

Influence of Cells and Plasma on *In vitro* Survival of Malaria Sporozoites.* (17379)

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It has been shown that the sporozoites of *Plasmodium cathemerium* 3H2, infective for the canary but not the hen, retain their infectivity during several hours in drawn canary but not drawn hen blood.¹ The present communication reports investigation of the effect of the cell and plasma portions of these bloods upon the retention of infectivity.

Mixtures of canary and hen whole bloods. Infected mosquitoes were ground in Locke's solution and added to the following freshly prepared mixtures: (a) one-tenth canary and nine-tenths hen blood; (b) five-tenths canary and five-tenths hen blood; (c) nine-tenths canary and one-tenth hen blood. After incubation of the blood-sporozoite mixtures with gentle agitation at 41.5°C for 1½ hours, portions representing one mosquito were injected intramuscularly into canaries, the peripheral blood of these birds being subsequently examined from the 5th to 16th days, inclusive. Birds failing to become positive were proved negative in all instances by subsequent infection from mosquito bites. Trials were made at such intervals that in most instances at least one insectary cycle had been completed between them.

In the trials employing mixture (a), 27% of the birds became infected (see Table I); mixture (b) infected 72% and mixture (c) 87%.

Canary and hen plasmas alone. Sporozoites were separately incubated in canary and hen plasmas. In two trials (See Table I), they failed to retain infectivity in either plasma.

Canary plasma alone and with canary cells. Four trials were made of sporozoites in canary plasma alone and in another portion of the same plasma to which the normal amount of canary cells was returned after 3 washings in

Locke's solution. None of the sporozoites retained their infectivity in the plasma alone (see Table I), but they did so in 100% of instances when the cells were present.

Canary plasma with canary cells, hen cells, and both. Seven trials were made of sporozoites in canary plasma to which the washed canary cells were returned, canary plasma to which an equivalent amount of washed hen cells were added, and canary plasma in which one-half the added washed cells were canary and the other half hen. When canary cells alone had been added, sporozoite infectivity was retained in 100% of instances (see Table I); when hen cells alone had been added, infectivity was lost completely; when the canary-hen cell mixture had been added, infectivity was retained to the extent of 74%.

Hen plasma with canary cells, hen cells, and both. These 5 trials constituted a test just the reverse of the above in that the fluid medium was hen instead of canary plasma. When hen cells were added there was no retention of sporozoite infectivity (Table I); when canary cells were added infectivity was retained 100%; when a mixture in equal parts of the two types of cells was added infectivity was 85% retained.

Canary plasma with canary white and red cells, separately. In 5 trials, after the initial separation of canary plasma and cells by centrifugation, the cells were washed 3 times in Locke's solution and then loaded through a long needle into the bulb and stem of a 2 cc Ostwald-Folin pipette modified by cutting off the delivery tip below the bulb, fusing the lower opening, and cutting down the suction stem to a size suited to the centrifuge. Spinning of this improvised centrifuge tube loaded with washed total blood cells delivered the white cells as "buffy layer" into the suction stem, whence they were removed with a thinly drawn capillary tube. One small drop of this buffy layer was blown into a 0.6 cc mixture,

* Research supported by National Institute of Health Research Grant 67 (C2).

¹ Beckman, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 172.

TABLE I.
Survival of Malaria Sporozoites in Various Whole Blood, Plasma and Cell Mixtures.

Mixtures of canary and hen whole bloods (C = canary, H = hen).			
Trial No.	1/10 C-9/10 H (birds infected)	5/10 C-5/10 H (birds infected)	9/10 C-1/10 H (birds infected)
1	1/4	2/3	4/4
2	3/5	5/5	5/5
3	2/5	4/4	5/5
4	1/5	3/5	3/5
5	0/8	7/8	8/8
6	1/8	1/8	5/7
7	4/8	7/7	6/7
Totals	12/43 (27%)	29/40 (72%)	36/41 (87%)
Canary and hen plasmas alone.			
Trial No.	C plasma (birds infected)	H plasma (birds infected)	
1	0/12	0/12	
2	0/12	0/12	
Totals	0/24	0/24	
Canary plasma alone and with Canary cells.			
Trial No.	Alone (birds infected)	With C cells (birds infected)	
1	0/6	6/6	
2	0/6	6/6	
3	0/6	6/6	
4	0/6	6/6	
Totals	0/24 (0%)	24/24 (100%)	
Canary plasma with Canary cells, Hen cells, and both.			
Trial No.	With C cells (birds infected)	With H cells (birds infected)	With C and H cells (birds infected)
1	5/5	0/5	4/5
2	5/5	0/5	2/5
3	5/5	0/5	1/5
4	4/4	0/4	4/4
5	8/8	0/7	8/8
6	7/7	0/7	6/8
7	8/8	0/8	7/8
Totals	42/42 (100%)	0/41 (0%)	32/43 (74%)
Hen plasma with Canary cells, Hen cells, and both.			
Trial No.	With C cells (birds infected)	With H cells (birds infected)	With C and H cells (birds infected)
1	4/4	0/4	4/4
2	4/4	0/4	4/4
3	4/4	0/4	1/4
4	4/4	0/4	4/4
5	4/4	0/4	4/4
Totals	20/20 (100%)	0/20 (0%)	17/20 (85%)
Canary plasma with Canary white and red cells, separately.			
Trial No.	White cells (birds infected)	Red cells (birds infected)	Plasma alone (birds infected)
1	5/5	3/5	0/5
2	5/5	3/5	0/5
3	5/5	3/5	1/5
4	8/8	3/8	0/8
5	5/7	1/8	0/8
Totals	28/30 (93%)	13/31 (41%)	1/31 (3%)

