# LOUPING ILL IN MAN

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The fact that the etiological agent of louping ill (1, 2), a natural disease of sheep in Scotland and the northern part of England, is a filterable virus (3) capable of producing in monkeys (4) and mice (5) a disease somewhat similar to poliomyelitis induced us to procure some of the virus for investigation (6). It was supplied to another laboratory of the Institute in which three of the workers, one after another, became sick. Inquiry disclosed the fact that an English investigator had also become ill after having worked with the virus. A preliminary note (7) has already been made regarding these cases. In the present paper a detailed description of the cases and a report of the results of the investigations undertaken to ascertain whether the virus of louping ill was the etiological agent involved are presented.

## *Report of Cases*

*Case 1.*—Dr. F., male, 28, became sick on Dec. 2, 1932, twelve weeks after the initiation of his work with louping ill virus. For 5 days he experienced general malaise, headache, and a temperature ranging between 101° and 101.5°F. Following this bout of fever the patient was afebrile for 8 days during which time he worked in spite of the fact that he did not feel as well as usual. On Dec. 15, he returned to bed because of a transient diplopia and a temperature of 102°F. From Dec. 15 until Dec. 18, the date of admission to the Hospital, the patient had a headache, fever, and recurring attacks of diplopia, and, in spite of drowsiness, was unable to sleep. On Dec. 17 he experienced several attacks of projectile vomiting which were unaccompanied by nausea.

Upon admission to the Hospital of The Rockefeller Institute the patient had a temperature of 102.1°F. and a pulse rate of 84. He was drowsy and had a headache. The general physical examination was negative with the exception of a marked diminution in the intensity of the deep reflexes and a diplopia caused by a weakness of the internal rectus muscle of the left eye. During the examination it was noticed that the patient's respirations were irregular and accompanied by sighs. There were 10,500 white blood cells per c.mm. of which 83 per cent were granulocytes. On Dec. 19, the day after admission to the Hospital, the patient

continued to have attacks of vomiting but at this time they were accompanied by nausea. No other significant changes were noticed in the patient's general condition. Spinal fluid was obtained for study. It was clear, but contained an increased amount of globulin and 61 white cells per c.mm. of which 88 per cent were mononuclear elements. Cultures of the blood and spinal fluid remained sterile. Mice injected intracerebrally with blood and with spinal fluid remained well. On Dec. 20, the patient received intravenously 50 cc. of a 50 per cent solution of glucose. Two similar intravenous injections were made the next day. Following the administration of the hypertonic solutions the patient's condition

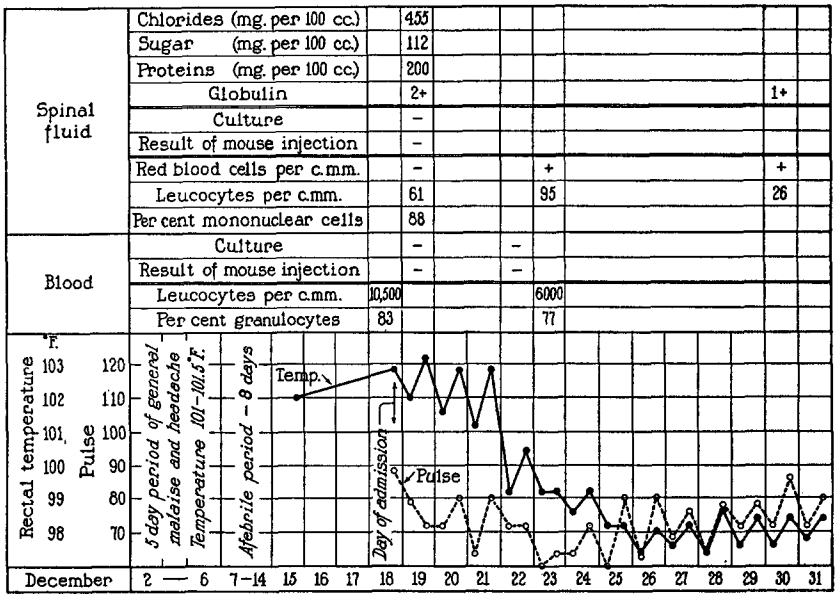


CHART 1. Graphic portrayal of Case 1.

improved; his headache was less severe, the nausea and vomiting ceased, he became jovial and regained the ability to sleep normally. A second specimen of blood was taken on Dec. 22; cultures remained sterile and mice inoculated intracerebrally with small amounts of it remained well. Spinal fluid obtained on the 23rd of December contained blood. That obtained on the 30th had a few blood cells, 26 leucocytes per c.mm., and an increased amount of globulin. The diplopia disappeared on the 22nd. The temperature returned to a normal level on the 23rd. Following this the patient made a rapid recovery since which time he has remained well. See Chart 1 for a summary of the case. At the time the patient was in the Hospital the physicians who saw him believed that he had epidemic encephalitis.

