MENINGITIS IN MAN CAUSED BY A FILTERABLE VIRUS

II. Identification of the Etiological Agent

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In the preceding paper (1) we described the clinical picture presented by two patients who were suffering from a nonbacterial lymphocytic meningitis, and the method by which a virus-like agent was isolated from each patient's spinal fluid. Then we showed that the two agents were immunologically identical and that they were etiologically related to the disease process in the individuals from whom they were obtained. In the present communication we shall describe experiments in support of the viral nature of the agent, state details concerning the range of susceptible hosts and the clinical and pathological picture developed in each, compare our active agent with known viruses that spontaneously affect the central nervous system of man or lower animals or that might have contaminated our materials because of their proximity in the laboratory, and, finally, discuss the relative importance of our agent as a cause of disease in human beings.

Viral Nature of the Transmissible Agent

Invisibility.—Numerous sections of organs from animals dead of infection with the active agent have been stained according to Giemsa's method and studied by means of the microscope. In no instance has it been possible to demonstrate the presence of ordinary bacteria, protozoa, or fungi.

Failure of Cultivation.—Infectious material was seeded in meat infusion broth and on blood agar. The cultures were then incubated either aerobically or anaerobically at 37°C. for 2 weeks. With the exception of a few obvious contaminants no organism of etiological significance was encountered.

Filterability.—Both strains of the active agent were tested by means of Seitz filters and graded collodion membranes. Inasmuch as the findings with the 2 strains were the same and since only the W. E. strain was tested with Berkefeld

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candles, the results of experiments on the filterability of this strain will be presented in full.

Filtration through Berkefeld Candles.—3 Berkefeld candles, V, N, and W, shown by air pressure to be free from leaks, were satisfied by the passage of 40 cc. of sterile broth through each of them.

A 2 per cent emulsion of mouse brains infected with the W.E. strain was prepared in a diluent of 10 per cent human ascitic fluid in equal parts of nutrient broth and Locke's solution. After thorough centrifugation at 2000 r.p.m., the supernatant fluid was removed and divided into 4 equal parts of about 15 cc. each.

1 portion of the supernatant fluid was passed through the V candle, and 0.03 cc. of the filtrate were then inoculated intracerebrally into each of 6 mice. 50 per cent of the mice died on the 7th day after inoculation and the remainder were sacrificed for passage. When the brains of the animals were found to be free from ordinary bacteria, a 10 per cent emulsion in Locke's solution was prepared from which decimal dilutions were made. Intracerebral inoculations into mice of dilutions up to 10^{-2} caused death in 100 per cent of animals after the usual incubation period.

The 3 remaining portions of the supernatant fluid, purposely contaminated with *B. prodigiosus*, were passed through the candles, 1 through the V used above, 1 through the N, and 1 through the W. Portions from each filtrate were separately cultured aerobically on blood agar and aerobically and anerobically in meat infusion broth. Portions from each filtrate were also inoculated intracerebrally into mice. The cultures of the filtrates showed no evidence of bacterial growth after incubation at 37°C. for 7 days. However, all the mice died within 8 to 12 days after inoculation.

From the results of the experiment just described it is obvious that the active agent passes Berkefeld candles V, N, and W that hold back ordinary bacteria.

Filtration through Seitz Filters.—A 2 per cent emulsion of mouse brains infected with the W.E. strain was prepared in a diluent consisting of equal parts of human ascitic fluid, nutrient broth, and sterile distilled water. The emulsion was centrifuged at 1500 r.p.m. for 30 minutes and the supernatant fluid was removed, a portion of which was reserved for titration of its potency. 70 cc. of the remaining supernatant fluid was forced, under 8 pounds of pressure, through a 60 mm. Seitz pad which had been satisfied by the passage through it of 100 cc. of sterile broth. A portion of the filtrate was reserved for titration of its potency, while the remainder was used for filtration through graded collodion membranes to be described later.

The ability of the active agent to pass through Seitz pads was estimated by a comparison of the results of the intracerebral titrations

of the unfiltered and filtered portions of the supernatant fluid. In the titrations, not only were the morbidity and mortality rates considered, but the end-points were more precisely determined by tests for the presence of immunity in the mice surviving the original inoculations. As is evident from the results shown in Table I, the active agent was present in dilutions of the unfiltered material up to 10^{-5} because 3 of 5 mice receiving inoculations of this dilution were immune, while demonstrable amounts of the agent were not present in dilution of the filtrate higher than 10^{-2}. Such results clearly indi-

TABLE I

Results of Filtration of W. E. Strain of Virus through a Seitz Filter

Titration of virus before filtration					Titration of virus after filtration				
Dilution of virus	Results of initial inoculation		Results of reinoculation with potent virus		Dilution of virus	Results of initial inoculation		Results of reinoculation with potent virus	
	Sick	Dead	Sick	Dead		Sick	Dead	Sick	Dead
Undiluted	6/6	3/6	Not done		Undiluted	6/6	1/6	1/6	0/6
10^{-1}	6/6	5/6	"	"	10^{-1}	6/6	2/6		
10^{-2}	6/6	5/6	"	"	10^{-2}	6/6	1/6	0/5	0/5
10^{-3}	6/6	4/6	"	"	10^{-3}	4/6	0/6	3/3	2/3
10^{-4}	1/6	1/6	1/4	1/4	10^{-4}	0/6	0/6	6/6	6/6
10^{-5}	0/6	0/6	2/5	2/5	10^{-5}	0/6	0/6	6/6	6/6
Uninoculated control mice			6/6	6/6	Uninoculated control mice			6/6	6/6

The results of the experiment are expressed as fractions, the denominators representing the number of mice inoculated, the numerators the number of mice either sick or dead.

cate that the agent was capable of passing a Seitz pad in spite of the fact that 99.9 per cent of it was held back under the conditions of the experiment.

