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.

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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.

according to the methods of this Division (*14*). Appropriate serum, antigen, complement, and cell controls were included and showed that nonspecific reactions did not occur.

were mixed at different volumes, and this difference was eliminated within 5–10 min when the volumes were rectified by the addition of saline. The phenomenon is therefore unlikely to be due to variation of one reagent, nor

TABLE 1

EFFECT OF RELATIVE CONCENTRATION OF ANTIGEN ON AMOUNT OF COMPLEMENT FIXED BY IDENTICAL AMOUNTS OF ANTIGEN AND ANTISERUM*

Antigen		Saline	Serum dilution (0.05 ml)				
Dose (γ)	Dilution	Amount pipetted (ml)	Amount pipetted (ml)	1:1,000	1:1,100	1:1,200	1:1,300	1:1,400
				Hemolysis (%)				
0.05	1:400,000	0.02	0.13	70	80	85	95	100
	1:800,000	0.04	0.11	65	75	80	90	100
	1:1,000,000	0.05	0.10	60	70	75	85	100
	1:1,200,000	0.06	0.09	50	60	70	80	100
	1:1,400,000	0.07	0.08	45	55	65	75	100
	1:1,600,000	**0.08**	0.07	**40**	**50**	**55**	**65**	**100**
	1:1,800,000	0.09	0.06	55	60	70	80	100
	1:2,000,000	0.10	0.05	60	80	85	85	100
	1:2,400,000	0.12	0.03	65	85	90	90	100
	1:3,000,000	0.15	0	70	90	95	100	100
0.02	1:1,000,000	0.02	0.13	55	65	75	90	95
	1:1,500,000	0.03	0.12	50	60	70	85	90
	1:2,000,000	0.04	0.11	45	55	60	80	85
	1:2,400,000	0.05	0.10	40	50	55	75	80
	1:3,000,000	**0.06**	0.09	**35**	**40**	**45**	**70**	**75**
	1:4,000,000	0.08	0.07	45	50	55	75	80
	1:5,000,000	0.10	0.05	50	55	65	80	85
	1:6,000,000	0.12	0.03	55	60	75	85	95
	1:7,000,000	0.14	0.01	65	70	80	90	100
	1:7,500,000	0.15	0	70	75	85	95	100
0.01	1:3,000,000	0.03	0.12	100	100	100	100	100
	1:4,000,000	0.04	0.11	95	100	100	100	100
	1:5,000,000	0.05	0.10	90	95	100	100	100
	1:6,000,000	**0.06**	0.09	**70**	**65**	**100**	**100**	**100**
	1:7,000,000	0.07	0.08	75	85	100	100	100
	1:8,000,000	0.08	0.07	80	90	100	100	100
	1:9,000,000	0.09	0.06	85	95	100	100	100
	1:10,000,000	0.10	0.05	90	100	100	100	100
	1:12,000,000	0.12	0.03	95	100	100	100	100
	1:13,000,000	0.13	0.02	100	100	100	100	100
	1:14,000,000	0.14	0.01	100	100	100	100	100
	1:15,000,000	0.15	0	100	100	100	100	100

* Six 50% units of complement (in 0.10 ml) were used throughout.

The amount of complement fixed, as measured by per cent hemolysis, increased regularly to an optimum point, then similarly decreased, depending on the relative concentration at which constant amounts of antigen and antibody first came into contact (Table 1). Hemolysis was minimal in each tube which received 0.08 ml of a 1:1,600,000 dilution (0.05γ), 0.06 ml of a 1:3,000,000 dilution (0.02γ), and 0.06 ml of a 1:6,000,000 dilution (0.01γ). With these reagents, therefore, the most complement was fixed when the reagents were first mixed in a total volume of approximately 0.21–0.23 ml, and this condition is a characteristic of the reaction in addition to the optimum antigen-antibody proportion of Dean (*3*). From these data, the optimum amount of antigen for the antiserum range tested was 0.02γ.

The tubes containing a given amount of antibody and polysaccharide antigen (in γ) differed from one another in no respect except that serum, complement, and antigen is it likely to involve complement in the brief period during which volumes are unequal, since fixation of complement is comparatively slow. Antigen-antibody reactions, however, are rapid (*5*), the size of antigen-antibody aggregates is a factor influencing the amount of complement fixed (*2, 4*), and the relative concentration of antigen and antibody is a factor determining the composition of precipitates (*6*) and amount and visibility (*7, 8, 12*) of precipitates. An hypothesis to explain the effect might be built on this basis, assuming that the size of the aggregates varied with composition and amount. An analogous effect has been observed with gold sols, the relative concentration in which reagents are mixed modifying the degree of coagulation of the sol (*9*).

