LANE MEDICAL LECTURES

VIRUSES AND VIRUS DISEASES

THOMAS M. RIVERS

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STANFORD UNIVERSITY PUBLICATIONS
UNIVERSITY SERIES

MEDICAL SCIENCES
VOLUME IV NUMBER 1

Lane Medical Lectures: Viruses and Virus Diseases

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STANFORD UNIVERSITY PRESS
STANFORD UNIVERSITY, CALIFORNIA
LONDON: HUMPHREY MILFORD
OXFORD UNIVERSITY PRESS
1939

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OXFORD UNIVERSITY PRESS

THE BAKER AND TAYLOR COMPANY
55 FIFTH AVENUE, NEW YORK

THE MARUZEN COMPANY
TOKYO, OSAKA, KYOTO, SENDAI

FIRST PUBLISHED, NOVEMBER 1, 1939

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THE LANE MEDICAL LECTURES

The Lane Medical Lectures were established in 1896 by Dr. Levi Cooper Lane of San Francisco, the founder of Cooper Medical College, which, in 1908, became the School of Medicine of the Leland Stanford Junior University. The University maintains the Lane Medical Lecture Fund, which is used to secure as lecturer at intervals of two years some eminent physician or scientist who has made a definite contribution in the field of medicine. The first series of lectures was given in 1896, and the total number, inclusive of the present series, is twenty-seven.

In 1927, action was taken by the School of Medicine to the effect that the formal lectures shall be published in a suitable journal, or in monograph or book form, according to arrangements to be made by the Committee on Special Medical Lectures and the lecturer. Prior to this date, only the lectures for the years 1900, 1904, 1906, 1917, and 1924 had been published, and some of these only in part. Subsequent to 1927, all the lectures have been published in full, including a posthumous series, not actually delivered, by Dr. Rudolf Magnus. It is hoped that regular publication of the Lane Medical Lectures will be a means of extending their scientific value and influence.

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1911. Ernest Fuchs, Professor of Ophthalmology in the University of Vienna. "The Importance of Ophthalmology in Its Relation to Systemic Diseases."

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1917. Simon Flexner, M.D., LL.D., Director of Laboratories, Rockefeller Institute for Medical Research, New York City. "Physical Basis and Present Status of Specific Serum and Drug Therapy."

1919. Alonzo Englebert Taylor, Professor of Physiological Chemistry, University of Pennsylvania, Philadelphia. "The Feeding of the Nations at War."

1921. L. Emmett Holt, M.D., Sc.D., LL.D., Emeritus Professor of Pediatrics, College of Physicians and Surgeons, Columbia University, New York City. "Growth and Nutrition."

1924. Ludwig Aschoff, M.D., Professor of Pathology, University of Freiburg, Germany. "Selected Topics in Pathology."

1925. Vittorio Putti, M.D., Professor of Orthopedic Surgery, University of Bologna, Director Rizzoli Institute of Bologna, Italy. "Selected Topics in Orthopedic Surgery."

1928. F. d'Herelle, M.D., Professor of Bacteriology, Yale University, New Haven, Connecticut. "Bacteriophagy."

1929. Walther Straub, M.D., Ph.D., Professor of Pharmacology, University of Munich, Munich, Germany. "Selected Topics in Pharmacology."

1930. Charles R. Stockard, M.D., Ph.D., Sc.D., Professor of Anatomy, Cornell University Medical College, New York City. "Constitution."

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1935. G. V. Anrep, M.D., D.Sc., F.R.S., Professor of Physiology, Egyptian University, Cairo, Egypt. "Studies in Cardiovascular Regulation."

1937. Eugene F. Du Bois, M.D., Professor of Medicine in the Cornell University Medical College, New York City. "The Mechanism of Heat Loss and Temperature Regulation."

1939. Thomas M. Rivers, M.D., Sc.D., Director of the Hospital of The Rockefeller Institute for Medical Research, New York City. "Viruses and Virus Diseases."

