advantage. Thus the variance ratio might be given for more intermediate degrees of freedom, the angular transformation assigned a more detailed table and Tables VIII and XI expanded. Useful new tables could include tests for the significance of runs; the range of the normal distribution in different-sized samples at various levels of significance, in terms of the standard deviation computed with varying degrees of freedom; other terms of value in statistical control of quality; and criteria for identifying discordant observations. These omissions, however, are not vital. The appearance of a second edition on good paper, in the same convenient format as before and with as many additions as have been made, must rank as a real achievement under present war conditions.

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CONNECTICUT AGRICULTURAL EXPERIMENT STATION

SPECIAL ARTICLES

A PROTECTIVE ANTISERUM AGAINST MOUSE PNEUMONITIS VIRUS[1]

A VIRAL agent causing pneumonitis in mice has been isolated in this laboratory on two occasions, in 1938[2] and in 1940, by intranasal "blind passage" of the normal appearing lungs of white mice. A general study[3] of its properties indicates that it is similar if not identical to certain other latent pneumotropic viruses found in mice.[4] The morphology of its inclusion bodies, as seen in sections of mouse lungs, and of its elementary bodies, as revealed by smears of mouse lung or infected chick embryo yolk sac, relate it very definitely to the viruses of psittacosis, other ornithosis strains, lymphogranuloma venereum, meningopneumonitis, trachoma, inclusion conjunctivitis and others. Some cases of primary atypical pneumonia of man are related to agents within this group.[5]

Although the serum neutralization test is applicable to the study of most viruses, difficulty has been encountered in the use of the test in this group of agents. Repeated attempts in this laboratory to induce neutralizing antibody in the rabbit against the mouse virus have failed. In general, it has been the experience of other investigators that infection or artificial immunization of animals with these agents gives rise to neutralizing antibody only to a slight extent or not at all. Similar results have been obtained with convalescent human serums, although exceptions are found in some of the reports regarding lymphogranuloma venereum serum,[6] and in the demonstration of neutralizing antibodies in monkey and human serums after artificial immunization with psittacosis virus.[7]

TABLE 1

COMPARATIVE SERUM NEUTRALIZATION TESTS ON RABBIT AND CHICKEN SERUMS

Serums	LD_{50}*	Average infectivity scores† at virus dilutions								
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Normal rabbit	$10^{-3.60}$	5.00	5.00	5.00	3.5	1.83	1.00	.67	.00	
Immune rabbit	$10^{-3.25}$	5.00	5.00	4.33	1.33	1.00	1.00	.00	.00	
Normal rooster	$10^{-4.50}$	4.83	4.83	5.00	5.00	3.00	1.20	1.00	.33	.00
Immune rooster	$> 10^{-1}$	2.00	1.00	1.00	.33	.00	.00	.00	.00	

Three-tenths cc volumes of the serums were added to equal amounts of virus dilutions. Infected mouse lung served as antigen in the rabbit serum test, and infected yolk sac with the rooster serum. After standing at 20° C for 1 hour, 0.03 cc from each tube was instilled intranasally into each of 6 mice. Mice were observed daily and survivors were autopsied on the 10th day.

* The 50 per cent. mortality dose computed according to the method of Reed and Muench (L. J. Reed and H. Muench, *Am. Jour. Hyg.*, 27: 493, 1938) from the record of deaths not included here.

† Computed by the method of Horsfall (F. L. Horsfall, *Jour. Exp. Med.*, 70: 209, 1939) which gives a numerical value to the amount of infection as determined by the extent of lung consolidation.

Recognizing the similarity of our mouse virus to the several strains infecting birds, we investigated the ability of chickens to produce antiserum against this virus. Repeated injection of infected mouse lung emulsion into roosters resulted in the appearance of neutralizing antibody of relatively high titer. Parallel inoculations of rabbits were made, but only traces of antibody were produced. The immunizing procedure for both species was the same and consisted of a series of 25 intraperitoneal and 3 intramuscular injections of mouse lung virus over a period of 15 weeks.

[1] This investigation was supported by the John Rockefeller McCormick Memorial Fund of the University of Chicago.

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[3] H. V. Karr, *Jour. Infect. Dis.*, 72: 108, 1943.

[4] A. R. Dochez, K. C. Mills and B. Mulliken, *Proc. Soc. Exp. Biol. and Med.*, 36: 683, 1937; K. Herzberg and W. Gross, *Zentralbl. f. Bakt. (Abt. I), Orig.*, 146: 129, 1940; K. Herzberg, *ibid.*, 177, 1940; R. Goonert, *Klin. Wchnschr.*, 20: 76, 1941; C. Nigg, SCIENCE, 95: 677, 1942.

