No constant differences between predegenerated and non-predegenerated, or between conditioned and unconditioned, grafts have as yet been ascertained, although such differences might be expected in view of the fact that the "devitalized" grafts are being repopulated by host cells. As in nerve grafting in general, success depends on the proper way of joining the graft to the stumps. The method of sutureless sleeve-splicing[2] has been instrumental in securing the success of the frozen-dried grafts in the present experiments.

Summary. Full functional recovery may be obtained in cats and monkeys after the bridging of a nerve gap of several centimeters by grafts of frozen-dried nerve, stored for several months and rehydrated before use.

2 Weiss, P., PROC. SOC. EXP. BIOL. AND MED., 1943, preceding paper.

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Neurotropic Virus Infections in Chicago, 1939-1941. Nine Cases of Lymphocytic Choriomeningitis.

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Ordinarily it is difficult or impossible to diagnose the various neurotropic virus infections of man on the basis of clinical or pathological findings because of their great similarity. For this reason, laboratory procedures must be employed for identification of the specific virus. During epidemics, it may be possible to identify such infections as poliomyelitis by means of characteristic findings. On the other hand, laboratory diagnosis becomes mandatory if two or more viruses are known to be endemic in the same region. Thus mixed epidemics of St. Louis encephalitis and western equine encephalomyelitis have been reported in certain of the Western states.[1-3] Laboratory aid is also essential for the diagnosis of lymphocytic choriomeningitis because of the protean clinical manifestations this virus produces and the fact that this infection is highly sporadic in man.[4]

1 Howitt, B. F., *Am. J. Pub. Health*, 1939, **29**, 1083.

2 Meiklejohn, G., and Hammon, W. McD., *J. A. M. A.*, 1942, **118**, 961.

3 Hammon, W. McD., and Howitt, B. F., *Am. J. Hyg.*, 1942, **35**, 163.

4 Armstrong, C., *Harvey Lectures*, Lancaster, Pa., Science Press, 1941, 39.

The present studies were carried out in order to identify possible etiologic viruses in 75 patients suffering from various sporadic central nervous system infections in the Chicago area. In most instances, specimens of both spinal fluid and defibrinated blood obtained during the acute stage were routinely inoculated into Swiss mice and guinea pigs. Blood specimens were defibrinated by means of glass beads. In fatal cases, small portions of tissue obtained from different areas in the brain and cord were triturated in a mortar with sand, and a 10% emulsion in buffered saline (pH 7.4) was prepared. Most specimens were inoculated into animals within an hour after collection from the patient. Usually each specimen was inoculated intracerebrally (0.03 cc) into each of 6 mice and both intracerebrally (0.2 cc) and intraperitoneally (1 to 4 cc) into each of 2 guinea pigs. All animals remaining normal for 30 days after the original inoculation were tested for immunity to lymphocytic choriomeningitis by intracerebral inoculation with approximately 10 MLD of known virus. Bacteria were ruled out by routine inoculation of various aerobic and anaerobic culture mediums. Evidence that our stock mice and guinea pigs were free from latent infection with the choriomeningitis

TABLE I.
Summary of Virus Studies of Various Central Nervous System Infections of Man in Chicago, 1939-1941.

Clinical diagnosis	No. of cases	Lymphocytic choriomeningitis: Virus isolated	Lymphocytic choriomeningitis: Positive neutralization test	Lymphocytic choriomeningitis: Positive complement fix. test	St. Louis encephalitis: Virus isolated	St. Louis encephalitis: Positive neutralization test	Western equine encephalomyelitis: Virus isolated	Western equine encephalomyelitis: Positive neutralization test
Preparalytic poliomyelitis	28	1	1 (8 wks)	1 (8 wks)	—	0	0	0
Paralytic poliomyelitis	11	1	1 (9 ")	1 (3 ")	—	—	—	—
Acute encephalitis	20	3	3 (8 ") (8 ") (20 ")	3 (3 ") (3 ") (12 ")	0 (5 fatal)	1 (5 mos.)*	0	0
Aseptic meningitis	15†	2	2 (8 ") (24 ")	2 (4 ") (24 ")	—	0	0	0
"Influenzal" infection	1	1	1 (10 ")	1 (10 ")	—	0	0	0
Normal adults	10	0	0	0	—	0	—	0
Total	85	8	8	8	0	1	0	0

* Patient had a "sleeping episode" 5 years previously when living in San Diego, California.
† Three cases were positive for choriomeningitis—virus was isolated and antibodies were detected in one; virus was isolated from the second, but no convalescent serum could be obtained; in the third, no attempt was made to isolate virus but antibodies were demonstrated.

virus has been published elsewhere.[5] Moreover, no stock animals were encountered that were immune to inoculation with fatal amounts of virus.