Filtration through Graded Collodion Membranes.—As more accurate information regarding the approximate size of particles can be obtained by filtration through collodion membranes of graded average pore diameter than through Berkefeld candles or Seitz pads, such membranes were employed in the investigation of the approximate size of our active agent.

For filtration through membranes[1] of average pore size ranging from 850 mμ

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[1] Experiments on filtration through graded collodion membranes were performed with the cooperation of Dr. J. H. Bauer of the International Health Division of the Rockefeller Foundation.

to 250 mμ, the supernatant fluid (with the active agent) described for the experiment with Seitz filters was employed. For membranes of average pore diameter ranging from 250 mμ to 50 mμ, the Seitz filtrate of that supernatant fluid was used. Before filtration each membrane was satisfied by the passage of 6 cc. of broth through it. The material was forced through the 850 mμ membrane under a pressure of 10 pounds, while a pressure of 30 pounds was used for the other membranes. The filtrate from each membrane was tested for the presence of the infectious agent by means of intracerebral inoculations into white mice, both the results of primary inoculations and those of reinoculations for tests of immunity in the survivors being taken into consideration.

TABLE II

Results of Filtration of W. E. Strain of Virus through Collodion Membranes

Average pore size of membrane		Results of inoculation with membrane filtrates		Results of reinoculation with potent unfiltered virus	
		Sick	Dead	Sick	Dead
	mμ				
Unfiltered superna-	850	6/6	3/6	Not done	
tant used for filtra-	650	6/6	4/6	" "	
tion	450	6/6	5/6	" "	
	250	6/6	3/6	" "	
Seitz filtrate used for	250	4/7	1/7	4/6	4/6
filtration	210	6/6	1/6	4/5	3/5
	150	0/6	0/6	6/6	6/6
	110	0/6	0/6	6/6	6/6
	85	0/6	0/6	6/6	6/6
	50	0/6	0/6	6/6	6/6
Uninoculated control mice..........................				6/6	6/6

The results of the experiment are expressed as fractions, the denominators representing the number of mice inoculated, the numerators the number of mice either sick or dead.

The results of the above experiment, summarized in Table II, indicate that all or some of the mice receiving filtrates from membranes with an average pore diameter of 210 mμ or greater became sick or died. When the survivors were reinoculated it was found (Table II) that immunity was present in some of the animals that had received filtrates from the membranes with average pore diameters of 250 mμ and 210 mμ but not in those inoculated with filtrates

from membranes of 150 mμ or smaller average pore diameters. According to Elford (2), particles, in order to pass through a membrane with pores having an average diameter of 210 mμ, cannot possess a diameter greater than one-half or three-quarters of that of the pores. Consequently, the particles of our active agent do not possess diameters greater than 100–150 mμ.

Results of experiments being conducted at the present time in which Berkefeld V filtrates, instead of Seitz filtrates, containing the active agent are passed through graded collodion membranes indicate that our virus is capable of penetrating membranes with average pore diameters of 150 mμ. V candles allow the passage of more virus than do Seitz pads, and, inasmuch as the estimation of the size of a virus is influenced by its concentration in the material filtered, the difference in the results of the experiments cited is adequately accounted for. Our active agent is obviously smaller than any known bacterium, and results of experiments in which attempts have been made to estimate its actual size will be given in a later paper.

Preservation of the Active Agent in Glycerol.—In infected mouse brains stored in 50 per cent glycerol in Locke's solution at 0°C., sufficient virus remained active for 217 days to kill 3 of 5 mice inoculated intracerebrally with a 10 per cent emulsion of them. Under similar conditions the virus in the brains of guinea pigs retained its activity for 235 days.

Preservation of the Active Agent in the Absence of Glycerol.—Virus was easily demonstrated in a 10 per cent emulsion made from infected mouse brains and kept under a vaseline seal at +5°C. for 56 days. Virus in a guinea pig brain stored in a Petri dish at +5°C. for 32 days was still active.

Preservation of the Active Agent by Means of Desiccation.—To a 10 per cent emulsion of infected mouse brains in Locke's solution sufficient sterile acacia was added to make a 2.5 per cent solution. This mixture was dried in the frozen state, sealed *in vacuo* (3), and stored at +4°C. for 49 days. Sufficient active virus was still present at the end of this time to kill 1 of 4 mice and immunize the other 3 when small amounts of the dried material resuspended in Locke's solution were inoculated intracerebrally.

The facts presented above regarding the invisibility, failure of cultivation, filterability, size, and preservation of our active agent clearly indicate that it should be classed with the viruses. Now we shall pass to a consideration of the clinical and pathological pictures produced by the virus in susceptible laboratory animals.

Response of Laboratory Animals to Inoculations of the Virus

Mice.—The following events take place in mice as a result of intra-cerebral inoculations of the virus.

Clinical Picture.—During the first 5 days after inoculation the mice appear well. Occasionally on the 5th, but more commonly on the 6th day, symptoms appear, at which time some of the mice may be found dead although none of them were obviously sick on the preceding day, while others with dirty, ruffled fur, half-closed eyes, and hunched backs remain motionless. When disturbed they occasionally leap up and down in the jar and fall over backwards; but the characteristic reaction, especially when the animals are susupended by the tail, is for them to exhibit coarse tremors of the head and extremities frequently going on to a series of clonic convulsions terminating in a tonic extension of the hind legs. In male mice an erection sometimes occurs during the convulsions. The convulsions, often the cause of death, may also occur spontaneously either in sick mice or even in those that appear to be normal. As a rule, the animals either die within 1 to 3 days after the onset of symptoms or quickly recover in 5 or 6 days. Paralyses have never been observed.