The phenomenon has been elicited regularly with several preparations of pneumococcus polysaccharides and, as mentioned above, with influenza virus A and B antigens prepared by various technics. It has not yet been

demonstrated with cardiolipin antigen (*10, 11*), since sufficiently accurate replicate dilutions could not be prepared from the concentrated antigen, and it is known that adding saline to a dilution already made up will alter the properties of a lipid antigen. It seems reasonable, however, to expect that it can be detected in complement-fixation tests with a wide variety of antigens. Studies of the further characteristics of the reaction and its application to the standardization of antigens will be presented in greater detail elsewhere.

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Effect of Low Temperatures on the Germination of Inbred Lines of Sweet Corn

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Commercial seed corn producers have for some time been using cold-room tests as indicators of the cold hardiness of their inbred lines of seed corn and to see how well they can withstand the attacks of soil pathogens under poor conditions for germination. Usually the seeds are sown in soil taken from a near-by cornfield known to contain plenty of detrimental soil organisms. They are then placed in a 50° F room for a given length of time (usually 8 days), after which they are removed to a warm room so that the uninjured seeds may germinate. The percentage germination, measured by plumule appearance above soil level, gives an estimate of the hardiness of the lines.

In order to test whether such conditions could be applied to inbred sweet corn, 5 Connecticut lines of inbred sweet corn, viz., C4, C40, P51, T51, and C95, were obtained from W. R. Singleton and sown in flats containing soil from a field recently under corn. Each flat contained 50 seeds of each inbred for each test. One series of flats was placed in a 40° F cold room and the other in a 50° F room, the soil being kept moist by watering when necessary. After definite intervals in the cold, a flat from each room was removed to a warm greenhouse and the resulting germination of each inbred line determined.

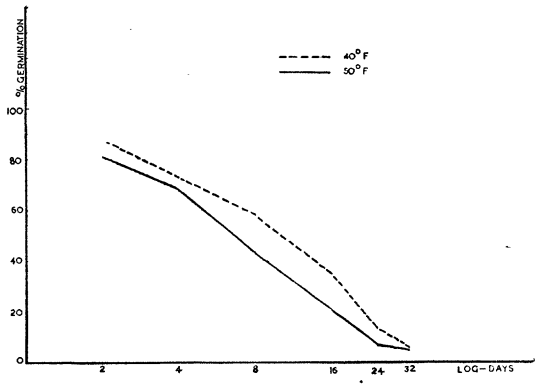

FIG. 1. The effect of cold room treatments on the subsequent mean percentage germination of 5 inbred lines of sweet corn.

The mean percentage germination of all the inbreds for each treatment was calculated and plotted against the logarithm of time of treatment. It is seen from Fig. 1 that germination drops off with increase of duration in the cold prior to plumule emergence, and that the 40° F treatment does not reduce germination as much as does 50° F. As the mean percentage germination of inbred lines in the control flat placed directly after sowing in the warm greenhouse was 84.8%, it appears from the graph that 8 days at 50° F are necessary to obtain a diminution of 50% in germination. Although this is the set of conditions commonly used by the corn companies for their estimates, it would seem that, for a more critical evaluation, a mean percentage germination of 35% is indicated. This would require 10 days in the cold room. In general, then, the empirical tests of the corn companies fit in well with the laboratory tests for the sweet corn inbred lines.

The difference between the effects of the 40° and 50° temperatures for equivalent lengths of treatment suggests that the causes of loss of germination are in part due to damage of the seed from cold during germination and in part to damage by the penetrating soil pathogens acting on the weakened seedling; these organisms are themselves partially inhibited at the lower temperature. The 40° room thus gives a truer picture of resistance of the inbreds to direct damage by cold. When using the above experimental conditions as a laboratory method for selecting cold hardy corn lines, the 50° room is preferable, for not only is it more critical, but there is selection of lines resistant to several different types of detrimental influences.

Further investigations on this aspect of cold treatment of sweet corn are now being made with funds provided by the Agricultural Research Council of Great Britain.