When Dr. F. became sick Mrs. C. carried on the work with louping ill virus until she (Case 2) became sick, Jan. 31, 1933, approximately 6 weeks after she began handling the infectious agent.

*Case 2.*—Mrs. C., 27, on Jan. 31 went to bed at home because of fever, headache, backache, and prostration. Nothing of significance except a reddened throat was observed by her husband who is a physician. The patient's condition remained unchanged until Feb. 5 when her temperature reached normal. During this period of her illness a marked leucopenia, 2000–4000 cells per c.mm., existed (Chart 2). She gradually improved and returned to work on Feb. 14 at which time the white blood cell count was 6400. On Feb. 18 the headache returned and increased in intensity until the 21st when it became very severe. In spite of the discomfort Mrs. C. continued to work until the afternoon of the 21st when she was carried home prostrated with fever, slight photophobia, and intense headache. On Feb. 22 the white blood cells numbered 12,000 per c.mm. of which 82 per cent were granulocytes. A blood culture taken at this time remained sterile. The fever continued and was accompanied by a slightly confused mental state. On Feb. 23 the patient was admitted to the White Plains Hospital,[1] White Plains, N. Y.

On admission to the Hospital the patient's temperature was 104°F. and the pulse rate was 90. She complained of headache, stiffness of the neck, sore throat, and nausea. Examination revealed that the patient was drowsy and had a stiff neck and an inflamed throat. Feb. 24, she was still nauseated and complained of transient blurring of vision. The white blood cell count was 17,350. Feb. 25, headache, nausea, and vomiting persisted. A consultant was called who, in view of the symptoms, the stiffness of the neck, and the blurring of the optic discs, made a diagnosis of tuberculous meningitis. A lumbar puncture was made. The spinal fluid was clear, reduced Fehling's solution, contained, in addition to an increased amount of globulin, 45 white cells per c.mm. No tubercle bacilli were found in the spinal fluid; cultures remained sterile; mice injected intracerebrally with small amounts of the fluid remained well. The patient's general condition improved rapidly, and, on Feb. 28, her temperature reached a normal level. Although her general condition improved, a definite retrobulbar neuritis developed. Mar. 3, spinal fluid was again obtained for examination. It was slightly blood-tinged; sugar was present; globulin was increased; leucocytes were 50 per c.mm. of which 60 per cent were mononuclear elements; cultures remained sterile. The patient continued to improve and the optic neuritis gradually subsided. Mar. 23, the patient was discharged from the Hospital. After a period of convalescence at home she returned to work and since that time has remained well. Chart 2 summarizes the essential features of the case. In view of the

[1] Mrs. C. was on Dr. W. W. Mott's service and it was through his courtesy and cooperation that we were able to obtain data regarding her illness.