half of which was canary plasma and half Locke's solution containing the sporozoites.

One small drop of red cells obtained from deep down in the bulb of the pipette was then

blown into another portion of the same plasma-Locke-sporozoite mixture. Incubation and subsequent injections as usual.

Survival of sporozoite infectivity occurred in 93% of instances when white cells alone had been returned to the plasma (see Table I); in 41% when red cells alone had been added; in 3% when presumably plasma alone was present.

Discussion. In the recent interesting paper of McGhee,² who succeeded in infecting Swiss mouse erythrocytes injected intravenously into chick embryos 2 days after infecting the embryos intravenously with *P. lophurae* trophozoites, it is suggested that perhaps the malaria resistant principle in certain species lies in the serum constituents rather than in the cells. The studies herein reported indicate that the reverse is the case with regard to sporozoite survival, at least in drawn blood. The sporozoites in these experiments did not survive in the plasma of either the resistant or the susceptible species, but they survived equally well in the plasma of either species provided cells of the susceptible species were present. They did not survive, however, in the plasma of the susceptible species if cells of the resistant species alone were added, though they survived extremely well in the plasma of either species if, in addition to cells of the resistant species, cells of the susceptible species were present. Apparently, therefore, insofar as one can draw conclusions from a study of two species of the same phylum, the survival of sporozoites in drawn blood depends upon the presence therein of cells of the susceptible species. This would appear to conform to the *in vivo* observation of Huff and Coulston,³ who were unable to prevent sporozoite infection of *P. gallinaceum*-susceptible chickens by inoculation of them with the whole blood, serum or plasma of non-susceptible ducks, but who did succeed in conveying to ducks some of the susceptibility of the chicken by inoculating them with chicken blood.

² McGhee, R. B., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 92.

³ Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1946, **78**, 99.

Whether it is the white or red blood cells of the susceptible species which make survival possible was not conclusively determined in these studies, though the evidence (Table I) favors the greater importance of the white cells. If a technic can be evolved which will deliver pure samples of white and red cells, it should be possible to settle the point. Contamination of the white cells with a small number of red cells would not probably be important in view of the evidence, but it appears that the presence of only a few white cells in the red cell sample could considerably facilitate survival because it required only one small drop of the buffy layer in 0.6 cc of diluent to enable practically complete survival to take place.

No evidence of the nature of the action of the canary cells in facilitating sporozoite survival *in vitro* was obtained. Prolonged study of stained smears made at various times during the incubation of the mixtures did not reveal sporozoites or anything resembling pre-erythrocytic forms in any of the cells. Immunologic studies were not performed.

Summary. In a study of the role of cells and plasma in retention of infectivity of sporozoites of *P. cathemerium* 3H2 in canary blood and the failure of such retention in hen blood, it was found that (a) survival occurs in hen whole blood provided a small amount of canary whole blood is present; (b) it does not occur in either canary or hen plasma alone; (c) it occurs in either canary or hen plasma if canary cells are present whether or not hen cells are simultaneously present but does not occur in either plasma if only hen cells are present; (d) it occurs in canary plasma to a greater extent if canary white cells (somewhat contaminated with canary red cells) are present than if canary red cells (somewhat contaminated with white cells) are present. The possible significance of the findings is discussed.

Technical assistance of Darrell B. Paul is gratefully acknowledged.

Received August 18, 1949. P.S.E.B.M., 1949, **72**.