[5] T. Francis and T. P. Magill, *Jour. Exp. Med.*, 68: 147, 1938; M. D. Eaton, M. D. Beck and H. E. Pearson, *ibid.*, 73: 641, 1941; J. E. Smadel, *Jour. Clin. Invest.*, 22: 1, 1943.

[6] E. Rodaniche, *Jour. Infect. Dis.*, 66: 144, 1940.

[7] T. M. Rivers and F. E. Schwentker, *Jour. Exp. Med.*, 60: 211, 1934.

Table 1 shows the results of neutralization tests in which a specimen of serum taken after the period of immunization is compared with serum from the same animal before immunization. Considerable protection was afforded by the immune chicken serum as shown by survival of all mice (L_{D50} of virus = $> 10^{-1}$), and much less extensive lung involvement than was seen in the controls. The protective effect of immune rabbit serum was very slight.

In the tests with chicken antiserum, virus propagated in the yolk sac of the chick embryo was used instead of mouse lung virus to avoid any non-specific effects due to antibody against mouse lung tissue. However, in one test neutralization occurred to the same degree when mouse lung virus was used with the chicken antiserum, and although flocculation occurred in the tubes containing low dilutions of serum, this did not appear to influence the result of the test. No flocculation was observed when rabbit antiserum was mixed with mouse lung virus.

The results recorded in Table 1 suggest that there is a distinct advantage in using the chicken as a source of antibody against this agent. Whether this advantage is due to a greater susceptibility resulting in an unrecognized infection, or whether other factors are responsible, we do not know. Several reports[8] have appeared recently suggesting that chicken serum has certain advantages over other types of serum for various purposes.

The anti-mouse virus chicken serum reacts also with egg-propagated virus to give a visible *in vitro* flocculation. The antigen for this test was prepared by differential centrifugation of infected yolk sac emulsion. By this means the elementary bodies, which appear to constitute infectious particles of virus, may be partially purified and concentrated. Dilutions of the immune chicken serum were made and antigen added as in an ordinary bacterial agglutination test. Final dilutions of serum were from 1:4 to 1:1,024. For suspending the antigen and making dilutions of serum, saline solution lightly buffered at pH 7.0 was used. After incubation at 50° C for 12 hours, flocculation was visible in the first 5 tubes (1:4 to 1:64), with the maximum at 1:16. No flocculation appeared in a parallel series of tubes with normal chicken serum. Although the flocculated matter, when stained, was seen to contain aggregated elementary bodies, much of the floccule consisted of a more highly dispersed lightly staining material. This was always encountered during attempts at purification of elementary bodies, and its removal proved to be extremely difficult. However, by continued manipulation, a few suspensions of elementary bodies were prepared in which this more highly dispersed material was not detectable. Floccules appeared when these suspensions were mixed with the chicken antiserum on a slide, and staining of such preparations revealed the floccules to be composed only of aggregated elementary bodies. Thus, the antiserum appears to contain an agglutinin for the elementary bodies and an antibody capable of flocculating the more disperse material.

Following the demonstration that this chicken antiserum neutralized virus in the ordinary *in vitro* test, several experiments were performed to test its prophylactic and therapeutic effect on the disease in the mouse. Table 2 gives a representative protocol. A definite protective effect is evident when a single dose of serum is given either before or after the virus inoculation. Other experiments show that even a greater therapeutic effect may be obtained when several serum injections are made during the 3 days following virus inoculation.

TABLE 2

PROTECTION OF MICE AGAINST MOUSE PNEUMONITIS BY ADMINISTRATION OF IMMUNE CHICKEN SERUM AT VARYING PERIODS BEFORE AND AFTER VIRUS INOCULATION

Serum administration, 0.03 cc intranasally	Result with normal serums	Result with immune serums
6 hours before virus inoc.	4.2	1.6
1 hour before virus inoc.	4.8	1.1
1 hour after virus inoc.	4.0	3.1
1 and 3 hours after virus inoc.	4.4	2.6
4 hours after virus inoc.	4.0	2.4

Virus inoculation made intranasally with 0.03 cc emulsion of infected yolk sac. All surviving mice autopsied on 5th day. Figures represent average infectivity scores (see legend, Table 1) of 8 to 11 mice.

The successful use of the chicken for producing antibody against this agent may indicate a satisfactory method of antiserum production against other viruses of this group. Serums of good neutralizing titer or suitable for *in vitro* flocculation tests would be of great advantage in clarifying the antigenic relationships within this group. It is also possible that such serums would prove of value in treatment of human infections with these agents. Further investigation along these lines is under way.

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ATTEMPTS TO PROTECT AGAINST INFLUENZA VIRUS WITH VARIOUS SULFONAMIDES, ACRIDINES AND ANTIBIOTICS[1]

SEVERAL sulfonamides, acridines and antibiotics

[1] The opinions advanced in this publication are those of