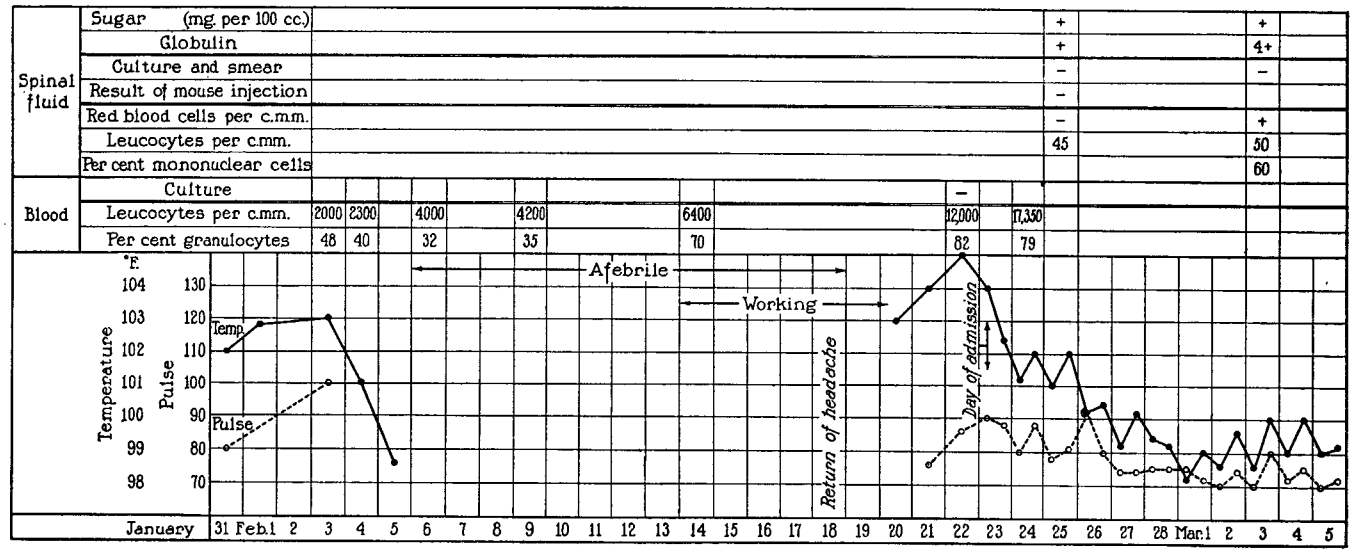


CHART 2. Graphic portrayal of Case 2.

fact that the patient made a speedy recovery the diagnosis of tuberculous meningitis was changed to acute encephalitis.

When Mrs. C. became sick on Jan. 31, Dr. W. took charge of the work on louping ill. He (Case 3) became ill on Feb. 8.

*Case 3.*—Dr. W., male, 39, on Feb. 8, after strenuous exercise followed by exposure to inclement weather experienced chilly sensations, dizziness, and elevation of temperature. Feb. 9, he worked in spite of dizziness, anorexia, and a temperature of 101°F. Feb. 10 to 17, he remained in bed because of headache, photophobia, and fever—temperature ranged between 101° and 103°F. Feb. 17 to 20, convalesced at home; headache and weakness persisted. Feb. 20 to Mar. 1, the patient was able to be at the laboratory but was weak and unable to use his eyes for close work. Mar. 1, the patient felt fully recovered and has remained well since that time. The physician in charge of the case made a diagnosis of influenza.

Upon inquiry it was found that an English investigator (Case 4) at the Lister Institute had an attack of encephalitis in 1932 after having worked with louping ill virus. Through the courtesy of Dr. Critchley, 137 Harley Street, London, we have been able to secure the data regarding this patient. The patient himself was in the United States during 1933, a year after recovery from his illness, and we were able to obtain a specimen of serum from him at that time for neutralization tests which will be described later in the paper.

*Case 4.*—Dr. H., male, 31, from May 10 to 14, experienced headache, soreness in throat, malaise, and muscular stiffness. He worked on May 14 in spite of a headache and a slight elevation of temperature, 100°F. From the 14th until the 19th the patient remained at home because of intense headache, drowsiness, nausea, vomiting, and fever—temperature ranged between 100° and 104.5°F. The pulse was slow. On the 17th the white blood cells numbered 16,000 per c.mm. of which 80 per cent were granulocytes.

In view of the patient's condition he was admitted to King's College Hospital, May 19. Upon admission his temperature was 103.4°F., pulse 64, respirations 20. He was drowsy and had a severe headache; speech was slow; neck was stiff; deep reflexes were diminished in intensity; abdominal reflexes were not obtained. The spinal fluid was under increased pressure and contained sugar, 667 mg. of chlorides per 100 cc., 110 mg. of protein per 100 cc., an increased amount of globulin, 32 red blood cells per c.mm., and 468 leucocytes per c.mm. of which 77 per cent were mononuclear elements. Cultures and smears of the fluid revealed no microorganisms. On May 20, 21, and 22, lumbar punctures were made (Chart 3), and the spinal fluid was found to be of the same general nature as that already de-

