Inhibition of Chicken Tumor I by Plasma from Chickens Infected with an Avian Malaria Parasite. (20356)

W. TRAGER AND R. B. MCGHEE.

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Certain similarities in the pattern of age resistance of chickens to tumor I of Rous(1,2) and to the malaria parasite *Plasmodium lophurae*(3) suggested that these two infections might influence each other in some way. No effect of the tumor on the course of the malarial infection was observed, but a series of experiments showed that the growth of the tumor was temporarily inhibited by the malarial infection(4). Preliminary experiments then indicated that plasma, taken from malarious chickens and injected intravenously into non-malarious chickens previously inoculated with a suspension of tumor tissue, might have an inhibitory effect on the tumor. This indication has been fully supported by the results of 5 successive experiments designed in the following way.

Materials and methods. For each experiment a group of day-old Rhode Island Red chickens was obtained and reared in a uniform manner. When the chickens were 2 weeks old, one of the authors (A) inoculated a suitable number of them with chicken tumor I (a strain which had been maintained in the laboratory of Dr. J. B. Murphy at the Rockefeller Institute and which was obtained through the courtesy of Dr. K. R. Porter). Each chicken received into each side of the breast muscle 0.2 ml of a suspension made by grinding in a glass grinder tumor tissue (from a young chicken inoculated 8 to 10 days previously) in nutrient broth in the proportion of 1 g of tissue to 2 ml of broth. The other author (B) then weighed the chickens and arranged them on the basis of weight into comparable groups. In each of the 5 experiments one such group was treated with normal plasma and a second with malarious plasma. In 4 of the experiments a third group was left without further treatment, and in one experiment a fourth group was treated with a second preparation of malarious plasma. The plasma samples were all prepared by worker A. For each treatment, 8 to 11 normal chickens of the same age as the tumor chickens, or 2 or 3 older chickens, depending on the experiment, were bled from the heart. For each 10 ml of blood, 1 ml of a solution containing, per 100 ml, 27 mg heparin and 0.85 g sodium chloride, was used as an anticoagulant. The individual blood samples were centrifuged and the plasmas were pooled. In a similar manner, pooled plasma was prepared from corresponding malarious chickens. These were in the 4th, 5th, or 7th day, depending on the experiment, of an infection with *P. lophurae*. Strain 12A of this parasite was used. It was passed in young chickens at 4- to 6-day intervals. Chickens to be used as donors of malarious plasma were inoculated with 0.2 ml, per 100 g body weight, of blood containing approximately 50 to 75 parasites per 100 red blood cells. The samples of pooled plasma, prepared by worker A, were turned over to worker B, who administered the treatments. Each treatment consisted of the slow intravenous injection of either 5 ml or 2.5 ml of the appropriate plasma to each chicken. On one or several days during the course of the experiment, worker A made an estimate of the relative size of the tumors. This estimate could not have been influenced in a subjective way, since worker A not only did not know which chicken received which treatment, but he also did not even know the arrangement of the chickens by groups. On the last day of the experiment (8 to 11 days after inoculation of the chickens with tumor tissue) blood films stained with Giemsa stain were prepared from the chickens which had been treated with malarious plasma. All of the chickens were then killed with chloroform, placed in paper bags, and left for several hours in a refrigerator. This treatment rendered the contents of the tumors less fluid. Worker A, still without knowledge of the grouping of the chickens, then cut into the breast muscle of each chicken, removed all of the loose mucoid tumor tissue which could be spooned and scraped

TABLE I. Effect of Intravenous Administration of Malarious Plasma on Mean Tumor Weight of Chickens Inoculated with Tumor I Tissue. 5 experiments.

Group	No. chickens	Treatment: Plasma	Treatment: Dose, ml	Treatment: On days*	Day* tumors weighed	Mean wt tumors, g	Diff. of mean: g	Diff. of mean: p‖	Ratio, mean tumor wt Group A/ mean tumor wt Group B
A	8	Normal‡	5	4–10	11	16	A – B = 7	.1	1.8
B	8	5-day m†‡	5	4–10	11	9			
A	10	Normal‡	5	4–8	9	24	A – B = 10	.02	1.7
B	10	5-day m‡	5	"	9	14	C – B = 10	.02	
C	10	0	—	—	9	24	C – A = 0		
A	10	Normal‡	5	1–8	9	13	A – B = 5	.1	1.6
B	10	5-day m‡	5	"	9	8	C – B = 15	<.01	
C	10	0	—	—	9	23	C – A = 10	.02	
A	10	Normal§	2.5	4–7	8	16	A – B = 5	.1	1.5
B	10	4-day m§	"	"	8	11	C – B = 6	<.01	
C	10	0	—	—	8	17	C – A = 1		
A	10	Normal§	2.5	4–7	8	18	A – B = 7	.05	1.6
B	10	4-day m§	"	"	8	11	C – B = 6	.05	
C	10	0	—	—	8	17	A – D = 5	>.1	
D	10	7-day m§	2.5	4–7	8	13	A – C = 1		

* After day of inoculation with tumor tissue.
† m = malarious.
‡ From chickens of same age as recipients.
§ From 3-month-old chickens.
‖ $p < 0.01$ for the differences between the means of Groups A and B in the entire series of 5 exp.

