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ENCEPHALITIS IN NORTH CHINA. RESULTS OBTAINED WITH NEUTRALIZATION TESTS

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The work of Muckenfuss, Armstrong and McCordock (1) and of Webster and Fite (2) has established the fact that the type of encephalitis prevalent in St. Louis during the summer of 1933, was due to a filtrable virus. The latter succeeded in transmitting the virus to mice. They showed by means of protection tests (3) that neutralizing antibodies were present in the sera of convalescent encephalitic patients. In this way Webster and Fite demonstrated that the epidemic in St. Louis was due to a single virus which differed from other viruses previously described (4). Furthermore the experiments of Webster and Fite, and Wooley and Armstrong (5) indicate that the St. Louis type of encephalitis is etiologically distinct from Economo's encephalitis. The results of neutralization tests suggest that the first appearance in the United States of the virus of the St. Louis type occurred in 1932, in Paris, Illinois.

Clinically, the St. Louis type of encephalitis closely resembles the so-called "B" type of Japanese encephalitis, first differentiated in the epidemic occurring in the vicinity of the Inland Sea, Japan, in 1924 (6). However, Webster and his coworkers (4) failed to demonstrate neutralizing antibodies in the sera of Japanese patients who had recovered from the "B" type of encephalitis either in 1924 or 1933.

In view of the fact that the results of Webster and his colleagues seemed to indicate that the St. Louis type of encephalitis differed from that prevalent in Japan, it was of interest to determine whether two cases of encephalitis observed in Peiping, China, belong to the Japanese or the St. Louis type. The majority of the cases of the St. Louis type and of the "B" type in Japan seem to run a short acute course. Recovery, if it takes place, is usually complete. The two cases to be reported here have to date failed to recover completely, and one of the patients had an unusually prolonged and severe course. It seems of

interest, therefore, to give brief summaries of the histories of these cases. Both patients were American women; one contracted the disease in North China during the summer of 1934, the other in Japan during the summer of 1935. (The second patient became acutely ill on the boat on her way from Japan to North China, one day after sailing from Kobe.)

Case 1

Mrs. W., a 27-year old American married woman, came to China for the first time in the fall of 1933. She lived in Peiping until June 15, 1934, when she went to a sea-side resort, in the province of Hopei, about 200 miles north of Peiping. She remained well until July 28, 1934. She was 7½ months pregnant at this time, but had no abnormal symptoms. On July 28th she developed a discrete erythematous rash which faded on pressure and which involved mainly the trunk. She was seen by a doctor who thought she had an allergic reaction probably due to eating crabs. On the following day, July 29th, the rash was fading, but she had an extremely intense headache, and seemed sick. On July 30th the headache had improved slightly, but she was somewhat irrational. The temperature fluctuated between 37° and 39.2° C. She never vomited. Her neck was slightly stiff on July 30th, but Kernig's sign was not definitely positive. On July 31st, the day of admission to the hospital in Peiping, the patient was irrational, the neck was less stiff, and the headache still present, though less intense than it had been on July 29th. No similar disease was noted in other members of the community.

Physical examination. The patient was a well-nourished and well-developed pregnant woman who was irrational. The temperature on admission was normal but rose to 38.4° C. within the first 12 hours. This was the highest point the temperature reached throughout the course of the illness. The reflexes were all hyperactive; no definite Kernig's sign was elicited.

Laboratory findings. The white cell count was 22,000 with a differential showing 94 per cent neutrophilic polymorphonuclears. The spinal fluid was under normal pressure, clear and colorless. The fluid contained 460 cells, 63 per cent of which were neutrophilic polymorphonuclears. Tests for increased globulin content were positive. A second lumbar puncture was done on the following day, August 1st; the total cell count was 380 with 49 per cent polymorphonuclears. The sugar content of the spinal fluid was found to be 98 mgm. per

cent, while the blood sugar was 118 mgm. per cent. The chloride content of the spinal fluid was 717 mgm. per cent.