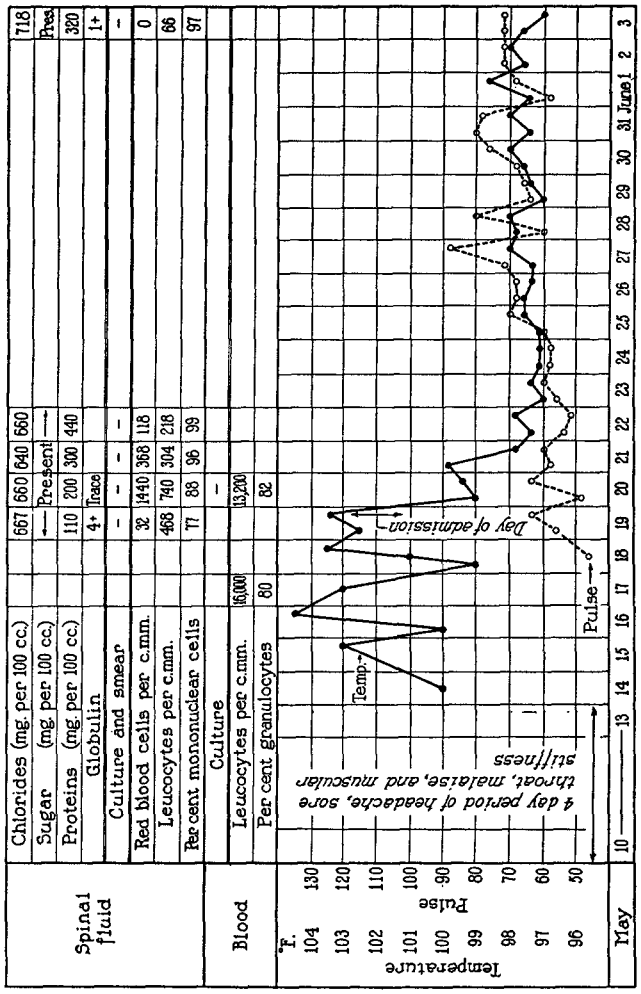


CHART 3. Graphic portrayal of Case 4.

scribed. The presence of blood cells should be noted, inasmuch as it was believed not to be due to the trauma of the puncture. In fact, several specimens of the fluid were brownish in color. May 20, the patient's general condition was improved; his fever was not as high as it had been; the tendon and abdominal reflexes were absent; the optic discs showed decided pallor. May 21, the patient's temperature reached the normal level and remained there. He felt better, was rational, and took a small amount of solid food. A marked tremor of the face, eyes, tongue, and hands was noticed. This persisted for several days and gradually disappeared. Tendon and abdominal reflexes were still not obtained. May 25, patient was entirely free from headache, was very alert, and wanted to get up. May 26, deep reflexes were still absent, but the abdominal reflexes had returned. The patient continued to improve, but on June 3 he developed a definite weakness of the muscles of the upper and lower parts of the left side of the face and some difficulty was experienced in blinking the left eyelid. Nothing else abnormal was noted. In fact, all the reflexes had returned to a normal state of activity. A lumbar puncture made at this time revealed a clear colorless fluid which contained sugar, 718 mg. of chlorides per 100 cc., 320 mg. of protein per 100 cc., an increased amount of globulin, no red blood cells, and 66 leucocytes per c.mm. of which 97 per cent were mononuclear cells. Cultures and smears of the fluid showed no bacteria. From June 3 until the time of discharge from the Hospital, June 18, the patient improved rapidly; the facial weakness gradually decreased and finally disappeared during convalescence at home. Since this illness the patient has been well. See Chart 3 for a summary of the significant features of the case. At first it was thought that the patient had tuberculous meningitis, but, in view of his rapid recovery, a final diagnosis of acute encephalitis was made.

## EXPERIMENTAL

There is no record in the literature of the occurrence of louping ill in man, yet the disease was considered in connection with the cases we have reported, and attempts to demonstrate the virus in the blood and spinal fluid of two of them were made without success. Failure to demonstrate the virus under such conditions, however, was not considered proof that the patients had not had louping ill, because it is not always possible to demonstrate the virus in the blood and spinal fluid of monkeys known to have the disease. Consequently, inasmuch as we had developed a test (6) for the presence of neutralizing or protective antibodies in the sera of monkeys immune to louping ill, we decided to apply the test to the sera of the four individuals who were suspected of having had the disease. In addition to these sera, we also tested the sera of other people working with the virus, as well as the sera of a number of individuals who had had no known contact

with the active agent. The manner in which the tests were conducted will be described. Then, several of them will be presented in the form of tables. Finally, a summary of the results will be presented.

### *Method of Conducting the Neutralization Tests*

The virus emulsions used in the neutralization experiments were prepared from pooled brains of mice killed at the height of a louping ill infection. After removal from the animals, the brains were stored for 24 hours in separate containers in an ice box while bits of each were tested by means of cultures for the presence of ordinary bacteria. The brains free from bacteria were pooled and ground in a mortar. Sufficient Locke's solution was then added to make a 20 per cent emulsion which was centrifuged at 2000–3000 R.P.M. for 10 minutes. Then decimal dilutions of the supernatant fluid were made with Locke's solution. Portions of each dilution were mixed with an equal amount of the serum the neutralizing properties of which were being investigated. The mixtures were allowed to stand for 2 hours at room temperature and then for 1 hour in a refrigerator at 0°C. 0.03 cc. of each of the mixtures were then injected intracerebrally, respectively, into each of six mice. The animals were observed for 18 days, and the number of deaths and the day of death of each animal were recorded. No mouse that succumbed sooner than the 4th day after injection was considered to have died of louping ill.