Blood sera obtained during the acute stage of the disease and again several weeks or months after the attack were tested for the presence of neutralizing antibodies against the following viruses: (1) the W. E. (Rivers) or J. P. strain[6] of lymphocytic choriomeningitis, (2) the D.219 or Hubbard strain of St. Louis encephalitis, and (3) the B.A.I. strain of the western type of equine encephalomyelitis. The protection tests with the choriomeningitis virus were performed by inoculating guinea pigs subcutaneously with mixtures of equal parts of undiluted serum and serial tenfold virus dilutions according to the technic already described in connection with other studies.[5] Howitt's technic[1] was followed in protection tests with the St. Louis encephalitis and western equine encephalomyelitis virus. The sera were also tested for the presence of complement fixing antibodies against the choriomeningitis virus. Essentially the same procedure described by Smadel, Baird, and Wall[7] was used in the complement fixation test. The complement fixing antigen consisted of saline extracts of pooled infected guinea pig spleens. Known negative and positive control serums were included in all neutralization and complement fixation tests.

Results obtained in the virus and immunologic studies of 75 patients are summarized in Table I. All but one of these patients were examined and diagnosed by Drs. S. O. Levinson and A. M. Wolf, who are consultants on poliomyelitis to the Department of Public Health of the State of Illinois. As shown in Table I, 9 cases were positive for lymphocytic choriomeningitis, 2 of which were laboratory infections. Virus studies on the 11 cases of poliomyelitis showing paralysis were included because of atypical clinical findings which made the diagnosis questionable in the minds of the clinicians.

The pertinent clinical data on these patients are given in Table II. In 8 of the positive cases, no specific neutralizing or complement fixing antibodies were detected in the acute stage of the disease, but they subsequently

5 Milzer, A., *J. Infect. Dis.*, 1942, **70**, 152.

6 Leichenger, H., Milzer, A., and Lack, H., *J. A. M. A.*, 1940, **115**, 436.

7 Smadel, J. E., Baird, R. D., and Wall, M. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 71.

TABLE II.
Clinical Data on Nine Proven Cases of Lymphocytic Choriomeningitis in Chicago.

Clinical diagnosis	Patient	Age	Color and sex	Max. cell count spinal fluid	% lympho-cytes	Date of onset
Preparalytic poliomyelitis	F.M.	10	W.F.	297	55	June 41
Paralytic "	R.M.	14	W.M.	265	80	Aug. 40
Encephalitis	B.D.	22	W.F.	2	100	May 41
"	A.M.*	23	W.M.	8	100	Avg. 39
"	J.A.	2	W.M.	31	70	Sept. 41
Aseptic meningitis	J.P.	24	C.M.	970	90	Oct. 38
" "	B.B.	19	W.F.	620	60	May 40
" "	F.R.*	26	W.M.	—	—	May 41
"Influenzal" infection	A.V.	34	W.M.	—	—	Sept. 40

*Laboratory infections.

appeared during convalescence as indicated in parenthesis in Table I. Although convalescent serum was not obtained in the ninth case (J.P.), the diagnosis was established by the isolation of virus during the acute stage and the absence of specific antibodies. The choriomeningitis virus was isolated from spinal fluid or blood taken from 8 of the patients during the attack; in the remaining case (F.R.), no attempt was made to isolate virus, but the development of specific antibodies during convalescence was demonstrated. This last patient became infected in the laboratory following an accidental puncture of the skin of the leg with a hypodermic needle contaminated with infectious material. The second laboratory infection (patient A.M.) which occurred apparently resulted from contact with infected rhesus monkey lice. A detailed clinical report of this latter case and two other cases of the present series (J.P. and B.D.) have appeared in separate publications.[8,6,9]

Other studies which were made show that there is complete cross-immunity between the eight isolated strains of choriomeningitis virus and the W.E. (Rivers) strain as demonstrated by resistance to intracerebral inoculations in mice which have been infected and recovered. However, the pathogenicity of the isolated strains varied considerably with infectivity titers ranging from 10^{-4} to 10^{-7} dilutions. Microscopic examination of stained sections of the brains of guinea pigs and mice that succumbed to the various strains showed the round cell infiltration of the meninges and choroid plexus comparable to that produced by the W.E. strain.[10] All of the isolated strains suspended in nutrient broth of pH 7.2 (5% brain tissue suspension) and centrifuged at 2,000 r.p.m. to remove large tissue particles readily pass through previously tested Berkefeld N and W candles.