TABLE II. Body Weights and Tumor Weights of Individual Chickens of Exp. 5 (Table I) on the 8th Day after Inoculation with Tumor Tissue.

Treatment							
Group A Normal plasma		B 4-day malarious plasma		C No treatment		D 7-day malarious plasma	
Body wt, g	Tumor wt, g	Body wt, g	Tumor wt, g	Body wt, g	Tumor wt, g	Body wt, g	Tumor wt, g
215	22	185	12	234	21	222	14
264	26	241	2	232	5	212	19
273	9	246	16	246	21	250	16
225	20	243	7	224	17	275	10
287	29	272	13	281	6	280	10
252	5	229	14	249	14	261	14
224	17	209	9	236	17	255	19
153	12	222	9	175	14	209	16
248	6	221	8	232	31	250	14
295	29	314	19	280	21	290	2
Avg							
244	18	238	11	239	17	250	13

out, and weighed it. In this way there was obtained a quantitative measure of the amount of tumor development.

Results. The details of the treatments and the principal results of the 5 experiments are summarized in Table I. The consistency of the results, as expressed in the ratio of mean tumor weight of chickens treated with normal plasma to mean tumor weight of chickens treated with 4- or 5-day malarious plasma, is obvious. This consistency is reflected in the high degree of statistical significance of the differences between these means for the entire series of experiments ($p < 0.01$ as estimated from a calculation of $t(5)$). The low statistical significance of the difference in mean tumor weight between Groups A and B (Table I) in certain individual experiments is largely a result of the well-known inherent variability in the response of unselected chick-

TABLE III. Estimated Sizes of Tumors of Chickens of Exp. 3 (Table I) during Treatment.

Group	Treatment	Sum of estimated sizes (± = 0.5, + = 1, 2+ = 2, etc.) on day after inoculation 6	7	8	9
A	Normal plasma	17	19	24	22.5
B	Malarious "	7.5	8	15	18
C	0	20	23	29	32

ens to inoculation with tumor I. This variability is well illustrated in the results of Exp. 5, presented in detail in Table II. Table II also shows that there were no significant differences between any of the groups with respect to final body weight ($p > 0.9$). This was true in the other 4 experiments as well.

In only one experiment (Exp. 3) was there any difference between the mean tumor weight of the untreated group (C) and that of the group (A) treated with normal plasma (Table I). This also happened to be the only experiment in which the treatments were begun at one day rather than at 4 days after inoculation with tumor tissue. Interestingly enough, the effect of the normal plasma became apparent late in the relatively long course of treatment, whereas the effect of the malarious plasma was most noticeable early in the course of treatment (Table III).

In all the experiments, the estimates of tumor size made on the living chickens during the course of treatment showed that the tumors tended to become apparent later and to grow more slowly in the chickens treated with malarious plasma than in those treated with normal plasma or left untreated. For example, in Exp. 2, on the 7th day after inoculation with tumor tissue, 3+ to 4+ tumors were present in 2 of the chickens in Group B, 7 of the chickens in Group A, and 7 of the chickens in Group C. Again, in Exp. 5, on the morning of the 6th day and hence after only 2 treatments, the numbers of chickens in each group of 10 which still showed small growths (rated as ± or +) were: A (normal plasma) 3; B (4-day malarious plasma) 6; C (untreated) 3; D (7-day malarious plasma) 4. The remaining chickens in each group had tumors rated as 2+ or 3+.

With the dosages of malaria parasites which were used to infect the donors of malarious plasma, the malarial infection generally reached its peak on about the 6th day in young chickens and the 5th day in the older chickens. Hence all of the donors of plasma for the Group B chickens of each experiment were used shortly before the infection would have reached its peak. Since plasma taken on the 7th day of infection was used in only one experiment (Exp. 5, Group D) no definite conclusions concerning it can be drawn. However, the intermediate type of result obtained with it, or the possible complete lack of significant effect, was in keeping with a number of preliminary experiments which indicated that the most effective plasma could be obtained shortly before, rather than at or after, the peak of the infection.