Pathological Picture.—Brain and Cord. In this strain of mice, only a slight congestion of the brain is observed in the gross. Stained sections reveal an engorgement of the surface vessels and an occasional hemorrhage in the meninges. The meninges of the brain, and to a less extent those of the cord, are infiltrated with mononuclear cells resembling lymphocytes (Figs. 1 and 2). At times a slight infiltration of mononuclear cells into the subependymal tissues and the choroid plexus is noted. Necrosis of nerve cells and perivascular cuffing are rarely if ever found in early lesions. If the animals are chronically ill or if they have been killed 2 to 3 weeks after recovery considerable perivascular cuffing may be present. *Liver and Spleen.* Sections of the liver reveal some engorgement of the capillaries, a definite increase in the number of Kupffer cells, and a few small areas of focal necrosis. Nothing particularly characteristic of the disease is seen in the spleen. *Lungs.* Regardless of the portal of entry of the virus, the lungs frequently show areas of discoloration and consolidation which are free from bacteria. Stained sections of these areas reveal an interstitial bronchopneumonia similar to that caused by a number of viruses. The small blood vessels are surrounded by numerous round cells. Involvement of the bronchioles, characterized by desquamation of the mucous membrane and cuffing with mononuclear· elements, occurs only where the pathological changes are most marked. In definitely consolidated portions of the lungs, the alveolar walls are densely infiltrated with mononuclear cells; the alveoli may be collapsed or may contain an exudate consisting of fibrin and a few cells which are usually of the mononuclear group. In some sections lungs present nothing more than distended capillaries and a few alveoli filled with red blood cells. *Other Organs.* The remaining organs present no characteristic changes.

Distribution of the Virus in the Mouse.—After a number of experiments in which blood and emulsions of organs from infected mice were inoculated into normal mice it was obvious that the virus could be recovered from the blood and hence equally well from the brain, liver, spleen, or lungs.

Effect of Route of Inoculation upon the Course of the Disease.—The course of events varied with the route of inoculation of the virus. (*a*) When the virus was introduced intracerebrally, the typical illness described above resulted 5 to 7 days after inoculation. (*b*) When 0.5 cc. of a virus-containing emulsion were administered intraperitoneally, the mice showed indefinite symptoms of illness after an incubation period of 8 days, and only a few died. When the liver of one of the sick animals was removed and an emulsion of it was injected intraperitoneally into normal mice, the animals did not become sick, but were later found to resist infection by the virus administered intracerebrally. (*c*) Subcutaneous inoculations of the virus produced no obvious illness in mice but immunity to virus given intracerebrally. (*d*) Virus administered intranasally to etherized mice resulted in no illness but solid immunity. (*e*) Intravenous injections of the virus in the form of a 10 per cent suspension prepared from an infected brain produced no evidence of illness, but, when an emulsion of the brains of these inoculated mice, removed 12 days after the injections, was administered intracerebrally to normal mice, the typical picture of the disease developed and the virus could be passed from them to another group of normal mice.

Contagiousness of the Disease.—Traub's (4) report of the discovery of a virus, later shown to be closely related to if not identical with our active agent, that was widely disseminated in his stock mice and which presumably spread in some manner from mouse to mouse, induced us to ascertain whether either infection or immunity could be produced in normal mice by contact with a mouse infected with our virus, precautions being taken when the infected animal died to prevent the normal contacts infecting themselves intranasally by devouring the corpse. 2 experiments were performed in the following manner. A mouse inoculated intracerebrally 4 days previously with the R.E.S. strain of virus was placed in a jar with 5 normal mice 4 weeks of age. The inoculated animal became sick on the 1st day after being placed with the normal mice, the 5th day after inoculation. It lived 3 days longer and was found dead on the morning of the 4th day. The normal contacts were observed for 3½ weeks and no evidences of illness were noted. Then they were inoculated intracerebrally with potent virus and found to be fully susceptible. The second experiment was conducted with the W.E. strain of virus in a manner similar to that just described. No infection or immunity was produced by close contact of the normal mice with the infected animal. The results of the two experiments clearly indicate that our virus does not spread with ease from mouse to mouse when normal animals are not allowed to devour an infected corpse.

Inheritance of Resistance to the Virus.—On two occasions, 4 weeks old mice from infected or recovered mothers have been inoculated intracerebrally with potent virus. In each instance all the animals became sick on the 5th or 6th day after

inoculation and were dead by the 7th. Thus it appears that no immunity to the virus is to be found in mice born of infected or immune mothers.

Guinea Pigs.—Having presented in detail the reactions of mice to the virus, we shall now describe those of guinea pigs.

Susceptibility.—Guinea pigs inoculated intracerebrally (0.1 cc.) or subcutaneously (0.25 cc.) with emulsions of infected mouse brains become sick and the majority of them die. The virus can be propagated indefinitely in this host by means of brain to brain or by brain to skin to brain passages.

Clinical Picture.—The clinical picture produced in guinea pigs by intracerebral inoculations of the virus is essentially the same as that caused by subcutaneous injections, with the exception that the former method of administration leads to a more acute illness. After intracerebral inoculation, the temperature of the pigs usually becomes elevated within 24 to 48 hours, while after subcutaneous administration the rise is, as a rule, delayed 3 to 6 days. The temperature once elevated usually remains high, often reaching 106–107°F., until shortly before death when it may drop suddenly to a subnormal level. The average duration of illness following intracerebral and subcutaneous inoculations is 9 or 10 and 12 to 16 days, respectively. When infected, the pigs lose weight rapidly and may develop labored breathing and a mild diarrhea. Marked terminal salivation is not unusual. Definite neurological manifestations are not observed.

