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Clinically Apparent and Inapparent Infection with Japanese B Encephalitis Virus in Shanghai and Tientsin.

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The occurrence of the Japanese B type of encephalitis in Peiping, China, has been reported in recent years at first on the basis of generally inconclusive neutralization tests,[1-4] and later in 1941 on the basis of unequivocal identification of a virus recovered from a fatal case.[5] During the summer of 1946, an outbreak of an illness among U. S. Marines stationed in Tientsin which was at first thought to be encephalitis and later regarded as poliomyelitis, led to certain investigations which are reported in this communication. Since we could obtain no history of outbreaks of "summer" encephalitis in Tientsin, it was deemed desirable to determine by means of neutralization tests whether or not there was evidence of inapparent dissemination of the virus in the native population. Accordingly, blood was obtained from 20 Chinese adults, aged 17 to 48, who had no history of encephalitis and stated that they had spent their entire lives in the Tientsin area. During this period there were also submitted to our laboratory acute and convalescent serum specimens from a case of acute encephalitis in a Chinese doctor in Shanghai, and, through the courtesy of Dr. Ming-Sing Hwang, we subsequently obtained blood from a group of Chinese adults in Shanghai who had no history of encephalitis.

Clinically Apparent Infection in Shanghai. According to Dr. M. S. Hwang, there is no record of an outbreak of encephalitis in Shanghai, although infrequent sporadic cases are seen,[6] and to the best of his or our knowledge, no case of encephalitis in Shanghai had been proved as being caused by the Japanese B encephalitis virus. During the summer of 1946, Dr. Hwang saw 2 other cases of acute encephalitis in addition to the patient to be reported here. While we were in Shanghai in the middle of August, 1946, Dr. Hwang "combed" the crowded hospitals for cases of acute encephalitis but all we were able to find on rounds were a few cases, in which the most probable clinical diagnoses were poliomyelitis, tuberculous meningitis, and measles encephalopathy. We are indebted to Major F. A. Mantz, M.C. of the Surgeon's Office of the China Service Command

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§ We wish to express our indebtedness to Brig. Gen. S. Bayne-Jones, at the time deputy chief of the Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Brig. Gen. J. I. Martin, U.S.A., Chief Surgeon of the U. S. Army Forces in the Pacific, Col. Crawford F. Sams, M.C., chief of the Public Health and Welfare Section of the Supreme Command for the Allied Powers, and Lt. Col. W. D. Tigertt, M.C., commanding officer of the 406th Medical General Laboratory, for making possible the work of this Commission.

1 Kuttner, A. G., and T'ung, T., *J. Clin. Invest.*, 1936, **15**, 525.

2 Chu, F. T., Wu, J. P., and Teng, C. H., *Chinese Med. J.*, 1940, **58**, 68.

3 Huang, C. H., and Liu, S. H., *Chinese Med. J.*, 1940, **58**, 427.

4 Huang, C. H., *Chinese Med. J.*, 1941, **59**, 34.

5 Yen, C. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 609.

6 Lowenberg, R. D., *Chinese Med. J.*, 1937, **51**, 989.

7 Mitamura, T., *Jintendo Ijikenkyu Zasshi*, 1943, No. 589, 1 (in Japanese; partial English translation provided by Dr. M. Kitaoka).

8 Sabin, A. B., *J. A. M. A.*, 1947, **133**, 281.

TABLE I.
Complement Fixation Tests on Acute and Convalescent Sera from a Case of Encephalitis (Dr. O. H. J.) in Shanghai (July, 1946).

Test	Exact units of complement	Temp. inactiv.	Serum days after onset	Complement fixation in mixtures with indicated antigens: Japanese B Serum 1:* 2	4	8	16	32	64	128	St. Louis Serum 1: 2	4	8	16	WEE Serum 1: 2	4	Saline Serum 1: 2	4	Jap B C-F Titer
I	1.7	60°C	7	4	4	4	4				3	1			1	0	2		AC
			21	4	4	4	4				4	2			±	±	±		AC?
II	1.7-2.0	65°C	7		4	3	1	±	0	0		0	0	0		0		0	1:8
			21		4	4	4	3	1	1		±	0	0		0		0	1:32

