results would also be possible. If one examines the data with the hypothesis that α-TPh has a direct inhibitory action on DPNase, one might consider that, if calcium were partially removed by phosphate, there would be less residual calcium available to α-TPh, more α-TPh uncombined with calcium, and a resulting greater DPNase inhibition. Conversely, if α-TPh were added to the buffer first, followed by calcium, the α-TPh would compete with phosphate for calcium and thus would have a better chance of reacting with calcium ions; the precipitation of α-TPh would be more complete, and less DPNase inhibition would result. Consequently, the direct inhibition of DPNase by α-TPh seems to us to be a valid possibility.

TABLE 1

INHIBITION OF DPNASE BY α-TPH AND EFFECT OF CALCIUM*

		Calcium added before α-TPh			Calcium added after α-TPh	
Molarity of α-TPh		5.6×10^{-4}	1.4×10^{-3}	2.8×10^{-3}	1.4×10^{-3}	2.8×10^{-3}
Molarity of $CaCl_2$	0	1.0	26.1	42.7	28.8	54.1
	3.3×10^{-4}	1.0	28.7		26.4	
	3.3×10^{-3}		15.5	41.4	3.5	8.8
	3.3×10^{-2}		10.0		4.0	

* Figures represent percentage inhibition of DPNase.

In the great majority of these experiments (10 out of 11) the addition of calcium to the control tubes, without α-TPh, produced no acceleration of DPN breakdown in the time intervals used. Swingle, *et al.* (*4*) have reported activation of DPNase in rat liver by calcium in experiments of 20-min duration. Since our first readings were made at 15 min, we may have failed to see the phenomenon. Of course, it is possible that heart-muscle DPNase does not require calcium for activation or, more probably, that sufficient calcium already is present. However, experiments using washed homogenates showed a great decrease in DPNase activity but no activation of the DPNase remaining was observed following the addition of calcium.

In conclusion, it may be said that α-tocopheryl phosphate has been shown by direct DPN estimation to inhibit DPNase. The observations of Ames that calcium relieves this inhibition have been confirmed. In our opinion, there are still bases for the possibility that α-TPh has a direct inhibitory effect on DPNase.

References

1. AMES, STANLEY R. *J. biol. Chem.*, 1947, **169**, 503.
2. GOVIER, WM. M., YANZ, N., and GRELIS, M. E. *J. Pharm. exp. Therap.*, 1946, **88**, 373.
3. LEVITAS, N., ROBINSON, J., ROSEN, FRED, HUFF, J. W., and PERLZWEIG, W. A. *J. biol. Chem.*, 1947, **167**, 169.
4. SWINGLE, K. F., AXELROD, A. E., and ELVEHJEM, C. A. *J. biol. Chem.*, 1942, **145**, 581.
5. WILLIAMSON, S., and GREEN, D. E. *J. biol. Chem.*, 1940, **135**, 345.

The Sex of the Host as a Factor in *Plasmodium gallinaceum* Infections in Young Chicks

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In chicks inoculated with *P. gallinaceum* Brumpt, the percentage of parasitized erythrocytes, the rate of endothelial invasion, and the therapeutic efficacy of quinine have been observed to differ significantly with the sex of the host.

Fourth-day parasite counts[1] were accumulated on 449 White Rock chicks inoculated at 6–8 days of age with

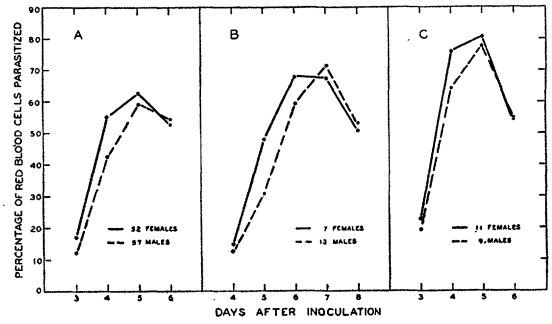

FIG. 1. Average daily parasite counts of male and female chicks infected with *P. gallinaceum*: (A) 109 White Rock chicks inoculated at 8 days of age with 16×10^6 parasitized red cells; (B) 20 White Rock chicks inoculated at 8 days of age with 1,000,000 parasitized red cells; (C) 20 Rhode Island Red chicks inoculated at 7 days of age with 16×10^6 parasitized red cells.

16×10^6 parasitized red cells (*1*). Analysis by sex showed that for 222 females the counts averaged 62.3% (S.E. = 1.2), whereas for 227 males the counts averaged 53.8% (S.E. = 1.2). Comparable sex differences were also found in the daily parasite counts in two different breeds of infected chicks (Fig. 1).

To determine whether the sex difference in parasite counts could be experimentally increased, male and female sex hormones of proven activity in chicks (*6*) were administered. Ninety infected chicks were divided into 3 equal groups. Each chick in group A was injected intramuscularly with 0.1 mg of testosterone propionate in 0.1 cc of corn oil daily for 6 days, beginning on the day before the inoculation. Chicks in group B were similarly injected with 0.1 mg of α-estradiol benzoate, and each of the controls in group C received 0.1 cc of corn oil alone daily for the same period. The difference in parasite counts in the two sexes was not significantly increased by the administration of male or female sex hormones under the conditions of these experiments.