Pathological Picture.—A mild meningeal reaction characterized by infiltration of mononuclear cells and an interstitial bronchopneumonia (Figs. 3 and 4) are found in pigs intracerebrally inoculated with the virus. Little or no pathological change has been seen in the brain and cord. After subcutaneous inoculations, the picture is similar to that just described with the exception that there is a minimum involvement of the meninges.

Distribution of Virus in the Body.—The blood of guinea pigs inoculated intracerebrally is infectious for mice at least as early as the 4th day after inoculation and remains so until the death of the animal. The brains of pigs that die as the result of subcutaneous administration of the virus are infectious for mice. Other organs were not tested, but the fact that the active agent is in the blood indicated that emulsions of all organs would also contain it.

Monkeys.—Although monkeys are susceptible to the virus, we have not made extensive investigations of its effects in them. The animals become sick after intracerebral inoculations and the virus can be propagated by brain to brain passages.

Clinical Picture.—4 to 7 days after intracerebral inoculations of 1.0 cc. of a 10 per cent emulsion of infected mouse brains, the monkeys develop fever, the temperature being 104°F. or more, that lasts 1 to 3 days. When the fever disappears the animals may begin to look sick, lose their appetite, and become less active.

One monkey appeared to develop a hyperesthesia, inasmuch as it sat in the cage on the smallest possible area of body surface and refused to be touched by its mate. Recovery usually takes place and is complete within about 3 weeks. A cisternal puncture was performed on 1 monkey 9 days after inoculation, and the cerebro-spinal fluid contained 1410 cells of which 97 per cent were mononuclear elements. Serum from recovered animals contains neutralizing antibodies.

Pathological Picture.—The only pathological changes of importance were found in the brain and cord. In the gross, the surface of these organs and the choroid plexus appear pinker than normal. Stained sections reveal a moderate amount of mononuclear infiltration in the meninges. There is also a marked involvement of the choroid plexus, a phenomenon either not observed or decidedly less definite in mice and guinea pigs. Between the walls of the blood vessels and the ependymal covering of the plexus occurs a dense infiltration of mononuclear cells (Figs. 5, 6, and 7). In places, the collection of cells causes sufficient tension to distort the cuboidal ependymal cells into long tenuous elements difficult to see (Fig. 7). There is a moderate amount of infiltration in the subependymal tissues and an occasional blood vessel is surrounded by a single layer of mononuclear elements. As yet, no lesions of importance have been found in the nervous system proper except in sections through or near the site of inoculation.

Distribution of Virus in the Body.—We have found that the brain, spinal fluid, and blood of a sick monkey contain the virus.

Rabbits.—Both strains of the virus were administered intracerebrally, dermally, and intradermally to several rabbits. No obvious illness was caused in the animals and the matter was not pursued.

From the data, it appears that mice, guinea pigs, and monkeys are susceptible to our virus and that it can be serially passed in them with ease, while rabbits show little or no susceptibility. The susceptible hosts display differences in their clinical symptoms but a similarity in the pathological pictures. The route of inoculation is sometimes of importance, because in the mouse clinical symptoms of the characteristic disease occur only after intracerebral administration of the virus, while in pigs both intracerebral and subcutaneous injections are equally efficacious. The most marked pathological changes are found in the meninges, choroid plexus, and the lungs. We have not as yet been able to demonstrate inclusion bodies in infected cells similar to those described by Traub (4).

Identification of the Virus

To identify our virus a comparison of its properties with those of a selected group of known viruses was made. The selection for com-

parison was conducted in the following manner. First, any virus that had been investigated in our laboratory or in other laboratories at the Institute in New York City or at Princeton was considered as a possible cause of the infection in the patients or a subsequent contaminant of our animal material. Secondly, any virus which, from its description, has characteristics closely resembling those of our active agent was critically examined in order to confirm or dispose of their identity.

Viruses Being Studied or Carried in Our Laboratory.—Virus III of rabbits, and the viruses of vaccinia, herpes simplex, infectious myxomatosis of rabbits, mumps, psittacosis, louping ill, and Rift Valley fever were for obvious reasons easily disposed of. Moreover, a known immune louping ill serum failed to neutralize our virus.

Viruses under Investigation in Other Laboratories of The Rockefeller Institute in New York City and Princeton.—B virus and the viruses of pseudorabies, equine encephalomyelitis, poliomyelitis, rabies, yellow fever, vesicular stomatitis, human influenza, swine influenza, lymphogranuloma inguinale, St. Louis type of encephalitis, Theiler's disease of mice, and rabbit pox were obviously not closely related to our active agent. Furthermore, convalescent serum from the two patients did not neutralize the virus of the St. Louis type of encephalitis.

Traub (4) recently described a virus indigenous to the stock mice in The Rockefeller Institute at Princeton which causes a malady indistinguishable from that induced by our active agent and produces pathological changes similar to those seen in our animals, with the exception that a slight amount of necrosis of nerve cells and a few intranuclear inclusion bodies were reported to occur in his infected animals.

Reports in the Literature of Viruses Similar to Ours.—As far as could be ascertained from the literature only one other virus, in addition to that described by Traub and noted above, has characteristics closely resembling those of our active agent. This virus was discovered by Armstrong and Lillie (5) and designated by them as the virus of lymphocytic choriomeningitis. Its properties, host range, and activities appear to be identical with those of our virus.