Course. The patient was restless and irrational the second day after admission. On the third day she became drowsy and was irritable when roused. On questioning she complained of diplopia. Papilledema was present on both sides, slightly more marked on the right. Retention of urine developed, and the patient had to be catheterized twice. A coarse intention tremor of both hands was noted on August 2nd which persisted for several weeks. The temperature became normal 48 hours after admission and showed no further elevation. Her mental condition improved very slowly. She became less drowsy and answered questions more readily. Perseveration was fairly marked, and there was also some tendency to confabulate. It was found that she was completely disoriented as to time and place and that her recent memory was greatly impaired. She did not remember that she was married, or realize that she was pregnant. She was emotionally unstable, laughing and crying without cause.

The patient remained in the hospital for almost 2 months. She went through a normal labor and was delivered spontaneously. The baby showed no abnormalities. There was very little change in the patient's mental condition following delivery. She did not remember going through labor, and did not realize that she had a baby. She continued to be emotionally upset. She was sure that she was going to die, and at intervals became very much frightened. She was discharged 19 days after delivery on September 26, 1934. At that time her physical condition was excellent, the tremor of her hands had subsided entirely. Her mental condition, however, was still extremely unsatisfactory.

Subsequent course. The patient moved away from Peiping, but it has been possible to keep in touch with her from time to time. According to her husband, who is a physician, her personality has undergone a marked change. She is now able to look after the baby and shows some interest in her immediate household. She has, however, become suspicious of her husband and takes little part in the community where she lives. She refused to return to the hospital for re-examination when she visited Peiping 8 months after discharge. It has been possible to obtain blood from her for neutralization tests on one occasion on January 4, 1936, about 1½ years after her recovery. The results of these tests will be reported in conjunction with the other cases.

Case 2

Onset: Mrs. G., a 32-year old American married woman, had been living in Tokyo, Japan, for 3 years prior to her coming to China for a visit in August 1935. She took the boat at Kobe on August 19, 1935. On August 20th she developed a temperature of 40.3° C. and complained of numbness of one hand. She had 3 severe vomiting spells. On August 21st her temperature was 38.8° C. and she complained of an intense headache. On

August 22nd the patient still complained of severe headache, and lapses of memory were noted. She developed shooting pains in the muscles of the right leg and became irrational. On August 23rd her temperature was 40° C.; she was unaware of her transfer from boat to train and did not recognize the names of friends.

Course in hospital: August 23, 1935 to December 18, 1935. The patient appeared critically ill, her temperature was 40.1° C. She was able to talk, but was irrational. Half an hour after admission the patient had a generalized convulsion lasting several minutes. On August 24th the eyegrounds showed mild optic neuritis. The most striking symptoms on admission were drowsiness and mental confusion. Signs of meningeal irritation only developed 48 hours later, and at the same time an increasing spasticity of the extremities was noted.

Laboratory findings. The white cell count was 16,500 with a differential showing 86 per cent neutrophilic polymorphonuclears. The spinal fluid was under increased pressure (375 mm. of water); clear and colorless; total cell count 228, with 75 per cent neutrophilic polymorphonuclears; tests for increased globulin content were positive; sugar 80 mgm. per cent.

The temperature fell from 40° C. to 38° C. in the course of 4 days. The patient's drowsiness increased, and she was no longer able to speak or move. Her attention could be aroused momentarily, and she would occasionally try unsuccessfully to obey commands to open her mouth and stick out her tongue. Her deep reflexes were all hyperactive, and no differences were noted on the two sides. The abdominal reflexes were absent. Marked cogwheel rigidity of all four extremities was present. No impairment of sensation could be determined. The Babinski sign was positive on the left, but only an equivocal response could be obtained on the right. She developed slight ptosis of the left eyelid which lasted for several weeks, and then cleared up. Rhythmic movements of the tongue and lower jaw were present for a short time. A coarse tremor of both hands, more marked on the left, persisted during most of the stay in the hospital and then diminished gradually.

The patient had an irregular fever, the temperature ranging between 38° and 39° C. for a period of 7 weeks. She had numerous furuncles during this time, but it was thought that the fever was related to the encephalitic process rather than to the condition of her skin.

The patient remained extremely drowsy for two months and had to be fed by nasal tube during most of that time. The rigidity of her extremities, particularly the lower, was marked. She held her legs persistently flexed, so that it was feared that she might develop permanent contractures. She was given atropine in increasing amounts until a maximum of 30 drops of 1-1000 atrophine sulphate was reached. The spasticity decreased gradually and did not return when the atropine was stopped. During the time that the patient was receiving atropine, there was some dryness of the throat, but her pulse rate showed no significant elevation.