[1] Smears of peripheral blood were air dried, fixed with methyl alcohol, and stained with Giemsa. When the parasite count was 30% and above, it was obtained by counting the parasitized cells in 100 erythrocytes. When lower counts were found, the number of erythrocytes examined was increased so that the probable error remained under 10% (*4*).

The incidence of exoerythrocytic schizonts (*5*) in the capillary endothelium of the brain of chicks examined (*2*) at various intervals after inoculation of sporozoites

TABLE 1

INCIDENCE OF EXOERYTHROCYTIC SCHIZONTS IN THE CAPILLARY ENDOTHELIUM OF THE BRAIN OF CHICKS EXAMINED 6–12 DAYS AFTER THE INTRAMUSCULAR INJECTION OF SPOROZOITES OF *P. gallinaceum*

	6th–10th day		11th day		12th day	
	No. examined	% positive	No. examined	% positive	No. examined	% positive
Females	61	65.6	41	90.2	40	92.5
Males	67	43.3	55	69.1	57	91.2
$\frac{\text{diff.}}{\text{S.E.}_{diff.}}$*		2.59		2.71		NS†

* $\frac{\text{diff.}}{\text{S.E.}_{diff.}}$ represents the ratio of the observed difference to its Standard Error. Values greater than 2.00 are considered significant.

† NS = not significant.

(*3*) is presented in Table 1. These data show that the female chicks evidence endothelial invasion earlier than the males.

Parasite-count differences between the males and females were observed also in 418 blood-inoculated chicks treated with a suppressive level of quinine hydrochloride

TABLE 2

FOURTH-DAY PARASITE COUNTS OF INFECTED CHICKS* TREATED WITH QUININE†

	No. treated	Average count	Chicks showing count of 30% and above	
			No.	%
Females	212	7.28%	15	7.1%
Males	206	4.60%	5	2.4%
$\frac{\text{diff.}}{\text{S.E.}_{diff.}}$		2.55		2.3

* All chicks received 16×10^6 parasitized red cells on the 7th or 8th day of life.

† 0.02 mg quinine hydrochloride/gm of body weight was given by capsule twice daily for 4 days, starting on the day of inoculation.

(Table 2). It should be noted further that, of the 20 chicks in this group that showed a parasite count of 30% and above (*i.e.* therapeutic failures or "breakthroughs"), 15 were females.

Thus, statistical analysis of the infections of young chicks inoculated with *P. gallinaceum* revealed significant differences attributable to the sex of the host. As compared with the males, the female chicks showed higher parasite counts, experienced earlier endothelial invasion, and were less effectively protected by quinine. Treatment with male and female sex hormones under the conditions specified did not increase the difference between the average parasite counts in the two sexes.

References

1. BENNISON, B. E., and COATNEY, G. R. *Publ. Hlth Rep.*, 1945, **60**, 127–132.
2. COATNEY, G. R., COOPER, W. C., and MILES, V. I. *Amer. J. Hyg.*, 1945, **41**, 109–118.
3. COATNEY, G. R., COOPER, W. C., and TREMBLEY, H. L. *Amer. J. Hyg.*, 1945, **42**, 323–329.
4. HARTMAN, ERNEST. *Amer. J. Hyg.*, 1927, **7**, 407–432.
5. JAMES, S. P., and TATE, P. *Parasitol.*, 1938, **30**, 128–139.
6. TABER, E., DAVIS, D., and DOMM, L. *Amer. J. Physiol.*, 1943, **138**, 479–487.

The Effect of Relative Concentration on Complement Fixation by Identical Amounts of Antigen and Antibody

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In the course of attempts to standardize influenza virus A or B antigens it was noted that there was sometimes considerable variation in the amount of complement fixed by two antigens prepared from the same strain by identical methods and titrated together in the same test. Investigation revealed the variation to be due to the fact that equivalent amounts of the same antiserum and antigen fix different amounts of complement when they are mixed at different volumes, even when the volumes are quickly equalized by the addition of appropriate amounts of saline. The phenomenon was also observed with soluble pneumococcus polysaccharides and their respective antisera, and, using these systems, it was found that there is an optimum volume, at which a maximum amount of complement is fixed, for given constant amounts of antibody and antigen. To show that this is true at several antigen-antibody ratios is the purpose of this preliminary report.

The technic employed was that of Rice (*13*). Experience brought out the necessity of taking pains in pipetting. To 0.05 ml of diluted pneumococcus type-32 rabbit antiserum[1] was added 0.10 ml of complement containing six 50% units, three different doses of purified (*1*) type-32 polysaccharide[2] solution in varying volumes of different dilutions, and sufficient 0.85% NaCl solution to make a total volume in each tube of 0.30 ml (Table 1). The reagents were added in the order given. The time which elapsed between the addition of the antigen and the adjustment of total volume to equality by the addition of saline was 5–10 min. After incubation at 3°–6° C for 24 hrs, 0.2 ml of sensitized sheep red blood cells was pipetted into each tube and the mixtures incubated at 37° C for exactly 15 min. The degree of hemolysis was read immediately by comparison with a color standard,

1 We wish to thank Miss Gretchen R. Sickles for the antiserum.

2 We are grateful to Dr. Rachel Brown for the purified pneumococcus polysaccharide.