

TABLE II.  
Effect of Penicillin on Equine-Encephalomyelitis  
in Chick Embryos.

| Dilution<br>of virus | Control | Treated |
|----------------------|---------|---------|
| 10-3                 | 8/8     | 7/7     |
| 10-4                 | 18/18   | 14/15   |
| 10-5                 | 8/8     | 6/8     |

Numerator: number dying; denominator: number inoculated.

Titer of virus in previous experiment: 10-6.

500 units penicillin injected into each egg just before virus inoculation.

other, 500 units of penicillin were mixed with the virus inoculum. In neither case could any difference between control and treated embryos be distinguished, either as to size or number of lesions when the membranes were examined at 48 hours.

The results of inoculation of chick embryos with psittacosis and with M.P. virus, and with penicillin plus these viruses, are given in Table I. It will be seen that with these 2 infections penicillin was effective in delaying, or with larger doses preventing death of the embryos within the period of observation. Not all embryos dying were tested for the

presence of virus but enough passages were made to learn that in a fair proportion of the embryos dying late after penicillin treatment, no detectable virus was present.

In one series of experiments embryos were inoculated with equine encephalomyelitis virus, and the results are recorded in Table II. The penicillin was entirely without effect on the course of the infection.

It is evident from these experiments that penicillin in the doses used was without effect on the multiplication of the viruses of vaccinia, St. Louis encephalitis or equine encephalomyelitis, or on the course of the disease induced in chick embryos by vaccinia or equine encephalomyelitis. Penicillin did have a very definite effect on the course of the disease induced in chick embryos by the viruses of psittacosis and of meningo-pneumonitis. It is to be noted, however, that the doses used were large. To maintain a comparable blood concentration of the drug would require the injection into man of several million units of penicillin daily. The practical importance of this observation may, therefore, be questioned.

## 14809

### Isolation of Influenza A Virus from Normal Human Contacts During an Epidemic of Influenza A.\*

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Various investigators have reported the occurrence of sub-clinical infections during epidemics of influenza A as evidenced by significant increases in neutralizing or complement fixing antibodies in the sera of persons who were exposed but who did not become

ill.<sup>1,2,3</sup> A review of the literature, however, has revealed no reports of the isolation of influenza virus from persons with subclinical infections following normal exposure to the disease.

In November, 1943, 171 typical clinical cases of influenza A occurred in a dormitory housing approximately 500 students at the

\* This study was supported by funds from the International Health Division of the Rockefeller Foundation.

<sup>1</sup> Francis, T., Jr., Magill, T. P., Rickard, E. R., and Beck, M. D., *Am. J. Pub. Health*, 1937, **27**, 1141.

<sup>2</sup> Stuart-Harris, C. H., *et al.*, *Medical Research Council, Special Report Series*, No. 228, 1938.

<sup>3</sup> Rickard, E. R., Lennette, E. H., and Horsfall, F. L., Jr., *Pub. Health Rep.*, 1940, **55**, 2146.

University of Minnesota. Provision for the medical care of the students was such that there was little probability that any individual suffering from the usual symptoms of influenza would escape observation. In order to study the incidence of subclinical infections 14 apparently normal students living in the dormitory were questioned in detail as to symptoms suggestive of influenza. None of these men considered himself to be ill and the few symptoms which were elicited were of such trivial nature as might be expected to be obtained by questioning any group of apparently normal individuals during the winter season.

From 13 of the 14 men naso-pharyngeal washings were taken at the time of questioning. A few cc of 20% normal horse serum-broth were instilled into the nostrils. After expectorating, the subject gargled with a small quantity of the same menstruum and expectorated into the container holding the nasal washings. The combined washings from the nose and throat were placed immediately on CO<sub>2</sub> ice where they were kept until examined. Blood specimens were obtained from 13 of the 14 men at the time the throat washings were taken and again at approximately two weeks later.

**Materials and Methods.** Throat washings were examined for the presence of influenza virus by the intra-allantoic inoculation of 11-day embryonated eggs with unfiltered washings.<sup>4</sup> The avian red blood cell agglutination phenomenon<sup>5</sup> was used as an indicator of the presence of virus. A minimum of 5 serial passages was performed with each washing before considering it negative.

Viruses isolated were identified as influenza A by comparing the red blood cell agglutination inhibition<sup>6</sup> titer of a pool of acute phase human anti-influenza A serum to the titer of a pool of convalescent phase serum from the same subjects. In every instance when these pools were tested against the unknown strains, there was an increase of at least 4 times in the titer of the convalescent pool as compared to the

acute phase pool. The average increase in titer against the 5 strains was 14 times.

Serum specimens were examined by the red blood cell agglutination inhibition test and end points were determined by the use of the photo-densitometer.<sup>7</sup> In all instances pre- and post-infection sera were examined in the same test. The antigens employed were 4 minimal agglutinating doses of the PR8<sup>8</sup> strain of influenza A virus and the Lee<sup>9</sup> strain of influenza B virus, both egg-adapted and contained in infected allantoic fluids.