In each test at least two control sera were used. These consisted of a serum (negative control) that did not neutralize the virus and of a neutralizing serum (positive control) either from an immune monkey or from an immune person. It must be remembered that one cannot use virus emulsions diluted with Locke's solution alone as negative controls, because it has been shown (6) that in emulsions diluted in such a manner the virus of louping ill deteriorates rapidly. The Rockefeller Institute strain of albino mice was employed in approximately one-third of the experiments, while the Swiss strain of albino mice was used in the others. The latter mice are very susceptible to the virus and are highly suitable for this type of work. The former are not 100 per cent susceptible to the active agent, yet they proved satisfactory for the tests even though the results obtained with them were not so striking as were those when Swiss mice were used.

### *Results of Neutralization Tests*

In the manner described above 17 tests were conducted in which sera from 63 individuals were examined for the presence of neutralizing antibodies for louping ill virus. In Table I, Test 2 is summarized, and from the results presented it is obvious that the sera from Individuals 1 and 8 are comparable to that of the negative control, Individual 19, while the sera from Individuals 5 and 6 are comparable

TABLE I

*Summary of Neutralization Test 2*

| Dilution of virus | No. of mice inoculated | No. of deaths | Percentage of deaths | Day of death | Average time of death |
|---|---|---|---|---|---|
| Immune monkey serum plus virus dilutions (positive control) | | | | | |
| $10^{-3}$ | 6 | 0 | | | |
| $10^{-4}$ | 6 | 0 | | | |
| $10^{-5}$ | 6 | 0 | | | |
| | 18 | 0 | 0 | | |
| Serum of Individual 1 plus virus dilutions | | | | | |
| $10^{-3}$ | 6 | 5 | | 7, 7, 7, 8, 9 | |
| $10^{-4}$ | 6 | 4 | | 6, 6, 7, 10 | |
| $10^{-5}$ | 5 | 5 | | 7, 8, 9, 10, 10 | |
| | 17 | 14 | 82 | | 8.0 |
| Serum of Individual 8 plus virus dilutions | | | | | |
| $10^{-3}$ | 6 | 6 | | 6, 6, 8, 8, 8, 12 | |
| $10^{-4}$ | 4 | 2 | | 7, 8 | |
| $10^{-5}$ | 5 | 3 | | 7, 8, 10 | |
| | 15 | 11 | 73 | | 8.0 |
| Serum of Individual 5 plus virus dilutions | | | | | |
| $10^{-3}$ | 5 | 1 | | 12 | |
| $10^{-4}$ | 6 | 0 | | | |
| $10^{-5}$ | 6 | 0 | | | |
| | 17 | 1 | 6 | | 12.0 |
| Serum of Individual 4 plus virus dilutions | | | | | |
| $10^{-3}$ | 6 | 0 | | | |
| $10^{-4}$ | 5 | 0 | | | |
| $10^{-5}$ | 6 | 0 | | | |
| | 17 | 0 | 0 | | |
| Serum of Individual 19 plus virus dilutions (negative control) | | | | | |
| $10^{-3}$ | 6 | 4 | | 6, 8, 10, 10 | |
| $10^{-4}$ | 5 | 3 | | 7, 7, 10 | |
| $10^{-5}$ | 5 | 3 | | 7, 10, 12 | |
| | 16 | 10 | 63 | | 8.8 |

Rockefeller Institute mice used in the test.

TABLE II

*Summary of Neutralization Test 17*

| Dilution of virus | No. of mice inoculated | No. of deaths | Percentage of deaths | Day of death | Average time of death |
|---|---|---|---|---|---|
| Immune monkey serum plus virus dilutions (positive control) | | | | | |
| $10^{-4}$ | 6 | 2 | | 9, 11 | |
| $10^{-5}$ | 4 | 0 | | | |
| $10^{-6}$ | 6 | 0 | | | |
| | 16 | 2 | 13 | | 10.0 |
| Serum of Individual 6 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 0 | | | |
| $10^{-5}$ | 6 | 0 | | | |
| $10^{-6}$ | 6 | 0 | | | |
| | 18 | 0 | 0 | | |
| Serum of Individual 2 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 0 | | | |
| $10^{-5}$ | 6 | 0 | | | |
| $10^{-6}$ | 6 | 0 | | | |
| | 18 | 0 | 0 | | |
| Serum of Individual 38 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 4 | | 8, 9, 9, 9 | |
| $10^{-5}$ | 6 | 2 | | 10, 11 | |
| $10^{-6}$ | 6 | 1 | | 13 | |
| | 18 | 7 | 39 | | 9.4 |
| Serum of Individual 17 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 6 | | 7, 7, 8, 8, 8, 9 | |
| $10^{-5}$ | 6 | 6 | | 7, 7, 8, 9, 9, 11 | |
| $10^{-6}$ | 6 | 6 | | 8, 8, 9, 9, 9, 10 | |
| | 18 | 18 | 100 | | 8.4 |
| Serum of Individual 7 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 6 | | 7, 7, 7, 8, 8, 9 | |
| $10^{-5}$ | 6 | 6 | | 7, 8, 8, 8, 9, 9 | |
| $10^{-6}$ | 5 | 5 | | 6, 8, 9, 9, 9 | |
| | 17 | 17 | 100 | | 8.0 |