It is a well recognized fact that occasionally similar clinical and pathological pictures may be produced by two or more different viruses. Therefore, it could not be assumed that our virus, the Traub virus, and the Armstrong-Lillie virus are identical because of a similarity in their attributes. Consequently, it was necessary to investigate the immunological relationship of the three viruses. With the cooperation of Dr. Traub and Dr. Armstrong the following cross neutralization and cross reinoculation experiments were performed.

*Demonstration of Similar Protective Antibodies in the Serum of Animals
Respectively Immune to Each of the Three Viruses*

The cross neutralization tests were performed independently by
Traub and ourselves. The method used by Traub was similar to
ours as already described (1) in connection with the investigations of
the neutralizing power of the serum of our two patients. In both
instances the serum-virus mixtures were tested in guinea pigs. From
Traub's results quoted by Armstrong and Dickens (6) and shown in
Table III, it can be seen that the serum from our immune monkey
and from Armstrong's immune monkey neutralized 100 lethal doses
of the Traub virus which had a titer of 10^{-3}. It is equally evident
from Table IV, in which our results are shown, that serum from
Traub's immune guinea pig, Armstrong's immune monkey, and one
of our patients (W.E.) during convalescence neutralized 100 infective
doses of our virus which also had a titer of 10^{-3}.

*Demonstration That Animals Immune to One Virus Resist Infection
with Either of the Others*

The results of the experiments summarized in Table V were ob-
tained independently by Armstrong, Traub, and ourselves. Arm-
strong and Dickens (6) showed that mice immune to the Rivers-
Scott virus were also resistant to the Armstrong-Lillie active agent,
whereas normal mice, both from The Rockefeller Institute, New York,
and the National Institute of Health, Washington, were susceptible.
Traub demonstrated that mice and a guinea pig immune to the
Rivers-Scott virus were equally resistant to his agent, while stock
guinea pigs and stock mice from New York were susceptible. We
showed that guinea pigs immune to the Traub virus were also im-
mune to the Rivers-Scott agent, whereas stock pigs were susceptible.

When Traub made his cross reinoculation experiment he also in-
cluded mice from the Princeton infected stock and found that 50
per cent of them were immune (Table V). The finding of such a high
percentage of immune mice in the Princeton stock and the absence
of immune animals from the stock used by us whether derived from
The Rockefeller Institute, New York, or from an outside dealer
(Freed) is additional evidence that the mice with which we are work-
ing are free from the virus.

TABLE III

Results of Neutralization Experiment Showing Immunological Identity of the Armstrong-Lillie, Traub, and Rivers-Scott Viruses

Dilution of virus	Serum of monkey immune to Rivers-Scott virus plus Traub virus	Serum of monkey immune to Armstrong-Lillie virus plus Traub virus	Serum of normal human being plus Traub virus	Serum of monkey immune to louping ill plus Traub virus	Serum of normal human being plus Traub virus
10^{-2}	No fever, survived	No fever, survived	Typical illness, died	Typical illness, died	Typical illness, died
10^{-3}	" "	" "	" "	" "	" "
10^{-4}	" "	" "	No fever, survived	No fever, survived	No fever, survived

The neutralization experiment was conducted by Traub in the manner described in the previous paper (1) except that the foot pad was the site chosen for inoculation.

TABLE IV

Results of Neutralization Experiment Showing Immunological Identity of the Armstrong-Lillie, Traub, and Rivers-Scott Viruses

Dilution of virus	Serum of human being (W.E.) immune to Rivers-Scott virus plus Rivers-Scott virus	Serum of normal human being plus Rivers-Scott virus	Serum of guinea pig immune to Traub virus plus Rivers-Scott virus	Serum of monkey immune to Armstrong-Lillie virus plus Rivers-Scott virus
10^{-1}	No fever, survived	Typical illness, died	No fever, survived	No fever, survived
10^{-2}	Transient fever, survived	" "	" "	" "
10^{-3}	No fever, survived	Typical illness, survived	" "	" "

The neutralization tests were conducted in the manner described in the previous paper (1).

TABLE V

Results of Reinoculation Experiments Showing Immunological Identity of the Armstrong-Lillie, Traub, and Rivers-Scott Viruses

Animals immune to Rivers-Scott virus

Reinoculations with Armstrong-Lillie virus*

Type of animal	Result (Dead)
12 mice immune to Rivers-Scott virus	0/12
12 normal mice Rockefeller Inst., N. Y., healthy stock	12/12
6 normal mice Nat'l Inst. of Health, Wash., healthy stock	5/6

Reinoculations with Traub virus†

Type of animal	Result (Sick)	Result (Dead)
10 mice immune to Rivers-Scott virus	0/10	0/10
12 normal mice Rockefeller Inst., N. Y., healthy stock	12/12	11/12
12 normal mice outside dealer (Freed), healthy stock	12/12	9/12
12 mice, Rockefeller Inst. at Princeton, infected stock	6/12	6/12
Guinea pig immune to Rivers-Scott virus	No fever, survived	
2 normal stock guinea pigs	Typical illness, died in 12 days	

Animals immune to Traub virus

Reinoculations with Rivers-Scott virus‡

Type of animal	Result
2 guinea pigs immune to Traub virus	No fever, survived
2 normal stock guinea pigs	Typical illness, died in 13 days

The results of the experiments in which mice were used have been expressed as fractions, the denominators representing the number of mice inoculated, the numerators the number of mice either sick or dead.

The inoculations were made intracerebrally in all animals.

* Tests were performed by Dr. Armstrong.

† Tests were performed by Dr. Traub.

‡ Tests were performed by Drs. Rivers and Scott.