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VIRUSES AND VIRUS DISEASES

INTRODUCTION

Certain general aspects of virus diseases and the agents inducing them will be discussed in this series of lectures. In the first lecture the discovery of a new virus disease in man, lymphocytic choriomeningitis, will be described, following which the methods of obtaining proof that it was a newly recognized disease and that the active agent recovered from the patients actually induced the disease in them will be outlined. In the second lecture characteristic pathological pictures induced by viruses will be discussed. The third lecture will be devoted to a consideration of serological and immunological phenomena associated with virus diseases. It is obvious that lectures two and three will have to do with the results of the activity of viruses in their hosts. A knowledge of the results of these activities leads one to a consideration of the nature of the responsible agents, and, in the fourth lecture, this matter will be covered, particularly in the light of recent work on bacteriophage, certain plant viruses, and the elementary bodies found in some of the virus diseases of animals. Physicians and laymen may be interested in all phenomena connected with virus diseases, but it is reasonable to believe that they are most interested in methods of treatment and prevention; consequently, in the last or fifth lecture, remarks dealing with prevention and treatment of virus maladies will be presented.

Many of you have already asked: What is a virus? What is a virus disease? What is there about virus diseases that induces investigators to place them in a group separate from maladies caused by the better-known infectious agents? A positive definition of a virus is difficult to give at the present moment. It is also difficult to state in a few words why a special group of diseases is spoken of specifically as virus maladies. However, during this series of lectures I trust that I shall be able to make clear, even though in a circuitous manner, that there is justification for speaking of a certain group of infectious agents as viruses and for classifying the maladies caused by them as virus diseases. In addition, I desire to put before you in a comprehensible manner some interesting problems at present under investigation in the virus field.

I. LYMPHOCYTIC CHORIOMENINGITIS

Many viruses share with other kinds of infectious agents the characteristic of limiting their attack to certain kinds of tissues or organs. For instance, some viruses attack nervous tissue and for that reason are spoken of as being neurotropic; but there is no evidence that they are regularly found in the spinal fluid of infected hosts. This is particularly true in regard to man, because, with a few exceptions, the presence of a virus in the spinal fluid has not been consistently noted in connection with diseases of the central nervous system. It occurred to us that eventually a virus would be found which attacks the meninges of man and which would be present with a degree of regularity in the spinal fluid during the course of the infection. With this idea in mind we were on the lookout for cases of meningitis in man in which the spinal fluid was bacteriologically sterile, in order that we might encounter and recognize a virus disease involving the covering of the central nervous system. After a number of negative attempts we succeeded in isolating a virus from the spinal fluid of two human beings and were able to demonstrate that it was the cause of the disease from which they were suffering (1–3).*

In this lecture I shall discuss in detail the clinical picture of the first patient (W.E.) from whom the virus was obtained and the manner in which the virus was shown to be the cause of his malady. The clinical picture of the disease in this patient is the one usually seen, but we have obtained the virus from three others (4), in one of whom the clinical course of the disease was quite different (5). Other investigators (6) have obtained a virus similar to ours from human beings and some of their cases have also shown unusual signs. In conclusion, a short discussion of this new disease, lymphocytic choriomeningitis, and its relation to Wallgren's acute aseptic meningitis and certain obvious virus infections of the central nervous system of man will be presented.

Case 1

W.E., N.Y. Hosp. No. 82075,† white male, 31 years of age, was admitted to the New York Hospital, December 22, 1934. He was employed as a painter at The Rockefeller Institute and had worked at different times in most of the laboratories. He had been well until eight days before admission, when he developed malaise and generalized aching; his young son‡ of 3 years had similar symptoms at the same time but rapidly recov-

* Figures in parentheses indicate items in "References" at the end of the section.

† We were enabled to study and publish a report on this case through the courtesy of Dr. Eugene Du Bois and Dr. Harold G. Wolff of the New York Hospital.

