THE PRODUCTION OF ERYTHROPHAGOCYTOSIS IN PERIPHERAL BLOOD

STUDIES IN AUTO-IMMUNE HAEMOLYTIC DISEASE AND CANINE TRANSFUSION REACTIONS WITH SOME EXPERIMENTAL OBSERVATIONS

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Phagocytosis of red cells by white cells in the peripheral blood has been observed fairly frequently during the last 50 years in isolated patients suffering from a wide variety of conditions. Systematic studies in any one group of diseases have, however, been few, and experimental observations to throw light on the cause are scanty. Recently there has been increased interest in the subject, especially in relation to the destruction of red blood cells in the auto-immune type of acquired haemolytic anaemia (Zinkham and Diamond, 1952) and paroxysmal cold haemoglobinuria (Jordan, Prouty, Heinle, and Dingle, 1952).

This report describes observations on the production of erythrophagocytosis by three different manoeuvres: (1) Incubation of peripheral blood from cases of auto-immune haemolytic disease; (2) incubation of the peripheral blood of dogs at various times after transfusion of incompatible plasma; (3) incubation of white cells with several red cell antigen-antibody mixtures.

Technically, the procedure was the same for whole blood and the artificial systems. An incubation period of one hour at 37° C. was adopted; usually there was no significant difference between the degree of erythrophagocytosis whether the specimens stood at room temperature or were incubated at 37° C., but the latter method was used to keep conditions standard. After incubation well-mixed samples were centrifuged at 1,000 r.p.m. for 10 minutes and cover-slip films, made from the buffy layers, were stained by Wright's method. Five hundred potentially phagocytic cells (neutrophils, monocytes, eosinophils) were examined and the number of each type actually phagocytic was noted. Heparin was generally used to prevent coagulation (1 mg. to 10 ml. of blood), but comparable results were obtained with defibrinated blood.

In most positive preparations many leucocytes contained vacuoles approximating in size to red cells. Not uncommonly various grades of loss of haemoglobin up to complete vacuolation were seen in groups of red cells contained within single phagocytic leucocytes.

As a control study, peripheral blood from 30 patients suffering from non-haematological disorders was examined in the same way. No definitely phagocytic white cells were seen.

Auto-immune Haemolytic Disease

This term has been applied to that type of acquired haemolytic anaemia in which auto-antibodies can be demonstrated in the serum or bound to the erythrocytes. Frequent observations were made in five successive apparently uncomplicated cases over periods varying from two to six months. Erythrophagocytosis was found, after incubation, in the peripheral blood of all five cases. In one case direct capillary blood films were positive on most occasions, but the number of phagocytic leucocytes was always slight compared with that after incubation. Monocytes were the important phagocytic cells; neutrophils and eosinophils were seldom involved. The maximum number of red cells ingested by a single monocyte was five.

During the course of the disease the degree of erythrophagocytosis could sometimes (Fig. 1) but not always, be related to the activity of the haemolytic process. In three cases positive results were obtained at all examinations irrespective of the clinical state (even during periods of remission) but in the other two patients phagocytosis eventually disappeared as the disease became quiescent. The latter patients both responded extremely well to treatment and the anaemia disappeared. There was no demonstrable relationship to the titre of the Coombs antiglobulin test, and neither hormone

Fig. 1.—The relationship between the haematocrit (%) and the percentage of erythrophagocytic monocytes in a case of auto-immune haemolytic disease.

therapy nor splenectomy had any constant effect. Phagocytosis of nucleated red cells was not encountered.

Canine Transfusion Reactions

Two dogs belonging to canine group A were transfused with canine anti-A plasma. The canine A iso-antibody acts as a haemolysin as well as agglutinin, and it fixes complement and sensitizes red cells for the anti-globulin reaction (Young, Christian, Ervin, Swisher, O'Brien, Stewart, and Yuile, 1951). Blood was withdrawn from both animals at five minutes, 20 minutes, four hours, and 24 hours after the end of the transfusion. Films were made immediately, and also after incubation of the blood and concentration of the white cells.

Both dogs developed haemoglobinaemia, hyperbilirubinaemia, leucopenia, anaemia, and spherocytosis. No evidence of the occurrence of erythrophagocytosis *in vivo* was found, but it developed prominently *in vitro* in the blood of both animals in the five-minute, 20-minute, and four-hour samples. Both the 24-hour specimens were negative after incubation. In one dog 12% of the neutrophils and 45% of the monocytes were phagocytic four hours after transfusion; these were the highest figures obtained in either animal. Nucleated red cells, although numerous in most preparations, were never involved in the phagocytic process.

Artificial Systems

Experiments involving the admixture of normal red cells with various antibodies were carried out in test-tubes. They were of two types: (1) Red cells were incubated with specific iso-antibodies in the presence of white cells. Iso-antibodies used were anti-A (dog), anti-A (human), anti-B (human), and anti-Rh. Several different antibodies of each type were used. (2) Serum or plasma from cases

of auto-immune haemolytic disease which showed erythrophagocytosis in the peripheral blood was incubated with normal red cells of the same blood group after the addition of white cells.