TABLE II—*Concluded*

| Dilution of virus | No. of mice inoculated | No. of deaths | Percentage of deaths | Day of death | Average time of death |
|---|---|---|---|---|---|
| Serum of Individual 45 plus virus dilutions | | | | | |
| $10^{-4}$ | 6 | 6 | | 7, 8, 8, 8, 9, 9 | |
| $10^{-5}$ | 6 | 6 | | 7, 8, 9, 9, 9, 9 | |
| $10^{-6}$ | 6 | 6 | | 7, 8, 9, 9, 9, 9 | |
| | 18 | 18 | 100 | | 8.4 |
| Serum of Individual 43 plus virus dilutions (negative control) | | | | | |
| $10^{-4}$ | 5 | 5 | | 7, 7, 8, 8, 8 | |
| $10^{-5}$ | 6 | 6 | | 6, 7, 8, 8, 9, 9 | |
| $10^{-6}$ | 6 | 6 | | 7, 9, 9, 9, 9, 10 | |
| | 17 | 17 | 100 | | 8.0 |

Swiss mice used in the test.

to that of the monkey immune to louping ill. Table II summarizes Test 17 and reveals (1) that the sera of Individuals 7, 17, and 45 are comparable to that of the negative control, Individual 43; (2) that the sera of Individuals 2 and 6 are comparable to that of the monkey immune to louping ill; (3) that the serum of Individual 38 possesses less neutralizing antibodies than do the other positive sera.

In Table III a summary of all the neutralization tests is presented. The sera for this work were collected from both sexes and from individuals 1 year to 68 years old. The majority of the people, however, were in the neighborhood of 35 years of age. A number of the sera were tested more than once and more than one specimen of serum was collected from six of the individuals. The sera from the different individuals were given numbers which have been arranged in the table according to whether the persons from whom the sera were collected had had close contact (Nos. 1–7), possible contact (Nos. 8–17), or no history of contact (Nos. 18–63) with the virus of louping ill. In addition to this arrangement, the nationality of the individuals, the diseases from which they were suffering or from which they had recently recovered are indicated. Finally the results—percentage of deaths of mice used—of the neutralization tests and our interpretation of them are given.

TABLE III

*Summary of Neutralization Tests*

| Person's No. | Nationality | History of contact with virus | Notes on person | Percentage of mice that died | | | Interpretation of results of neutralization tests |
|---|---|---|---|---|---|---|---|
| | | | | Negative control | Positive control | Test serum | |
| 1 | American | Close contact | Normal | 63 | 0 | 82 | − |
| | | | | 100 | 6 | 100 | |
| | | | | 100 | 0 | 100 | |
| | | | | 78 | 0 | 72 | |
| 2 | " | " " | " | 72 | 0 | 29 | + |
| | | | | 100 | 0 | 38 | |
| | | | | 100 | 0 | 12 | |
| | | | | 88 | 0 | 0 | |
| 3 | " | " " | Recovered from acute encephalitis | 100 | 19 | 37 | + |
| | | | | 100 | 13 | 0 | |
| | | | | 67 | 12 | 6 | |
| | | | | 88 | 0 | 0 | |
| 4 | English | " " | " " | 63 | 0 | 0 | + |
| | | | | 88 | 0 | 12 | |
| | | | | 86 | 0 | 14 | |
| | | | | 88 | 0 | 12 | |
| 5 | American | " " | " " | 63 | 0 | 6 | + |
| | | | | 88 | 0 | 0 | |
| | | | | 88 | 0 | 0 | |
| 6 | " | " " | Recovered from headache and fever | 100 | 0 | 25 | + |
| | | | | 100 | 0 | 0 | |
| | | | | 88 | 0 | 0 | |
| | | | | 100 | 13 | 0 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 7 | American | Close contact | Normal | 100 | 13 | 100 | — |
| 8 | " | Possible contact | " | 63 | 0 | 83 | — |
| 9 | " | " " | " | 72 | 0 | 72 | — |
| 10 | " | " " | " | 72 | 0 | 78 | — |
| 11 | " | " " | " | 72 | 0 | 67 | — |
| 12 | English | " " | " | 100 | 11 | 94 | — |
| 13 | American | " " | " | 78 | 28 | 74 | — |
| | | | | 100 | 6 | 100 | |
| 14 | " | " " | " | 100 | 0 | 100 | — |
| 15 | " | " " | " | 100 | 0 | 100 | — |
| 16 | " | " " | " | 100 | 0 | 75 | — |
| | | | | 100 | 6 | 100 | |
| 17 | " | " " | " | 100 | 13 | 100 | — |
| 18 | " | No history of contact | " | 100 | 0 | 100 | — |
| 19 | " | " " " " | " | 82 | 0 | 63 | — |
| 20 | Australian | " " " " | " | 83 | 28 | 78 | — |
| 21 | Chinese | " " " " | " | 100 | 0 | 100 | — |
| 22 | " | " " " " | " | 88 | 0 | 86 | — |
| 23 | " | " " " " | " | 100 | 0 | 86 | — |
| 24 | " | " " " " | " | 100 | 0 | 88 | — |
| 25 | Egyptian | " " " " | " | 100 | 0 | 100 | — |
| 26 | " | " " " " | " | 100 | 0 | 100 | — |
| 27 | " | " " " " | " | 100 | 0 | 100 | — |
| 28 | " | " " " " | " | 100 | 0 | 100 | — |
| 29 | South American | " " " " | " | 100 | 0 | 100 | — |
| 30 | " " | " " " " | " | 100 | 0 | 100 | — |
| 31 | " " | " " " " | " | 100 | 0 | 100 | — |
| 32 | " " | " " " " | " | 100 | 0 | 100 | — |
| 33 | American Negro | " " " " | Syphilis of aorta | 86 | 0 | 88 | — |
| 34 | " " | " " " " | " " " | 86 | 0 | 88 | — |
| 35 | " " | " " " " | Arteriosclerosis | 100 | 0 | 100 | — |
| 36 | " " | " " " " | " | 100 | 0 | 100 | — |
| 37 | " " | " " " " | Pseudobulbar palsy | 100 | 0 | 100 | — |