‡ Unfortunately we have been unable to obtain permission from the parents to investigate the antiviral content of the child's blood.

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ered without obvious involvement of the central nervous system. The patient remained in bed during the day following the onset of illness and then returned to work in spite of a persistent feeling of fatigue and muscular pain. His condition remained unchanged during the next three days, at the end of which time he developed a severe constricting headache and epigastric pain accompanied by loss of appetite. Attempts to take nourishment, even orange juice, resulted in vomiting, which, however, was not projectile. The vomitus was brown and contained a small amount of bright blood on one occasion. On the day of admission, eight days after the first symptoms and three days after the onset of the severe headache, he became drowsy and when examined by a physician was found to have a stiff neck, a Kernig sign, and a temperature of 102° F. A tentative diagnosis of post-influenzal encephalitis was made, and he was sent to the New York Hospital.

Physical examination.—Upon admission the patient's rectal temperature was 104° F., pulse 110, respirations 34, and blood pressure 100/80 mm. Hg (Fig. 1). He was semistuporous but could be aroused sufficiently to

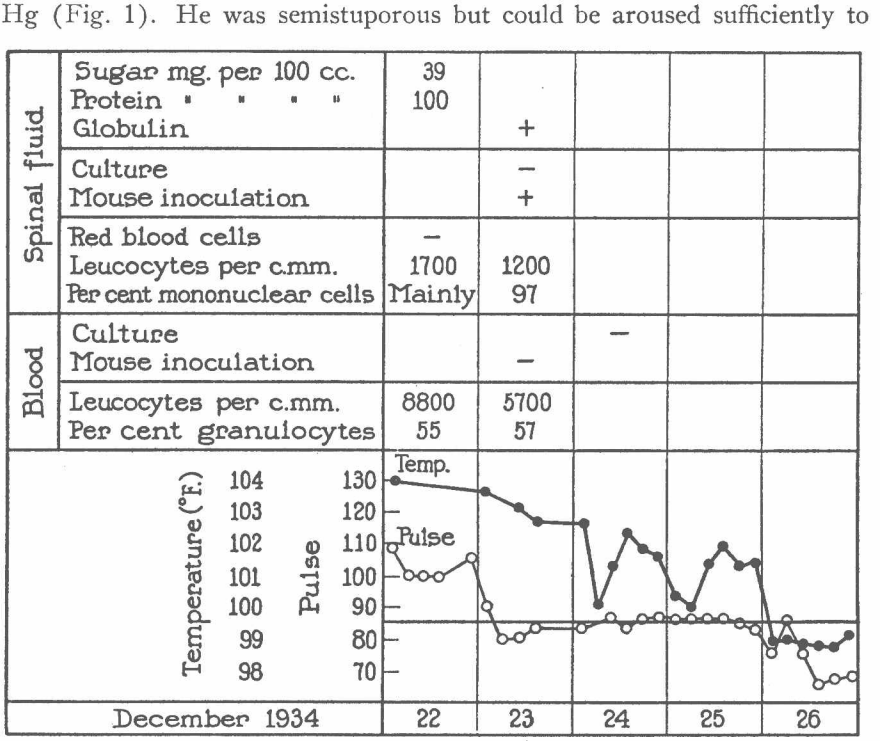

FIG. 1.—Summary of clinical findings on Case 1 (W.E.) during first five days in hospital. (From T. F. McN. Scott and T. M. Rivers, *Journal of Experimental Medicine,* 1936, **63**, 397.)

answer questions; he moaned at intervals and kept his eyes tightly shut; he tended to lie on his right side with thighs flexed on the abdomen, a position the legs still maintained when he was turned on his back. No lead line was present; there was no cutaneous evidence of herpes, and, apart from dry, crusted lips, the rest of the physical examination, except for the nervous system, was negative.