A comparison of the reactions of laboratory animals to our virus, the Traub virus, and the Armstrong-Lillie virus, and the results of cross neutralization and cross reinoculation experiments clearly indicate that the three viruses are closely related if not identical.

DISCUSSION

The immunological identity of the 3 strains of virus independently discovered permits us to use data already published by each of the other workers concerning the different strains in attempts to determine the extent of the distribution of the virus among laboratory animals and its importance as a cause of disease in human beings.

The virus under consideration was isolated in 3 different laboratories from 3 different hosts. Armstrong and Lillie (5) recovered the active agent from the brain of a *Macacus rhesus* monkey well along in a series of monkeys originally inoculated with material from the brain of a person who had died of the St. Louis type of encephalitis. Later Armstrong and Wooley (7) found evidence of immunity to the virus in some of their stock monkeys and even isolated it from the brain of an animal dying of experimental poliomyelitis. Traub (4) discovered his strain of this virus in albino Swiss mice and at first thought them to be the natural host. We have definitely demonstrated that our strain of the virus was obtained from the spinal fluid of human beings in whom it caused a meningitis.

Sufficient evidence is not yet available to estimate the ease with which infection can spread from animal to animal within a given species, or from one species to another. The fact that the malady has persisted for quite a while in stock mice at Princeton, certainly indicates that the virus can spread spontaneously from mouse to mouse. But, judging from the results of our experiments detailed above, its contagiousness is not very great. Armstrong did not encounter immune monkeys at the beginning of experiments on this virus, but at a later period he did. This may have been due to coincidence, but was more probably consequent on a spontaneous spread of the disease, once established, among the stock monkeys.

In regard to the spread of the disease, caused by the new virus, from lower animals to man or *vice versa*, the evidence is limited, but that which is available seems to indicate that it is more likely to go from man to lower animals than in the opposite direction.

None of the investigators actively engaged in work with the virus has become sick. Armstrong and Wooley (7) have reported that the serum of four workers constantly in contact with infected monkeys contains no neutralizing antibodies; Traub (8) states that, after working at least 2 or 3 months with the virus, two investigators had no neutralizing antibodies in their serum; and we have found that an individual in contact with the virus for 9 months still possesses no protective antibodies. Finally, Traub has allowed us to see evidence, which he will publish later, in favor of the idea that susceptible animals may contract the disease from a human carrier.

The evidence regarding the source of the infection of our patients is not conclusive. In the case of W.E. we can state definitely that it is unlikely that he came in contact with the virus from an animal source, because, so far as is known, there was no infected stock in the Institute where he was working at the time he became ill. R.E.S., however, worked with a stock of mice, which was later found to be infected, for 3 months prior to the onset of his illness, and therefore may have contracted the disease in that manner. Nevertheless as stated above, the disease does not readily spread to man from animals.

It seems that a source of virus in lower animals, if one exists, would be of little menace to man. Thus one is left with the probability that the source of infection for man is a human one. Evidence in favor of such an idea is difficult to obtain, yet that which is available does not nullify it and consists in finding either the virus or neutralizing antibodies against it in human beings geographically scattered who do not present histories of previous contact with infected lower animals.

We undoubtedly obtained the virus from the spinal fluids of two patients, one of whom gave no history of contact with infected animals. Armstrong and Lillie's (5) original strain C.G. may have come from the brain of a human being, because, at the time of its isolation, there was no evidence of a latent infection with the virus in their stock monkeys. Unfortunately, it is far from being clear that their second strain A.O. came from man, because, in the same paper (7) in which its isolation was described, Armstrong refers to the discovery of another strain in a monkey dying of experimental poliomyelitis.

The evidence regarding the presence of neutralizing antibodies in the serum of individuals geographically scattered is more striking. Armstrong and his colleagues (6, 7) have found protective antibodies

in the serum of 6 people living in different parts of the country from
California to the District of Columbia. 4 of them gave histories of
a previous illness similar to that observed in our patients, 1 had re-
covered from the St. Louis type of encephalitis and also possessed
antibodies against the virus of that malady, and 1 had no history of
any recent previous illness except grippe. Traub (8) found antiviral
substances in the serum of 2 of the men working in the animal house
at Princeton. It is true that 3 of the 8 individuals with antibodies
in their serum were located in the laboratories at Princeton or Wash-
ington, yet the others were so geographically scattered that one nec-
essarily considers the possibility of an endemic focus of the virus in
human beings.

The clinical picture of the disease definitely known to be caused by
the virus, that in our two patients and in the one cited by Armstrong
and Dickens (6), is seen not infrequently, and in 1925 Wallgren (9)
suggested that it probably was characteristic of a clinical entity.
For such an entity he adopted the name "acute aseptic meningitis,"
and laid down the following criteria for its diagnosis. Sudden onset
of signs of meningitis associated with a lymphocytic pleocytosis in
a spinal fluid free from bacteria, a short benign course, absence of a
focus of infection in the neighborhood of the brain, e.g., sinusitis,
and absence from the community of diseases known to be capable of
producing a meningeal irritation.

The problem would be considerably simplified if the discovery of
the new virus provided an etiological agent for Wallgren's clinical
entity. Facts, however, indicate that the virus is the cause of only
a few cases diagnosed as acute aseptic meningitis. We (1) have
failed to find the active agent in the spinal fluid of 7 patients suffering
from an illness that satisfied Wallgren's criteria[2] and 3 more indi-
viduals with a lymphocytic meningeal reaction concerning which
sufficient details are not available to determine whether it definitely
met the requirements. Furthermore, we have been unable to dem-
onstrate neutralizing antibodies against our virus in the serum col-

[2] The figures include the results of studies of an epidemic of acute aseptic
meningitis in Philadelphia during July and August, 1935. The privilege of in-
vestigating these cases was afforded by Stokes (10).

lected from 17 patients recovered from diseases diagnosed as acute aseptic meningitis,[2] and from 5 more individuals convalescent from a lymphocytic meningitis of unknown origin.