Plasma obtained by the centrifugation of blood heavily infected with malaria parasites may be expected still to contain a few parasitized erythrocytes. These might be capable of initiating a malarial infection in the chickens which received large amounts of such plasma. Therefore the blood films prepared from these chickens on the last day of each experiment were each examined for malaria parasites for a period of 5 minutes. In this way, a few of the chickens were found to have very light infections. For example, in Exp. 3, in which the greatest amount of plasma was given, one parasite was found in the smear from one chicken and 2 parasites in the smear from another. It was known from the earlier work with chickens infected simultaneously with *P. lophurae* and tumor I that such light malarial infections had no effect on the tumor growth(4). *P. lophurae* was a fortunate experimental material from this point of view, for in chickens large doses of this parasite are required to produce a severe infection. Hence a few stray parasites introduced with the plasma in most cases produced no patent infection and could not have been responsible for the effects which were observed.

Discussion. It is evident that the plasma of chickens near the peak of an acute infection with *P. lophurae* exerts an inhibitory effect on tumor growth when it is injected into young chickens previously inoculated with chicken tumor I tissue. A simple hypothesis to ex-

plain this result would assume that there appears in the plasma of the malarious chickens an increased concentration of some nonspecific reaction product which has growth-inhibiting properties. An increase in lipoproteins has in fact been demonstrated in birds undergoing malarial infections(6). This may provide a clue as to the type of substance to be looked for.

It is of interest to note the recent observation(7) that the life of leukemic mice was prolonged when they were infected with *Plasmodium berghei,* a malarial parasite of certain rodents.

Summary. Two-week-old chickens were inoculated in the breast muscle with a suspension of chicken tumor I tissue. They were subsequently left untreated or given a series of intravenous injections of either normal chicken plasma or plasma from corresponding chickens which were near the peak of an acute infection with *Plasmodium lophurae.* On the day after the last treatment the tumors which had developed were weighed. In a series of 5 experiments, the mean tumor weight of the chickens left untreated or treated with normal plasma was 1.5 to 1.8 times greater than that of the chickens treated with malarious plasma.

1. Rous, P., and Murphy, J. B., *J. Exp. Med.,* 1914, v20, 419.

2. Duran-Reynals, F., *Yale J. Biol. and Med.,* 1941, v13, 61.

3. Trager, W., and McGhee, R. B., *J. Exp. Med.,* 1950, v91, 365.

4. Trager, W., *Fed. Proc.,* 1951, v10, 374.

5. Fisher, R. A., *Statistical Methods for Research Workers,* Oliver and Boyd Ltd., Edinburgh, 1944.

6. Trager, W., *J. Exp. Med.,* 1947, v85, 663.

7. Nadel, E. M., Personal communication.

Received May 27, 1953. P.S.E.B.M., 1953, v83.

Serum Complement Levels in Dogs Undergoing Kidney Homotransplantation.* (20357)

C. B. FAVOUR, JOSEPH E. MURRAY, C. T. WEMYSS, JR., A. COLODNY, AND BENJAMIN F. MILLER.

From the Departments of Medicine and Surgery, Peter Bent Brigham Hospital and Harvard Medical School, Boston.

Success with corneal(1), bone(2), and aortic(3) homotransplantation has further stimulated interest in the possibility of skin and renal transplantation in human medicine. Advances in surgical technics which have made possible autotransplantation of kidneys in experimental animals(4) as well as temporary homotransplantation in man(5) have focused new attention on the phenomenon of individual tissue specificity(6).

The mysterious failure of skin and kidney homotransplants days to weeks following an initial period of functional survival is suggestive of an immune response to an antigenic stimulus. Coincident with the development of experimental renal lesions induced by large doses of serum globulin a temporary depression of serum complement levels has been observed(7). Similar depressions of complement have also been noted in nephrotoxic nephritis(8) and human glomerulonephritis (9,10).† In the present study serial serum complement levels were determined in a group of dogs undergoing kidney transplantation. A comparison of these findings with patterns

* This research was aided by grants from the Bristol-Myers Pharmaceutical Co. and the National Institutes of Health of the U. S. Public Health Service.

† Suppression of circulating complement is not unique for renal disease. Studies of C. A. Ianewav and co-workers (12) have shown this phenomenon to be a feature of "serum sickness" reactions produced by relatively pure antigens (albumin) which involve the arterioles. The most marked and sustained serum complement suppression observed in humans occurs in disseminated *lupus erythematosis* (11) with or without obvious renal disease.