Neurological examination.—Mentally he was coherent in spite of drowsiness; he was somewhat confused as to time relationships but could handle simple arithmetic. There were photophobia and some weakness of convergence; otherwise the cranial nerves were normal, and there were no changes in the optic fundi. There was definite but not marked nuchal rigidity, but Kernig's sign was absent. Motor power, sensation, and position sense appeared normal. The biceps reflexes were moderately active; the triceps reflex was obtained on the right but not on the left side. Both knee jerks and ankle jerks were moderately active. Plantar, abdominal, and cremasteric reflexes were present and normal.

Course.—A lumbar puncture performed on admission revealed a slightly turbid spinal fluid that was under increased pressure and contained 1,700 cells per c.mm., mainly lymphocytes (Fig. 1). During the twenty-four hours after admission the knee jerks disappeared and the plantar reflexes became equivocally extensor in type. Within four days, however, the knee jerks returned, the left more fully than the right, and the plantar reflexes were only transitorily abnormal. During the first four days in hospital the lumbar puncture was repeated twice, following which the patient's general condition improved rapidly. At the end of this time his temperature had fallen to the normal level (Fig. 1) and he had no definite symptoms, although his neck was still stiff on examination. During the first sixteen days in the hospital the patient's bowels were moved only with the aid of enemata, but at the end of that time he began to have spontaneous bowel movements and thereafter the intestinal motility remained normal. By January 26, forty-three days after onset, the cells in the spinal fluid had decreased in number until there were only 25 per c.mm. At that time the patient was allowed to get up. On February 6, the spinal fluid showed only 8 cells per c.mm. and a heavy trace of globulin. On February 7, fifty-six days after onset, since a complete examination showed a normal central nervous system, the patient was discharged from the hospital. After he had been back at work for eight months a neurological examination revealed no abnormalities. The patient is still working at The Rockefeller Institute and up to the present time, four years after his illness, he has developed no evidence of sequelae.

Spinal fluid.—Dec. 22, 1934: slightly turbid, under increased pressure; cells, predominantly lymphocytes, 1,700 per c.mm.; no organisms could be

found in stained preparations or in cultures; protein 100 mg. per 100 cc.; sugar 39 mg. per 100 cc. *Dec. 23:* turbid, under pressure of 115 mm. of water; 1,200 cells per c.mm., 97 per cent being lymphocytes. *Dec. 24:* clear, under pressure of 150–170 mm. of water; 1,100 cells per c.mm., 95 per cent of which were lymphocytes; Nonne-Apelt test was positive; a portion of the fluid was injected into a guinea pig to test for the presence of tubercle bacilli, and the animal was found dead sixteen days later with pulmonary congestion but no evidence of tuberculosis. *Dec. 31:* slightly turbid; 740 cells per c.mm.; protein 100 mg. per 100 cc.; spinal fluid sugar 43 mg. per 100 cc.; blood sugar 83 mg. per 100 cc. *Jan. 7, 1935:* clear; 100 cells per c.mm. *Jan. 19:* pink, 35 cells per c.mm.; *Jan. 26:* clear, with a faint yellowish tinge; 25 cells per c.mm.; protein 70 mg. per 100 cc.; sugar 68 mg. per 100 cc.; colloidal gold test yielded 5554342100, but the fluid was xanthochromic; Wassermann test on 0.1 cc. of fluid was negative. *Feb. 6:* clear; 8 cells per c.mm.; an excess of globulin was present.

Examination for tubercle bacilli in the sediment from three samples of spinal fluid was negative. There was never any demonstrable abnormality in the hydrodynamics of the spinal fluid.