Method.—In both groups of experiments a volume of serum or plasma four times that of the cells was used because smaller amounts failed to produce erythrophagocytosis with some antibodies when the larger volumes were successful. The leucocyte-erythrocyte-antibody mixtures were prepared as follows, taking an A+anti-A system as example. Leucocytes, erythrocytes, and plasma from 10 ml. of fresh heparinized normal group A blood were separated by centrifugation. The buffy coat was placed in 2.5 ml. of warm saline and incubated at 37° C. until required. The red cells were washed three times with warm saline and reagents were then added to four tubes as indicated in Table I.

TABLE I
PREPARATION OF TUBES FOR TESTING AN A+ANTI-A SYSTEM

Reagents	Tube Number			
	1	2	3	4
Packed group A erythrocytes (ml.)	0.5	0.5	0.5	0.5
Leucocyte-saline mixture (ml.)	0.5	0.5	0.5	0.5
Group O or B plasma or serum (ml.)	2	2		2
* Heated group O or B plasma or serum (ml.)			2	
Guinea-pig serum (ml.)		0.5		
* Heated guinea-pig serum (ml.)				0.5
Saline (ml.)	0.5		0.5	

* At 56° C. for 5 min.

The guinea-pig serum was freshly reconstituted pooled lyophilized serum and was used in a 1 in 2 dilution; 0.5 ml. contained approximately 40 units of haemolytic complement. The tubes were mixed and incubated for one hour. Tube 4 was not used in all experiments. This method usually produced a good concentration of intact leucocytes in the cover-slip preparations.

Iso-antibodies.—Positive results of varying degree were obtained with all the antibodies tested. Neutrophils, monocytes, and occasionally eosinophils were involved in the phagocytic process and with some antibodies more than 90% of potential cells were actually phagocytic. Erythrophagocytosis occurred independently of haemolysis, varied with the titre of the antibody, persisted even after the serum was heated, and was inhibited to some extent by added guinea-pig serum. The results obtained with three A+anti-A systems are

TABLE II
OCCURRENCE OF ERYTHROPHAGOCYTOSIS WITH THREE
A+ANTI-A SYSTEMS

System	Tube Number								
	1			2			3		
	1	2	3	1	2	3	1	2	3
Haemolysis	+++	+	—	++++	+	—	—	—	—
% of Cells Phagocytic									
Neutrophils	97	61	41	78	17	6	96	48	10
Monocytes	71	50	70	38	13	8	75	84	45

shown in Table II. The same A cells were used in all three experiments. The source of A antibody in systems 1 and 2 was serum from a group O subject who had proved to be a dangerous blood donor for A recipients (Ervin and Young, 1950); it was an immune type iso-antibody with a titre of 1 in 4,096. Unaltered serum was employed in system 1 and, for comparison, another batch was diluted (antibody titre 1 in 32) for use in system 2. The A antibody in system 3 was one naturally occurring in a group B subject. Table III shows the results obtained with two canine anti-A sera, each acting against different canine group A cells, and with two anti-Rh sera using the same O Rh-positive cells. The first anti-Rh serum contained a saline agglutinin and the second an incomplete antibody.

TABLE III
OCCURRENCE OF ERYTHROPHAGOCYTOSIS

System	Tube Number							
	1		2		3		4	
	1	2	1	2	1	2	1	2
(a) With two canine A+anti-A systems								
Haemolysis	—	—	++	++	—	—	—	—
% of Cells Phagocytic								
Neutrophils	2	4.5	0	1.5	1	4		
Monocytes	17	21	0	3	5	10		
(b) With two Rh+anti-Rh systems								
Haemolysis	—	—	—	—	—	—	—	—
% of Cells Phagocytic								
Neutrophils	4	3	0.5	0	1.5	1.5	0.5	1
Monocytes	11	9	0	1	6	4	2	1

A striking feature in several of the preparations was great distension of the red cells after ingestion by white cells. This was most marked with very high titre anti-sera. In one instance (A antibody of titre 1 in 4,096) the diameter of the erythrocytes increased up to three times and the

cytoplasm and nucleus of the leucocytes enveloped them as a thin rim. Ultimately, and especially if several red cells were ingested, the leucocytes burst. As many as eight red cells were seen within a single phagocytic cell.

Auto-antibodies.—Serum from three cases of auto-immune haemolytic disease which showed erythrophagocytosis in the peripheral blood was tested against normal red cells of the same blood group. Erythrophagocytosis was found in two instances but not in the third. The degree of phagocytosis was much less than in the iso-antibody systems, but the effects of heating the serum and adding guinea-pig serum were the same as in the previous experiments. In one of the systems the percentages of phagocytic cells were as follows: Tube 1, neutrophils 6, monocytes 16; tube 2, neutrophils 0.2, monocytes 0; tube 3, neutrophils 1.5, monocytes 3.2. The red cells became sensitized, as demonstrated by the anti-globulin test, in only one of the two positive systems.

Summary and Conclusions

Erythrophagocytosis develops with regularity when heparinized samples of peripheral blood from cases of auto-immune haemolytic disease are incubated.

It can easily be produced artificially by incubating red and white blood cells with anti-sera containing specific iso-antibodies.

Serum from cases of auto-immune haemolytic disease can sometimes influence normal red cells in such a way that they are ingested and destroyed by white cells.

There is no clinical evidence that ingestion of red cells by circulating phagocytic cells is important as a cause of anaemia in auto-immune haemolytic disease. It is, at present, simply an interesting biological phenomenon which provides visual information on the destruction of red cells.

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