The protean clinical manifestations of lymphocytic choriomeningitis noted in the tables have also been reported by others.[4,11] A few established cases of paralysis, which were initially diagnosed as poliomyelitis, have occurred in England.[12,13] Several of the English patients developed complete paralysis of both lower extremities as well as facial and bladder paralysis. Our patient (R.M.) had paralysis of the lower abdominal muscles. Ordinarily, lymphocytic choriomeningitis infection is systemic in character and rarely involves the central nervous system. Therefore, it does not deserve serious consideration in the differential diagnosis of poliomyelitis. This is substantiated by Armstrong's finding[4] that approximately 11% of 2,000 serums taken at random from the general population contained neutralizing antibodies against the choriomeningitis virus, although only very few of the positives gave a history of any central nervous system infection. Furthermore, one

8 Milzer, A., and Levinson, S. O., *J. A. M. A.*, 1942, **120**, 27.

9 Treusch, J. V., Milzer, A., and Levinson, S. O., *Ach. Int. Med.*, 1943, **72**, 709.

10 Rivers, T. M., and Scott, T. F. McN., *J. Exp. Med.*, 1936, **63**, 415.

11 Howard, M. E., *Yale J. Biol. and Med.*, 1940, **13**, 161.

12 Findlay, G. M., Alcock, N. S., and Stern, R. O., *Lancet*, 1936, **1**, 650.

13 MacCallum, F. O., and Findlay, G. M., *Lancet*, 1939, **1**, 1370.

of our patients (A.V.) had an "influenzal" infection without symptoms of central nervous system involvement. For this reason, no spinal tap was done, but the diagnosis was established by isolation of virus from the blood and the finding that specific choriomeningitis antibodies subsequently developed. This case was similar to the two described by Armstrong.[14,15]

Another point of interest is that 2 of our patients became ill in June and August (Table II). These cases are the first recorded for these months. All of the proven cases in the literature to date have occurred during the cooler portions of the year with the greatest incidence during October, November, and December.[15]

Tests for St. Louis encephalitis and western equine encephalitis gave negative results in all except one instance (Table I). This was one of the choriomeningitis patients (B.D.) who also had neutralizing antibodies for St. Louis encephalitis. This patient had suffered from a "sleeping episode" 5 years previously when she lived in a part of California where St. Louis encephalitis is known to be endemic.[16] The choriomeningitis virus was isolated from the spinal fluid on 2 occasions during her illness, and she subsequently developed an increased titer of both complement fixing and neutralizing antibodies for lymphocytic choriomeningitis. Further details of this case will appear in another publication.[9]

There are only two references in the literature to our knowledge concerning the presence of St. Louis encephalitis and western equine encephalomyelitis in Chicago. Wooley and Armstrong[17] reported that of 7 random sera collected in Chicago, 6 gave no protection and one gave questionable protection against the St. Louis encephalitis virus. On the other hand, 11 of 20 sera collected in Paris, Illinois, where the first cases of the 1933 St. Louis epidemic occurred, showed definite protection. More recently the occurrence of a sporadic case of western equine encephalomyelitis in a patient who had not been outside of metropolitan Chicago for many years has been described by Richter.[18] This author also tested the sera of 11 other patients with acute encephalitis in addition to twelve random controls for western equine encephalomyelitis neutralizing antibodies but obtained negative results.

Summary. Virus studies were carried out on 75 patients suffering from various sporadic central nervous system infections of unknown etiology. Nine cases of lymphocytic choriomeningitis proven by virus isolation or detection of specific antibodies are presented. The cases were clinically divisible as follows: (1) encephalomyelitic type, (2) meningeal type, and (3) an "influenzal" type without neurologic signs. Negative results were obtained in tests for St. Louis encephalitis and western equine encephalomyelitis.

14 Armstrong, C., *Pub. Health Rep.*, 1941, **56**, 907.

15 Armstrong, C., *Mil. Surgeon*, 1942, **91**, 129.

16 Howitt, B. F., *Am. J. Pub. Health*, 1942, **32**, 503.

17 Wooley, J. G., and Armstrong, C., *Pub. Health Rep.*, 1934, **49**, 1495.

18 Richter, R. B., *J. A. M. A.*, 1942, **119**, 486.