As the spasticity cleared up, the patient complained of

numbness of the 4th and 5th fingers of the left hand which persisted for several weeks and then disappeared. Slight hypo-esthesia over the dorsum of the 4th and 5th fingers and also over the dorsum of the left foot and the lateral aspect of the left leg was noted for a short period. With the decrease of the spasticity of the left leg, it became apparent that the peroneal and tibial muscles of the left leg, and the intrinsic muscles of the left foot were paralyzed. There was also weakness of the left gastrocnemius and left quadriceps. The patient's left foot was put in a splint on November 8, 1935, and she received daily massage and light treatments. The anterior tibial group of muscles showed slight atrophy. The left patellar reflex became hyperactive as compared to the right, and the left Achille's reflex hypoactive.

Mental status. The patient's mental condition was very poor when the fever subsided and she was able to talk. She was completely disorientated as to time and place and confabulated. Her recent and distant memory were both impaired. There was a striking improvement in her mental state before she was discharged. Her distant memory seemed normal, but her memory for recent events remained unreliable. She showed little insight into her illness or her financial situation.

On her discharge December 18, 1935, she returned to America and her family report that she seems to be improving. The muscular power of her left leg is somewhat better, and she is now able to walk without crutches. Except for inability to concentrate and lack of initiative, no striking personality changes have been observed by her relatives. Her memory is improving slightly.

Two samples of her blood were obtained for neutralization tests: one early in the course of the disease in September 1935, the other during convalescence in December 1935.

In addition to these cases which appeared to be clinically typical of epidemic encephalitis, it seems of interest to report a third case which was thought to be possibly vaccine encephalitis. In spite of the fact that encephalitis of both the "A" and "B" type is common in Japan, cases of encephalitis of any kind are not common in China. Although vaccination is generally practised throughout China, vaccine encephalitis is practically unknown. Only one case of possible encephalitis following vaccination has been reported, which occurred in a Chinese infant, aged 1½ months (7). The findings in this case, however, were not typical, since the vaccination failed to take. As far as we have been able to determine, no case of vaccine encephalitis has ever occurred in the absence of a successful vaccination. Although it is impossible to prove the diagnosis of vaccine encephalitis in a single case if

recovery takes place, it was thought of interest to exclude epidemic encephalitis as a possible cause by neutralization tests.

Case 3

The patient, a Chinese girl of 8, was admitted on September 17, 1935, for coma of 4 hours' duration. She had been vaccinated for the third time on September 7, 1935, with a definite "take." The first vaccination was done shortly after birth. A very small faint scar was visible on the leg. The second vaccination done sometime later, had failed to take. At the time of admission, she showed a good take with a scar 1 cm. in diameter.

The patient had been well until September 16th, 9 days following vaccination, when she complained of headache. She vomited once on September 16th and again the morning of September 17th. She was given aspirin, but it failed to relieve the headache. At 11 o'clock on September 17th, the patient was found to be comatose.

Physical examination. On admission, the patient was stuporous and could not be aroused. She was restless, throwing herself from side to side, with teeth clenched. Her temperature was 37.8° C. on admission and rose to 39° C. within 8 hours. Her extremities were flaccid, the deep reflexes active and equal. The abdominal reflexes were absent. The Babinski sign was positive on both sides. No impairment of sensation was noted. Her neurological signs were found to vary considerably from time to time. Her eyegrounds were normal.

Laboratory findings. The white cell count was 11,000 with a differential showing 89 per cent neutrophilic polymorphonuclears. The pressure of the spinal fluid was normal. The fluid was clear and colorless and contained 67 white blood cells, 88 per cent of which were lymphocytes. The globulin content of the spinal fluid was slightly increased. The sugar content of the spinal fluid was 91 mgm. and chlorides 699 mgm. per cent. Her fasting blood sugar on the same day was 154 mgm. per cent.

Course. The patient was given glucose intravenously and fluids by nasal tube. Lumbar punctures were performed daily for the first 3 days. The patient regained consciousness at the end of 48 hours, and was talking normally on the third day. Her recovery was rapid and complete. Blood for neutralization tests was obtained shortly after recovery and again in March 1936, 5 months after discharge.