It has already been indicated that our virus produces for the most part a reaction in the meninges. Many viruses that attack the central nervous system also cause some reaction in the meninges, but, in addition to this type of damage, these active agents tend to injure some particular part of the brain or cord with a resultant set of symptoms and signs held to be more or less characteristic of certain maladies, e.g., the anterior horn cells are especially picked out by the virus of poliomyelitis, while the nuclei of cranial nerves are likely to be damaged in Economo's disease. In regard to the new virus, however, there is no evidence from the meager clinical data or from the more extensive experimental material that any part of the nervous system, except the meninges and choroid plexus, is especially involved. Hence signs of a meningitis are the sole clinical manifestation of its activity.

Signs of a meningitis may be the only clinical manifestation of abortive attacks of poliomyelitis, of the St. Louis type of encephalitis, or of epidemic encephalitis in its meningeal form. Moreover, meningeal signs may occasionally be the outstanding feature of other virus diseases, such as herpes zoster and mumps, in which their usual manifestations are absent or poorly developed. From what has been said, it is obvious that incorrect diagnoses are likely to occur not infrequently until a more general effort is made to determine the etiological agent functioning in each instance.

SUMMARY

Evidence was presented substantiating the idea that our active agent is a virus. The reactions produced in laboratory animals by the active agent were described, and a comparison of it with other viruses was made. The results of experiments indicating the immunological identity of our virus, Armstrong and Lillie's virus of lymphocytic choriomeningitis, and Traub's virus were given in detail. Finally, the evidence in regard to its natural host and its importance as a factor in diseases of human beings was presented and discussed.

BIBLIOGRAPHY

1. Scott, T. F. McN., and Rivers, T. M., *J. Exp. Med.*, 1936, **63**, 397.
2. Elford, W. J., *Proc. Roy. Soc. London, Series B*, 1933, **112**, 384.
3. Rivers, T. M., and Ward, S. M., *J. Exp. Med.*, 1935, **62**, 549.
4. Traub, E., *Science*, 1935, **81**, 298.
5. Armstrong, C., and Lillie, R. D., *Pub. Health Rep.*, *U. S. P. H. S.*, 1934, No. 35, 1019.
6. Armstrong, C., and Dickens, P. F., *Pub. Health Rep.*, *U. S. P. H. S.*, 1935, No. 25, 831.
7. Armstrong, C., and Wooley, J. G., *Pub. Health Rep.*, *U. S. P. H. S.*, 1935, No. 16, 537.
8. Traub, E., personal communication.
9. Wallgren, A., *Acta pediat.*, 1925, **4**, 158.
10. Stokes, J., personal communication.

EXPLANATION OF PLATES

PLATE 30

FIG. 1. Section of brain from an infected mouse showing cellular infiltration of the meninges and absence of changes in the brain substance. Hematoxylin and eosin. × 40.

FIG. 2. Higher magnification of the meninges of an infected mouse showing that the exudate consists chiefly of mononuclear cells. Hematoxylin and eosin. × 250.

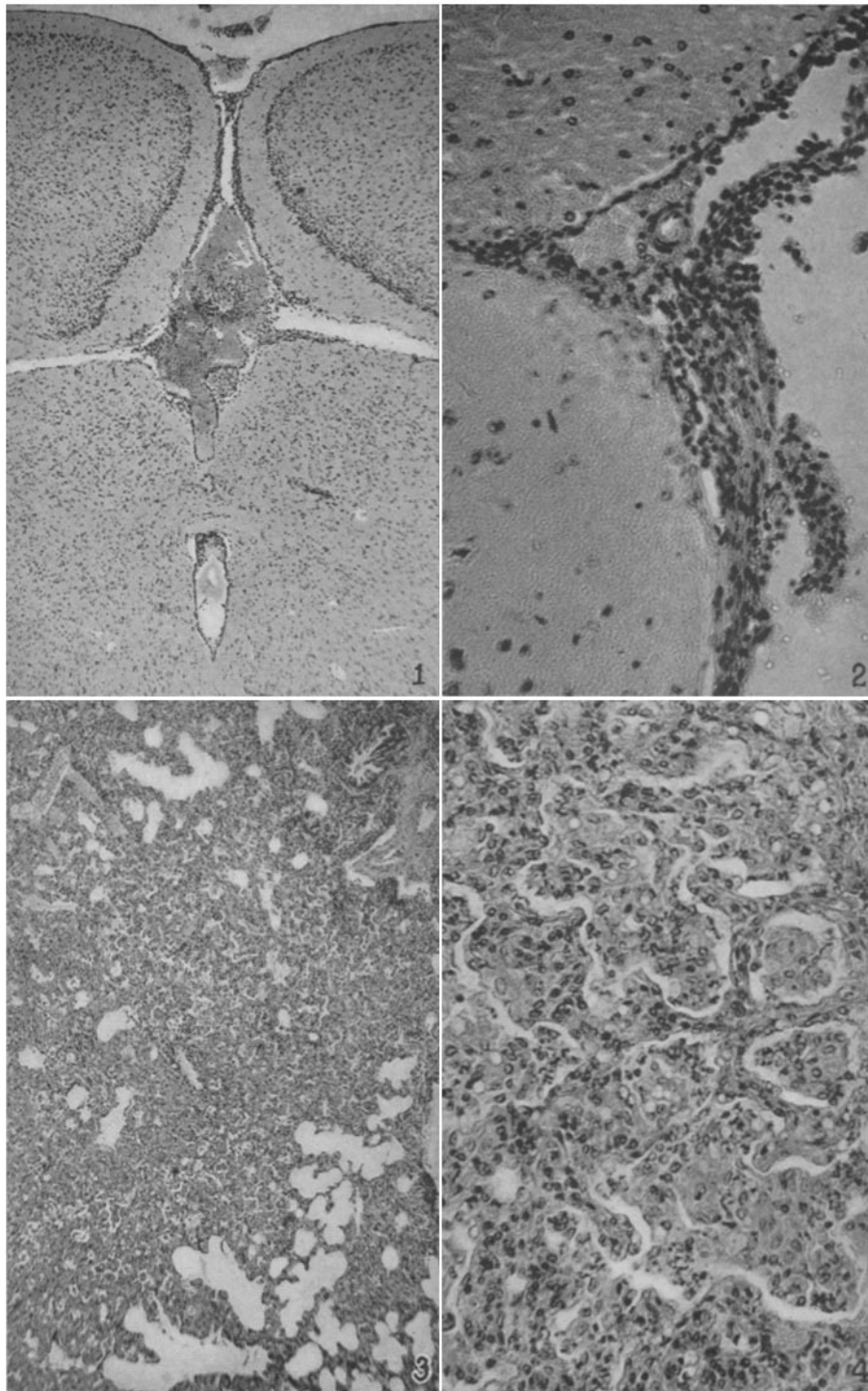FIG. 3. Section of lung from an infected guinea pig showing pneumonic consolidation. Hematoxylin and eosin. × 40.