**Results.** Details of virus isolation and serum examination have been summarized in Table I. It may be noted that 9 of the 13, or 69%, of the pairs of sera examined showed significant, or greater than fourfold, increases in antibodies to influenza A. None of the sera was positive to influenza B. Influenza A virus was isolated from 5 of the 13, or 38%, of the throat washings examined.

These percentages of positive virus isolation and antibody response were very similar to those obtained in the examination of material taken from clinical cases of influenza which occurred in the same dormitory. From the clinical cases 86 throat washings were examined and influenza A virus was isolated from 32, or 37%, of the specimens. Of 71 pairs of acute and convalescent serum specimens examined from the clinical cases in 61, or 86%, significant increases in antibodies for influenza A virus were observed.

**Discussion.** Of considerable interest is the finding that in the sera of three individuals from whose throat washings virus was isolated no significant increase in antibodies was noted. Although the importance of subclinical infection in the immunization to and the dissemination of influenza has long been appreciated, the observations herein recorded further emphasize the necessity of considering this factor in attempting to control the spread of the disease. The ease with which virus was isolated from the throats of apparently normal subjects suggests that such persons may be of equal, if not greater, importance in spread-

<sup>4</sup> Rickard, E. R., Thigpen, M. P., and Crowley, J. H., *J. Immunol.*, 1944, **49**, 263.

<sup>5</sup> Hirst, G. K., *Science*, 1941, **94**, 22.

<sup>6</sup> Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

<sup>7</sup> Hirst, G. K., and Pickels, D. G., *J. Immunol.*, 1942, **45**, 273.

<sup>8</sup> Francis, T., Jr., *Science*, 1934, **80**, 457.

<sup>9</sup> Francis, T., Jr., *Science*, 1940, **92**, 405.

TABLE I.  
Serological Findings and Virus Isolation from Apparently Normal Contacts.

| Case No. | Serological diagnosis influenza A |                             |                                       | Virus isolation<br>Egg passage<br>in which<br>agglutination<br>was first<br>observed | Symptoms              |
|----------|-----------------------------------|-----------------------------|---------------------------------------|--------------------------------------------------------------------------------------|-----------------------|
|          | Acute<br>phase titer              | Convalescent<br>phase titer | Significant<br>increase<br>antibodies |                                                                                      |                       |
| 1        | 1:208                             | 1:1450                      | +                                     | Fourth                                                                               | Feeling onset of cold |
| 2        | 1:223                             | 1:1270                      | +                                     | Neg.                                                                                 | Slight cough          |
| 3        | *                                 | *                           | *                                     | Third                                                                                | Slight coryza         |
| 4        | 1:91                              | 1:315                       | +                                     | Neg.                                                                                 | Chronic cough         |
| 5        | 1:147                             | 1:16,400                    | +                                     | Neg.                                                                                 | No symptoms           |
| 6        | 1:447                             | 1:722                       | —                                     | Fourth                                                                               | " "                   |
| 7        | 1:91                              | 1:2900                      | +                                     | Neg.                                                                                 | Slight sore throat    |
| 8        | 1:84                              | 1:97                        | —                                     | Third                                                                                | Chronic cough         |
| 9        | 1:21                              | 1:356                       | +                                     | Neg.                                                                                 | Slight cough          |
| 10       | 1:362                             | 1:891                       | —                                     | Neg.                                                                                 | No symptoms           |
| 11       | 1:132                             | 1:722                       | +                                     | Neg.                                                                                 | " "                   |
| 12       | 1:223                             | 1:1024                      | +                                     | *                                                                                    | " "                   |
| 13       | 1:223                             | 1:1270                      | +                                     | Neg.                                                                                 | Slight coryza         |
| 14       | 1:223                             | 1:512                       | —                                     | Third                                                                                | No symptoms           |

\* Not examined.

ing infection than are the clinical cases.

**Summary.** By the direct intra-allantoic inoculation of unfiltered throat washings influenza A virus was isolated from 5 of 13 apparently normal contacts during an epidemic of

influenza A. Significant increases in neutralizing antibodies were demonstrated in the sera of 9 of 13 such contacts by means of the red blood cell agglutination inhibition test.

## 14810

### Agglutination Tests with Streptococcus No. 344 in Primary Atypical Pneumonia.\*

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The discovery by Thomas, Mirick, Ziegler, Curnen, and Horsfall<sup>1</sup> that sera of patients convalescent from primary atypical pneumonia frequently agglutinate an indifferent streptococcus, labeled No. 344, has provided yet another method for investigating this disease. The fact that the streptococcus agglutinins

persist for a long time when stored at 4°C has made it possible to investigate sera from patients with primary atypical pneumonia received at this laboratory since the summer of 1941. Results of the tests, together with certain observations correlating laboratory and clinical findings, form the subject of this report.

**Methods and Materials.** The method described in the original report was followed without modification. Titers of 10 or more were considered significant.

Sera were collected from students admitted to Cowell Memorial Hospital, University of California, Berkeley, and to the University of

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<sup>1</sup> Thomas, L., Mirick, G. S., Curnen, E. C., Ziegler, J. E., and Horsfall, F. L., Jr., *Science*, 1943, **98**, 566.