15	0	9	16
		b	1	22	2	0	15	10	0	8	17	0	11	14
St.	2 years	a	23	2	0	23	2	0	16	9				
		b	6	17	2	6	16	3	0	20	5			
Sp.	5 years	a	3	12	10	1	16	8	0	13	12	0	7	18
He.	8 weeks	a	3	19	3	6	19	0	5	20	0			
		b	5	20	0	13	12	0	12	8	5			
Ku.	2 years	a	3	18	4	13	12	0	3	22	0			
		b	5	20	0	10	15	0	0	23	2			
D'A.	12 months	a	8	8	9	0	10	15	1	7	17			
		b	23	2	0	13	10	2	7	10	8			
Co.	18 months	a				10	12	3	11	10	4	10	7	8
		b				19	5	1	5	11	9	17	6	2
Ke.	6 years	a	9	16	0	2	23	0	3	21	1	5	14	6
		b	7	17	1	0	25	0	0	23	2	1	17	7
Ca.	5 months	a	0	17	8	2	20	3	0	24	1	0	15	10
		b				11	13	1	8	17	0	1	24	0
Average number of cells			6.8	14.5	3.6	7.7	14.4	2.9	3.2	15.4	6.3	3.7	12.2	9.0

TABLE III

The effect of intramuscular injection of 20 cc. of adult whole blood upon phagocytosis of H. pertussis

Patient receiving transfusion	Age	Cells of newborn infants	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed											
			Saline control			Serum of patient before transfusion			Serum of patient after transfusion			Serum of donor		
			0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Ru.	15 months	a b	14 0	2 2	9 23	9 14	6 3	10 8	11 7	4 3	10 15			
McS.	2 months	a	8	12	5	11	14	0	5	16	4	1	18	6
Fe.	12 months	a b	2 7	22 18	1 0	0 2	23 22	2 1	0 2	25 21	0 2	2 0	22 24	1 1
DeC.	18 months	a b	3 15	21 10	1 0	0 16	20 9	5 0	0 3	25 21	0 1	0 0	18 24	7 1
Ta.	3 years	a b	4 17	20 8	1 0	0 2	13 17	12 6	1 1	17 9	7 15	0 0	12 15	13 10
Average number of cells			7.7	12.7	4.4	6.0	14.1	4.9	3.3	15.7	6.0	0.4	19.0	5.6

selected in order to eliminate the possibility of the effect of an unrecognized attack of pertussis, and because it was easier to obtain low titers as controls. Tests of the effect of the addition of normal saline as well as of the donor's serum are included for comparison.

It is evident that serum taken after the transfusion stimulated the leukocytes of the newborn infant to a greater degree of phagocytosis than did the serum taken before the transfusion. The donor in each instance was a parent whose history of having had the disease was not determined. The amount of blood given in each case was approximately 10 cc. per kilogram of body weight.

In Table III, the effects of serum samples taken before and after the intramuscular injection of 20 cc. of whole blood into each of 5 infants is recorded. The donor in each instance was a parent whose history of the disease was not known. While the effect upon phagocytosis is not as marked as in the case of those receiving transfusions, it is apparent that a definite increase has occurred.

Table IV includes results obtained by the intramuscular injection of 10 cc. of hyper-immune human serum. In this experiment the whole blood

TABLE IV

The effect of the intramuscular injection of 10 cc. of hyper-immune adult serum upon phagocytosis of H. pertussis

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Before injection			After injection		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
Fa.....	3 months	0	21	4	0	21	4
Cr.....	10 months	10	15	0	0	25	0
Ta.....	18 months	1	18	6	0	13	12
St.....	2 years	0	4	21	0	15	10
Br.....	9 months	0	19	6	0	19	6
Fo.....	3 weeks	5	20	0	5	19	1
McS.....	2 months	5	18	2	0	21	4
MI.....	Newborn	10	15	0	4	21	0
Mit.....	Newborn	19	6	0	22	3	0
Ro.....	Newborn	2	21	2	7	18	0
Vl.....	Newborn	14	11	0	9	14	2
Er.....	Newborn	10	15	0	0	25	0
Ee.....	Newborn	19	4	2	0	15	10
Average number of cells.....		7.3	14.4	3.3	3.6	17.6	3.8

of the recipient before and after receiving the injection was tested directly. The hyper-immune serum was prepared by immunizing normal young adults by three weekly injections of Phase I *H. pertussis* antigen, each injection representing five

billion organisms. The donor was bled two weeks after the final injection, the serum harvested and preserved by the addition of 0.25 per cent tricresol. Kendrick (5) has reported excellent prophylactic results from the use of similar serum in exposed infants. The increase in phagocytosis brought about by this serum is not as great as that produced by transfusion, but consists chiefly in a shift from "none to slight" to "definite."

The effect of injecting 10 cc. of placental extract was studied in 3 infants and, as shown in Table V, a definite increase in the phagocytic

TABLE V
The effect of intramuscular injection of 10 cc. of placental extract upon phagocytosis of *H. pertussis*

Patient	Age	Distribution of polymorphonuclear neutrophils according to the number of organisms phagocytosed					
		Before injection			After injection		
		0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"	0 to 4 organisms "none to slight"	5 to 19 organisms "definite"	20+ organisms "marked"
	months						
McS.	2	0	14	11	1	7	17
So...	3	2	13	10	0	8	17
Ka...	9	1	24	0	0	15	10
Mi.*	2	5	16	4	0	8	17

* Received 20 cc. immune rabbit serum.

power of the blood resulted. In 1 infant, who received 20 cc. of immune rabbit serum, a similar response occurred. This immune rabbit serum was prepared by injecting rabbits intravenously with *H. pertussis* vaccine made from Phase I organisms; standardized to contain ten billion organisms per cc., and killed with merthiolate (1 to 10,000). Three weekly injections of 0.5 cc., 1.0 cc., and 1.5 cc. of the vaccine were given, and the rabbit was bled ten days after the last injection. The serum was separated and preserved by the addition of 0.25 per cent tricresol.

SUMMARY

The opsono-cytophagic reaction of the blood has been used to test the effect of the injection of immune blood in pertussis.

In 10 of a group of 11 infants and young children, the blood in each instance showed a marked increase in phagocytic power against *H. pertussis*, when a small amount of adult immune serum was added.

In a group of 9 infants, the degree of phagocytosis was definitely increased as a result of intravenous blood transfusions.

In 5 infants who received 20 cc. of adult immune blood, and in 13 infants who received 10 cc. of hyper-immune human serum intramuscularly, there was definite but less striking increase in phagocytosis.

Placental extract injected intramuscularly into 3 infants in dosages of 10 cc. produced a definite increase in the phagocytic power of the blood. A similar result was obtained when 1 infant was injected with 20 cc. of immune rabbit serum.

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