FIG. 4. Higher magnification of section shown in Fig. 3 illustrating the interstitial character of the pneumonia. Hematoxylin and eosin. × 250.

PLATE 31

FIG. 5. Section of choroid plexus of an infected monkey. The arrows indicate the portions shown under higher magnification in the next two figures. Hematoxylin and eosin. × 40.
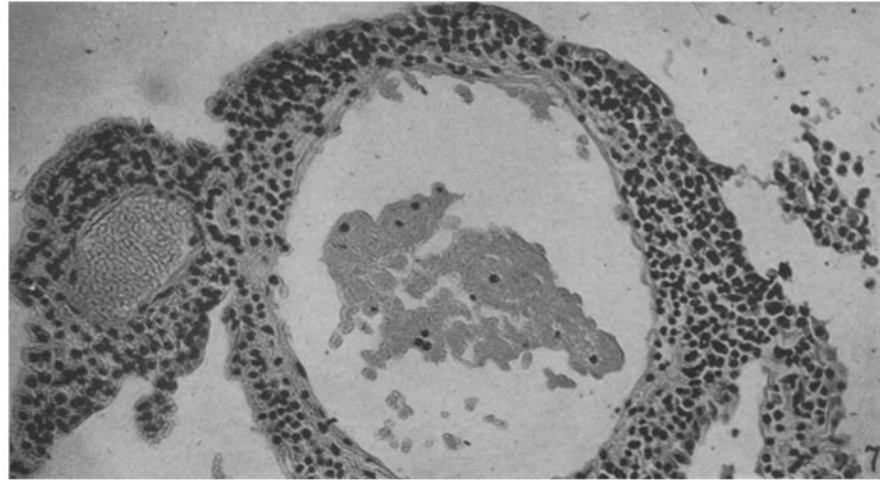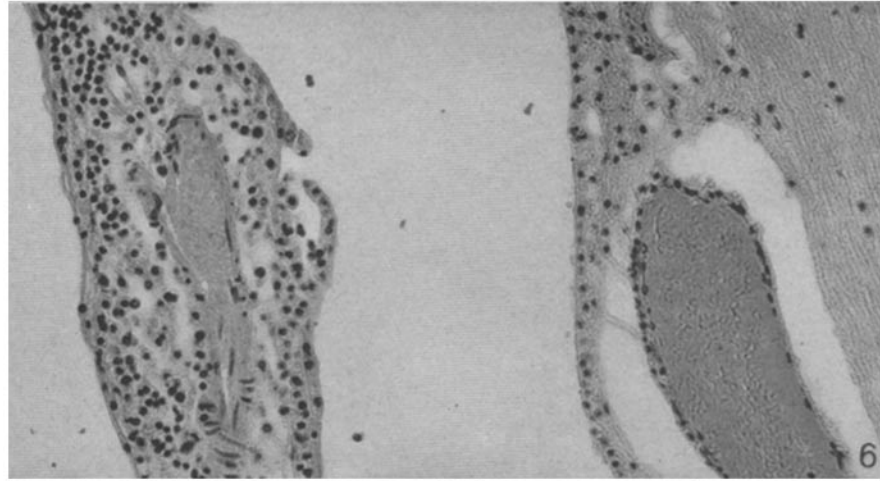
FIG. 6. Higher magnification of section shown in Fig. 5 illustrating the dense mononuclear infiltration around a blood vessel in the choroid plexus as contrasted with the comparative absence of infiltration surrounding a blood vessel of similar size in the subependymal tissues, which also show a mild diffuse inflammatory reaction. Hematoxylin and eosin. × 250.

FIG. 7. Higher magnification of another portion of section shown in Fig. 5 illustrating the distortion of the cuboidal ependymal cells by the perivascular inflammatory exudate. Hematoxylin and eosin. × 250.

(Rivers and Scott: Virus meningitis in man. II)

(Rivers and Scott: Virus meningitis in man. II)