TABLE III—*Concluded*

| Person's No. | Nationality | History of contact with virus | Notes on person | Percentage of mice that died | | | Interpretation of results of neutralization tests |
|---|---|---|---|---|---|---|---|
| | | | | Negative control | Positive control | Test serum | |
| 38 | American Negro | No history of contact | Obesity, pulmonary edema | 88 | 0 | 12 | + |
| | | | | 100 | 19 | 35 | |
| | | | | 100 | 13 | 39 | |
| 39 | American | " " " " | Epidemic encephalitis | 100 | 11 | 100 | − |
| 40 | " | " " " " | " " | 100 | 11 | 100 | − |
| 41 | " | " " " " | " " | 100 | 17 | 84 | − |
| 42 | " | " " " " | " " | 100 | 17 | 67 | − |
| 43 | " | " " " " | Postinfection encephalitis | 100 | 0 | 100 | − |
| 44 | " | " " " " | Encephalitis? | 100 | 11 | 88 | − |
| | | | | 100 | 0 | 100 | |
| 45 | " | " " " " | Benign lymphocytic meningitis | 100 | 13 | 100 | − |
| 46 | " | " " " " | Pertussis, vaccinia | 100 | 17 | 100 | − |
| 47 | " | " " " " | Varicella | 78 | 28 | 71 | − |
| 48 | " | " " " " | Measles, vaccinia | 100 | 17 | 100 | − |
| 49 | " | " " " " | Measles | 78 | 28 | 83 | − |
| 50 | " | " " " " | Measles, pneumonia, vaccinia | 78 | 28 | 71 | − |
| 51 | " | " " " " | Pneumonia | 88 | 0 | 88 | − |
| 52 | " | " " " " | " | 86 | 0 | 88 | − |
| 53 | " | " " " " | Aplastic anemia | 88 | 0 | 100 | − |
| 54 | " | " " " " | " " | 88 | 0 | 86 | − |
| 55 | " | " " " " | Rheumatic fever | 86 | 0 | 75 | − |
| 56 | " | " " " " | " " | 86 | 0 | 75 | − |
| 57 | " | " " " " | " " | 100 | 0 | 100 | − |
| 58 | " | " " " " | " " | 88 | 0 | 100 | − |
| 59 | " | " " " " | Rheumatic heart disease | 88 | 0 | 88 | − |
| 60 | " | " " " " | Carcinoma of stomach | 100 | 0 | 100 | − |
| 61 | " | " " " " | Hypertension | 86 | 0 | 83 | − |
| 62 | " | " " " " | Nephritis | 86 | 0 | 86 | − |
| 63 | " | " " " " | " | 88 | 0 | 62 | − |

+ indicates that the serum of the individual neutralized the virus of louping ill.

− indicates that neutralization did not occur when the individual's serum and the virus were mixed.