Attempts to demonstrate a virus in the spinal fluid

The spinal fluid was inoculated intracerebrally into 2 rabbits and into 2 mice. All the animals remained well. One rabbit was killed on the 10th day, the brain was removed with sterile precautions and emulsified. The emulsion was injected intracerebrally into another rabbit. This rabbit had received several applications of coal tar over

a small area of skin. The brain emulsion was injected intradermally into normal and tarred skin. No reaction took place and the rabbit remained well. The patient's spinal fluid was also rubbed into the scarified cornea and injected intradermally and by scarification into the tarred and normal skin of another rabbit. This animal also failed to show a reaction of any kind.

Neutralization tests

The virus used for neutralization tests was obtained from 2 sources.

Japanese virus. Dr. Hashimoto of St. Luke's International Clinic succeeded in isolating a virus, transmissible to mice, from a fatal case of encephalitis in Tokyo in September 1935. He was kind enough to send us two infected mouse brains. We were able to transmit the virus from mouse to mouse without difficulty by intracerebral inoculation. The virus was active in a dilution of 10^{-4} or 10^{-5} , and the mice died with typical symptoms of encephalitis on the 5th to 7th day.

St. Louis virus. Through the kindness of Dr. Webster of the Rockefeller Institute, specimens of mouse brain infected with St. Louis virus number 3 were sent to us. The virus was active in high dilution, and Swiss mice (derived from the stock of the Rockefeller Institute) as well as local stock laboratory mice were found to be susceptible.

Case 1. Neutralization tests were carried out with both the St. Louis and the Japanese viruses according to the technique described by Webster and his coworkers (4) with the serum of Case 1, obtained about $1\frac{1}{2}$ years after recovery. The serum obtained from Case 3 in March 1936, 5 months after recovery, was tested at the same time. The serum obtained from a child who had had as far as we could tell, no contact with encephalitis, was used as a control. Serum from one patient thought to have post-measles encephalitis, and also from another child with obscure symptoms suggesting subacute encephalitis, were included in some of the tests.

The same technique was used throughout the tests. The brain of one or more mice which had died with typical symptoms following the intracerebral injection of either the Japanese (Hashimoto) virus or the St. Louis virus were emulsi-

fied in broth so as to make a suspension of approximately 10 per cent by weight. In some instances the brains of the mice were stored in 50 per cent glycerine for 1 to 4 days before use. Dilutions ranging from 10^{-2} to 10^{-5} were made in broth from the 10 per cent emulsion. Equal quantities of the sera to be tested and the virus dilutions were mixed and incubated at 37° C. for 2 hours. Four mice were then injected intracerebrally with 0.03 cc. of each virus-serum mixture. Usually 12 mice were used for each serum. No significant difference in susceptibility was noted between Swiss mice and stock laboratory mice. Both types of mice were used in these experiments. Only a few representative protocols are given.

TABLE I
*Protection test: Japanese virus (Hashimoto).
January 4, 1936*

Serum \ Dilution	10^{-2}	10^{-3}	10^{-4}	Result
Case 1 Bled January 4, 1936...	4, 14*	6	11	Protection (8 survived)
Case 2 Bled December 3, 1935	7, 7, 7, 7	7, 8, 8	6, 9	No protection (3 survived)
Questionable post-measles encephalitis.	6, 6, 6	6, 6, 7, 15	5, 15, 16	No protection (2 survived)
Non-contact serum...	6, 7, 7	6, 7, 7, 8	6, 6, 8	No protection (2 survived)

* Duration of life of mouse in days; blanks indicate that mice remained well; mice were observed for a period of 2 weeks or longer.

TABLE II
*Protection test: Japanese virus (Hashimoto).
February 13, 1936*

Serum \ Dilution	10^{-2}	10^{-3}	10^{-4}	Result
Case 1 Bled January 4, 1936...	8* (Only 3 mice used for this dilution)	2	2	Protection (7 survived)
Non-contact serum...	6, 6, 6, 6	6, 6, 6, 7	6, 6, 7	No protection (1 survived)

* See footnote to Table I.

The serum from Case 1 was found to protect against both the Japanese and the St. Louis virus. The sera obtained from Case 3 and the non-contact serum failed to protect. These experiments were repeated with the same results. The sera from the case of possible post-measles encephalitis and that from the case of atypical encephalitis also failed to protect.