Blood.—Dec. 22, 1934: hemoglobin 100 per cent; red blood cells 5,300,000; white blood cells 8,800; differential count yielded 40 per cent adult and 15 per cent immature polymorphonuclear neutrophils, 35 per cent lymphocytes, 9 per cent monocytes, and 1 per cent eosinophils. *Dec. 23:* white blood cells 5,700; differential count yielded 45 per cent adult and 12 per cent immature polymorphonuclear neutrophils, 44 per cent lymphocytes, 8 per cent monocytes, and 1 per cent eosinophils; Kline test was negative; urea nitrogen 11 mg. per 100 cc.; blood sugar 71 mg. per 100 cc. *Dec. 24:* blood for culture was taken and remained sterile for five days at 37° C. *Jan. 29, 1935:* convalescent serum failed to neutralize the virus of the St. Louis type of encephalitis.*

Urine.—Normal.

Search for an Etiological Agent

The clinical picture presented by the patient suggested that he was suffering from a meningitis, while the type of cellular reaction evident in the spinal fluid, in which ordinary bacteria were not demonstrated, led us to believe that the etiological agent might be a virus. In order to test this idea blood and spinal fluid were collected from the patient on the day after admission for inoculation into animals.

Spinal fluid.—Four or 5 cc. of slightly blood-stained spinal fluid were obtained on December 23, 1934, and used, within one to two hours after

* Dr. L. T. Webster made the tests for the presence of neutralizing antibodies against the St. Louis type of encephalitic virus.

collection, for the inoculation of mice.* Each of six mice received 0.03 cc. of the spinal fluid intracerebrally and 0.5 cc. intraperitoneally. One of the animals died on the third day after inoculation; however, it was found to have a streptococcal infection of the brain and was discarded. Of the remaining five mice, two became sick on the sixth day after inoculation and were sacrificed for passage, one died on the ninth day and was discarded, and two became sick on the ninth day but recovered. The brains of the animals that were killed on the sixth day were removed aseptically. Aerobic and anaerobic cultures made from the brains in broth showed no growth after incubation for 48 hours at 37° C. At the end of this time, the brains, which in the interval had been kept at 5° C., were ground with Locke's solution into a 20 per cent emulsion which was used for the inoculation of five normal mice, 0.03 cc. and 0.25 cc. of the emulsion being given intracerebrally and intraperitoneally, respectively, to each of the animals. One mouse died as a result of the inoculations. Of the other four, two died and two appeared sick on the seventh day after inoculation. The sick mice were sacrificed and their brains were removed. The brain from one of the mice that died was also removed. All of the brains were found to be free from ordinary bacteria by means of aerobic and anaerobic cultures in broth. Then 0.03 cc. of a 20 per cent emulsion of these brains were inoculated into each of five normal mice by the intracerebral route alone. All five animals became ill on the sixth day after inoculation. Since that time the active agent has been passed serially through mice and guinea pigs by means of intracerebral inoculations of emulsions containing the virus, which have been shown to be free from ordinary bacteria.

Blood.—Blood drawn on December 23 into a suitable amount of a sterile solution of heparin was used within one to two hours after collection for the inoculation of six mice, each animal receiving 0.03 cc. intracerebrally and 0.75 cc. intraperitoneally. The mice were observed closely for a month but showed no symptoms of ill health, and, when inoculated later with potent virus, were found to be susceptible.

Other Cases

On December 27, 1934, five days after the first patient was seen in the New York Hospital, a second patient (R.E.S.) was admitted to the Rockefeller Hospital. The history, symptoms, signs, and clinical course of the disease were so similar to those of the first patient that a detailed account of them will not be given. In a manner similar to that described above, virus was obtained from the spinal fluid of this patient but not from the blood. Since that time we have obtained virus from the spinal fluid of three other patients (4), one in Princeton, New Jersey, and two in Balti-

* Albino Swiss mice, usually 4 to 5 weeks old, were used.

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more, Maryland. From one of the patients in Baltimore, virus was also obtained from the blood. The clinical course of the disease in one (5) of the patients in Baltimore was different from that seen in the others and will be spoken of later. The viruses obtained from these five patients have been shown to be immunologically identical by means of neutralization and reinoculation tests.