An examination of Table III reveals the following facts: No evidence was obtained to indicate that nationality and race determine the presence or absence of neutralizing properties of serum for louping ill virus. The sera of individuals sick of or recovered from nephritis, hypertension, arteriosclerosis, syphilis, carcinoma of the stomach, rheumatic heart disease, rheumatic fever, aplastic anemia, pneumonia, measles, pertussis, varicella, vaccinia, pseudobulbar palsy, epidemic encephalitis, postinfection encephalitis, benign lymphocytic meningitis, do not neutralize the virus of louping ill. Although the age and sex of the persons from whom the sera were collected are not shown in the table, it can be stated that these factors have no apparent effect upon the presence or absence of significant neutralizing antibodies against the active agent. The striking fact obtained from a study of the results in the table is that close contact of individuals with the virus is in some manner associated with the appearance in their sera of neutralizing properties. For instance, of seven people who had been in close contact with the active agent, five possess neutralizing sera. Of these five, three (Cases 1, 2, 4) had had encephalitis, one (Case 3) had been sick with an influenza-like disease, and one had not been consciously ill while working with the virus. Of ten individuals who might have had contact with the active agent but who had not worked with it, none possesses a neutralizing serum. Of 46 people who gave no history of contact with the virus, only one, No. 38, possesses a serum with neutralizing antibodies and these upon repeated tests were found to be less active or less abundant than were those of the other positive sera. There is no obvious explanation of why this serum should have been positive.

## DISCUSSION

Several interesting features of the cases presented in the first part of the paper deserve comment. It should be noted (Charts 1, 2, 3) that all of the patients had a pulse-temperature disproportion and that a small amount of blood was present in a number of the specimens of spinal fluid which the operators believed was not due to the trauma of the needle. One patient was thought to have had epidemic encephalitis, two were considered to have had tuberculous meningitis. Since all of them recovered promptly and completely it is unlikely that these

diagnoses were correct. All of the physicians who examined the patients, however, agree that the individuals had an encephalitis. Two (Cases 1, 2) of the four patients had an influenza-like disease followed by a short period of fair health before the onset of symptoms and signs of encephalitis. A third patient (Case 4) was sick for 4 days, although he continued to work, before the onset of the severe symptoms of encephalitis. The fourth patient (Case 3) had what seemed to be nothing more than a severe attack of influenza. It has been stated (8) that louping ill virus may at times produce in sheep an infection without much apparent involvement of the central nervous system. Consequently, one wonders whether the primary illness in three (Cases 1, 2, 4) of the patients and the illness of the fourth (Case 3) represent systemic infections which in three instances were followed by involvement of the central nervous system. In sheep and monkeys, ataxia due to involvement of the cerebellum is a prominent feature of louping ill. The cases presented in this paper evidenced no signs of ataxia. This fact, however, does not preclude the possibility that they represent instances of the disease in man.

The question of whether the attacks of disease in man described by us represent instances of infection with louping ill virus now arises. In fact, one might suggest that they represent cases of encephalitis similar to those that occurred in St. Louis during the summer of 1933. This is not true, however, because sera from our cases, which neutralize the virus of louping ill, do not neutralize the St. Louis virus (9). Unfortunately, a definite answer to the question raised cannot be given, because louping ill virus was not recovered from any of our cases. Nevertheless, the circumstances under which they occurred and the results of the neutralization tests make it likely that they represent such an infection. If the cases represent louping ill infections in man, the frequency with which they occurred in one laboratory was probably due to the fact that the intranasal instillations of the virus in large numbers of mice, practiced in that laboratory, led to the exposure of the workers to large doses of the virus suspended in the air in droplets of moisture.

The situation described by us is somewhat unique, because louping ill virus has been in America for only a short period of time, and, so far as is known, is to be found in only four laboratories. Therefore, the

facts obtained by means of our neutralization tests may be of significance in relation to certain general phenomena of immunity and for this reason may be interpreted in a broader way than merely as an attempt to diagnose the cases presented. For example, recently it has been suggested (10) that the neutralizing antibodies against poliomyelitis virus in the sera of adults who have not had obvious attacks of poliomyelitis are due to serological maturation instead of contact with or of subclinical infections with the virus. From the results of our experience with neutralization tests in connection with louping ill virus it appears that the antibodies which we demonstrated are not likely to arise in the absence of the active agent.

## SUMMARY

Four instances of infection in man which are believed, because of the circumstances under which they occurred and in view of the results of neutralization tests, to represent cases of louping ill have been described. Evidence obtained by the neutralization tests is in favor of the idea that the antibodies against louping ill virus demonstrated in certain sera were most likely the result either of contact with or of infection with the active agent.

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