WEE = Western equine encephalitis virus. AC = Serum anticomplementary.
Saline = Mixture with physiological salt solution instead of antigen to check on anticomplementary properties of the serum.
* The serum dilutions indicated here are the original dilutions added to the mixture and should be multiplied by 4 for the final dilution in the mixture prior to the addition of the sensitized cells. In test II the serum dilutions began at 1:4 because there was not enough serum to start with lower dilutions.
4 = Complete fixation; 3,2,1 = Different degrees of partial fixation; ± = Questionable trace; 0 = No fixation, or complete hemolysis. The original dilution of serum giving 2 plus (approximately 50%) fixation represents the titer.

and Dr. M. S. Hwang for the clinical history and serum specimens of the patient to be reported.

The patient was Dr. O.H.J., a 29-year-old male, Chinese physician who had not been outside of the environs of Shanghai in "recent" months; he was attending physician at a tuberculosis clinic and in "robust" health until the onset of the present illness. On 12 of July, 1946, 24 hours after the extraction of several teeth, there was a sudden onset of severe, generalized headache and fever. The headache became progressively worse and upon admission to the hospital on 14 of July he was said to be distinctly somnolent, and except for the fever (102°-104°F by mouth) and a relative bradycardia (90-110), the general physical and neurological examinations and an X-ray film of the chest revealed no abnormalities. Daily examinations of the cerebrospinal fluid showed a pleocytosis ranging between 195 and 300 cells per cu mm with 79 to 87% lymphocytes, sugar of 78 to 95 mg %, negative Pandy and Nonne-Appelt tests for protein, and no bacteria on smear. On 17 of July, instead of somnolence the patient exhibited a muttering delirium with only occasional lucid moments, pronounced nuchal rigidity, left central (supranuclear) facial palsy, somewhat hyperactive reflexes, and the Babinski sign was positive bilaterally. A well marked ophthalmoplegia was subsequently noted on the left side. On 19 of July the fever, relative bradycardia and general condition were still the same, and although the diagnosis of acute encephalitis was favored, basilar meningitis was also considered and large doses of penicillin were administered between 17 and 30 of July. Improvement began about 22 of July and by 5 of August, all abnormal neurological signs had disappeared and with the exception of extreme generalized asthenia the patient appeared well and had no complaints.

The first blood specimen was obtained on 19 of July, 7 days after onset, and the serum reached the Tokyo laboratory on 24 of July when it was frozen and stored in an insulated box containing solid CO_2. The second specimen was taken 2 of August, 21 days after onset, and reached the Tokyo laboratory on 6 of August, when it was frozen in the same manner. The protocols of the complement fixation tests with these sera are shown in Table I. Both sera were somewhat anticomplementary in the first test after the routine inactivation at 60°C for 20 minutes, probably due to the lack of refrigeration during the 4- to 5-day interval prior to their arrival in Tokyo. In the second test after heating at 65°C for 20 minutes, specific fixation was demonstrable with the Japanese B virus antigen and the titer in the con-

valescent serum (1:32 of the original serum) was 4 times greater than that obtained with the acute specimen. The intracerebral neutralization test in mice yielded an equivocal index of 32 with the acute specimen of serum and a distinctly positive index of 80 with the convalescent serum. Thus, the results of both types of tests, but especially those of the complement fixation test, warrant a serological diagnosis of Japanese B encephalitis.

Clinically Inapparent Infection in Tientsin and Shanghai. The blood specimens from the Chinese adults without history of encephalitis were iced immediately after they were obtained, and the sera were frozen and stored in a box containing solid CO_2 4 to 6 days later. The neutralization tests were carried out with the undiluted, unheated sera against varying dilutions of the Nakayama strain of Japanese B encephalitis virus, and the results are shown in Table II. Thus, 17 (89%) of the 19 sera from Chinese residents of Tientsin, aged 17 to 57, and 11 (85%) of the 13 sera from Chinese residents of Shanghai, aged 16 to 51, contained distinctly significant titers of neutralizing antibody for the virus. In 1943, Mitamura[7] reported that 83% of 104 "normal" human sera (age not indicated but Dr. M. Kitaoka informed us that they were "mostly" adults) obtained from Middle China (Nanking and Shanghai) in 1941 neutralized the Japanese B virus, as compared with 83% of 116 human sera from Tokyo in 1936 (one year after the largest epidemic) and 0.8% of 525 human sera from Hokkaido, Japan in 1937 where no epidemic of the disease had been observed up to that time. In 1945, 90% of natives on Okinawa, aged 20 to 60, were found to have neutralizing antibodies for this virus.[8]

