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

have been tested in this laboratory to determine their effectiveness in conferring protection against influenza virus. It has been reported by other workers that certain sulfonamides used in conjunction with azochloramide,[2, 3, 4] pyridium[5] or neoarsphenamine[6] against bacteria gave better results than when either compound alone was used. Accordingly, experiments were conducted to determine if the same combinations would prove effective against influenza virus, although it has already been demonstrated that many sulfonamides,[7, 8] some acridines[8] and penicillin[8] administered alone were ineffective as protective agents.

The PR–8 strain of influenza A virus was used throughout our experiments. Each sulfonamide listed below was tested alone and in combination with azochloramide, pyridium and neoarsphenamine. Two procedures were followed:

(1) Equal volumes of drug solution and mouse lung virus suspension were mixed so that the final concentration of virus was 10 MLD/0.05 cc. The mixture was then held at refrigerator temperature (4° C.) for 2 hours, after which period 0.05 cc was inoculated intranasally into each of six white mice.

(2) Daily intraperitoneal injections of the compound under test were given to white mice for a period of 12 days. On the second day of the series each animal received an intranasal inoculation of 0.05 cc of mouse lung virus suspension containing 10 MLD's. Those animals which died during a 10-day period of observation were immediately autopsied. If more than one half of the total lung tissue was consolidated the mice were considered to have died from influenzal infection. On the 10th day after intranasal inoculation of virus all surviving mice were sacrificed and the extent of pulmonary consolidation recorded.

The following compounds were studied:

SULFONAMIDES

Drug	Dosage Administered Daily (contained in 0.2 cc)
1. Sulfanilamide	1 mg
2. Sulfathiazole	1 mg
3. Sulfapyridine	1 mg
4. Sulfadiazine	1 mg
5. Benzenesulfamido-m-ethylphenol[9]	4 mg
6. 2-amino-5-azobenzenesulfonamido-pyridine[9]	4 mg
7. Carbonyldisulfanilamide[9]	3 mg
8. Thionyldisulfanilamide[9]	4 mg
9. Azobenzenesulfonamidotrypaflavine hydrochloride[9]	1 mg
10. 2-ethoxy-6,9-diaminoazobenzene-sulfonamido-acridine[9]	1 mg
11. Caproyl-p-benzenesulfonamide[9]	4 mg
12. Caproaldehydesulfonamide[9]	4 mg
13. O-hydroxybenzyl-p-aminobenzene-sulfonamide[9]	4 mg
14. Sulfuramine[9]	1 mg
15. 2-ethoxy-6,9-diaminobenzeneamido-thiazol-acridine[9]	4 mg

ACRIDINES

1. Trypaflavine[9]	2 mg
2. Proflavinehydrochloride[9]	2 mg
3. Rivanol[9]	2 mg

ANTIBIOTICS

1. Penicillin[10]	5 mg (650 Oxford Units)
2. Tyrothricin[11]	5 mg
3. Tyrocidin[11]	5 mg
4. Gramicidin[11]	5 mg
5. Subtilin[11]	5 mg

OTHER COMPOUNDS

1. Azochloramid	0.02 mg
2. Pyridium	0.02 mg
3. Neoarsphenamine	0.001 mg

None of the compounds tested under the conditions of these experiments, alone or in combination, was effective in preventing influenzal infection in mice.

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[9] Obtained from Dr. Frederick Proescher, pathologist, Santa Clara Hospital, San Jose, California.

[10] Obtained from Merck and Company, Rahway, N. J.

[11] Obtained from the Western Regional Research Laboratory of the Agricultural Research Administration, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture.

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