TABLE III
Protection test: *St. Louis virus*. March 19, 1936

Serum	Dilution	10 ⁻³	10 ⁻⁴	10 ⁻⁵	Result
Case 1 Bled January 4, 1936...	18, 18*				Strong protection (10 survived)
Case 3 Bled March 17, 1936...	5, 6, 7, 7	3, 6, 6, 7	6, 7, 7		No protection (1 survived)
Non-contact serum	5, 5, 7, 8	6, 6, 7, 7	7, 7, 7, 15		No protection (None survived)
Atypical encephalitis...	4, 5, 5, 6	6, 6, 8	6, 6, 6, 7		No protection (1 survived)

* See footnote to Table I.

TABLE IV
Protection test: *St. Louis virus*. April 1, 1936

Serum	Dilution	10 ⁻³	10 ⁻⁴	Result
Case 1 Bled January 4, 1936.....	4, 9*		1, 4	Protection (4 sur- vived)
Case 3 Bled March 17, 1936.....	7, 7, 7, 8	5, 6, 6, 6		No protection (None survived)

* See footnote to Table I.

Case 2. Neutralization tests with the sera obtained from Case 2 were only made with the Japanese virus, since the supply of this patient's sera was exhausted before the St. Louis virus was available. Both the serum obtained during the acute stage and that obtained during convalescence failed to protect.

Cross-protection tests. The fact that the serum of Case 1 protected against both the Japanese (Hashimoto) and the St. Louis virus seemed to indicate that these two viruses might be closely related. It was thought of interest to see whether mice that had survived inoculation with one of these viruses were immune to intracerebral injection of the other virus. Five mice which had been inoculated with the Japanese virus on March 19th and had survived, were inoculated with St. Louis virus diluted 10⁻³ on April 1st. All 5 mice remained well, whereas 4 control mice inoculated with the same dilution of virus died with typical symptoms 7 days after inoculation.

A few mice which had survived intracerebral injection with the St. Louis virus were reinoculated intracerebrally with the Japanese virus diluted 10⁻⁴. The mice which had been previously inoculated with the St. Louis virus survived, whereas the control animals died on the 6th to 7th day following injection.

DISCUSSION

Case 1, as far as we could determine, contracted encephalitis in China. She had, to our knowledge, no contact with any individuals who had recently come from Japan. No other cases of encephalitis developed in the community where she was living. This patient's serum, obtained 1½ years after recovery, contained neutralizing antibodies both for the virus isolated in Japan in 1935 by Hashimoto and for the virus isolated by Webster in America during the St. Louis epidemic of 1933. Mice that survived inoculation with the Japanese virus, were immune to subsequent intracerebral injections with the St. Louis virus, and *vice versa*. Due to a shortage of mice, the number of animals in the cross protection tests is small, but the results are suggestive. These experiments seem to indicate that the Japanese virus isolated in 1935 and the St. Louis virus isolated in 1933 may be closely related.

The failure of the serum from Case 2 to protect against the Japanese virus is of interest because this patient obviously contracted encephalitis in Japan. It may be that a later bleeding will show protective antibodies.

The lack of protective antibodies in the serum obtained from Case 3 seems to exclude the virus of epidemic encephalitis, either of the St. Louis or the Japanese type, in this case. Attempts to demonstrate vaccine virus in the spinal fluid of this patient failed. Although vaccine encephalitis is rare following re-vaccination, it has been observed (8). The incubation period following vaccination was typical in this patient.

Both the cases of epidemic encephalitis described, seem to show clear-cut sequelae. In Case 1, personality changes and emotional instability have persisted for more than 1½ years. In Case 2, there was an unusually severe and protracted course. This patient retains a foot-drop and an impaired memory.

SUMMARY

1. Two cases of epidemic encephalitis have been observed in North China which were followed by clear-cut sequelae.

2. The serum of one of these cases contained protective antibodies both against the St. Louis type of encephalitis virus and against a virus iso-

lated during the 1935 encephalitis epidemic in Japan by Hashimoto.

3. A small number of cross protection tests suggest that injections with the Japanese (Hashimoto) virus protect against the St. Louis type of virus, and *vice versa*.

4. A case of possible vaccine encephalitis in a Chinese child is described.

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