Evidence that the Active Agent Was Obtained from the Patients' Spinal Fluids

The virus obtained from the first two patients, who were seen at about the same time, presented us with certain problems. From the results of our preliminary work we were led to believe that the active agent producing the transmissible disease in mice had been obtained from the two patients, and that their blood streams were free from demonstrable amounts of the agent at the time tests were made. In spite of our belief that the viruses were obtained from the spinal fluids of the patients, evidence had to be brought not only that such was the case but that, even though the viruses were in the spinal fluid, they were etiologically related to the disease from which the patients were suffering. That such evidence had to be sought was emphasized by the facts: (*a*) that Theiler (7) in 1934 described a spontaneous virus disease in mice characterized by paralysis of the extremities; (*b*) that Traub (8) in 1935, shortly after we had obtained our active agent, reported that he was able by the injection of inert materials into the brains of normal-looking stock mice to demonstrate the presence of a latent virus capable of causing neurological symptoms similar to those shown by our sick mice; and (*c*) that Flexner (9) obtained the virus of herpes simplex from the spinal fluid of a patient being treated for meningo-vascular syphilis but showing no evidence of acute involvement of the central nervous system.

A spontaneous disease lying dormant in a colony of mice may manifest itself in several ways, e.g., by the occasional presence of a sick animal, or by the presence of a certain number of immune individuals. If the virus is likely to remain inactive in the nervous system until activated by some insult to the brain, then evidences of infection should be induced by the intracerebral injections of a variety of materials. With regard to the presence of immune mice in the colony, there are at least two possibilities: In the first place, the virus may not be entirely inactive and may cause a very mild disease from which recovery resulting in immunity takes place. Secondly, inoculations of inert or noninfectious materials may activate the latent virus to such an extent that it is capable of producing only a subclinical infection but one sufficient to render an animal resistant to potent virus administered subsequently. In either case, immune animals should

be encountered in a fortuitous manner among the mice used either for primary inoculations or for reinoculations. The results of many experiments clearly showed that neither illness caused by a virus similar to that recovered from the two patients nor immunity to it was fortuitously encountered in our stock of mice during the period of investigation.

Further evidence that we obtained the virus from the spinal fluid of the first two patients instead of picking it up from the mice used in the work was afforded by the solid immunity exhibited by the mice that received injections of the spinal fluid and developed no definite signs of illness or became sick and recovered. This immunity was demonstrated by reinoculation of the surviving mice with homologous strains of virus. In the reinoculation test for immunity, the results of which are summarized in Figure 2, we included, in addition to the mice that had received admission

Work with W.E. Virus			Work with R.E.S. Virus		
Original Inocula	Reinoculated, Intracerebrally, with W.E. Virus		Original Inocula	Reinoculated, Intracerebrally, with R.E.S. Virus	
	Sick	Dead		Sick	Dead
Admission spinal fluid, W.E.	0/2	0/2	Admission spinal fluid, R.E.S.	0/5	0/5
Admission blood, W.E.	6/6	5/6	Spinal fluid taken 4 weeks after onset, R.E.S.	6/6	5/6
Infected mouse brain, intranasally	0/5	0/5	Blood taken at onset, R.E.S.	11/11	9/11
Infected mouse liver, intraperitoneally ...	0/4	0/4	Admission blood, R.E.S.	3/3	0/3
None, control	5/5	4/5	Infected mouse liver, intraperitoneally	0/5	0/5
			Infected mouse liver, subcutaneously	0/5	0/5
			None, control	6/6	2/6

FIG. 2.—Results of reinoculation experiment supporting other evidence that the viruses were obtained from W.E. and R.E.S.'s spinal fluid. 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. (From T. F. McN. Scott and T. M. Rivers, *Journal of Experimental Medicine,* 1936, **63**, 397.)

spinal fluid and had recovered, several other groups of mice, some of which several weeks previously had been given materials known to contain

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