Our own data taken together with those reported by Mitamura indicate that the virus of Japanese B encephalitis had undergone as extensive dissemination among the population of North and Middle China, where only very few cases of encephalitis have been observed, as in those areas of Japan where the severest epidemics have occurred. Since antibodies can be detected in 80 to 90% of adults and since even the severest epidemics have attacked only a few thousand out of many millions of people, it is obvious that infection with this virus must be inapparent in the vast majority of human beings in Japan as well as in China, and the question really is whether epidemics might have occurred in China but had gone unrecognized. It is generally admitted in China, that only a small proportion of the population seeks the advice of, or is seen by, western-trained Chinese doctors and the possibility is granted that large numbers of cases of encephalitis could be missed. However, there are many good hospitals with thousands of patients in the large population centers of Shanghai, Peiping, Tientsin, etc. and an unusual increase in the number of cases during the summer and autumn, although it would only be relative rather than absolute, as far as numbers are concerned, could hardly go unrecognized. During the summer of 1938, Chu, Wu and Teng[2] saw 10 cases of acute encephalitis on the Pediatric Service of the Peiping Union Medical College within the short period of 24 days from July 29 to August 22, and 3 additional cases were seen in older individuals.[3] In 1939, 16 cases of acute encephalitis were admitted to the Peiping Union Medical College, and 12 of these came in during July, August and September.[3] During our visit to the Central Hospital in Peiping in August, 1946 we had an opportunity to see 2 patients in the acute stage of encephalitis with clinical manifestations and laboratory findings that could be entirely compatible with the Japanese B type of encephalitis. It is a pleasure to record here the excellence and completeness of the clinical and laboratory observations which we found on the patients at the Central Hospital. Dr. William H. L. Chung, the medical director and chief of the medical service at the Central Hospital, very kindly supplied us with the data on the cases of encephalitis admitted to this hospital during the 4 years of 1942 to 1945, which are shown in Table III. While the number of cases admitted during the 4 months of June to September is regularly as great or greater than during the other 8 months of the year, suggesting

TABLE II.
Intracerebral Neutralization Tests in Mice with Japanese B Encephalitis Virus on Sera from Normal Chinese Residing in Tientsin or Shanghai.

City	Test	Serum: No.	Serum: Age of donor, years	Mortality at indicated final dilutions of virus: 10^{-6}	10^{-7}	10^{-8}	10^{-9}	LD_{50}	Neutralization index
Tientsin	A	Rabbit control		4/4	5/5	3/4	0/4	8.3	—
		1	48	1/4	0/5	0/5		5.7-?	400+
		2	35	0/5	0/5	0/5		5.5-?	630+
		3	40	3/3	4/5	1/5		7.5	6
		4	20	0/5	0/5	0/4		5.5-?	630+
		5	37	0/5	0/5	0/5		5.5-?	630+
		6	35	0/3	0/5	0/5		5.5-?	630+
		7	24	0/4	0/5	0/5		5.5-?	630+
		8	47	0/4	0/5	0/5		5.5-?	630+
		9	21	0/4	0/5	0/4		5.5-?	630+
	B	Rabbit control		5/5	5/5	1/5	1/5	7.7	—
		10	26	0/5	0/4	0/3		5.5-?	160+
		11	57	0/5	0/5	0/5		5.5-?	160+
		12	20	0/5	0/5	0/5		5.5-?	160+
		13	17	0/5	0/4	0/5		5.5-?	160+
		14	37	0/4	0/3	0/5		5.5-?	160+
		15	37	0/5	0/5	0/5		5.5-?	160+
		16	44	0/5	0/5	0/4		5.5-?	160+
		17	37	0/4	0/3	0/5		5.5-?	160+
		18	18	4/5	0/4	0/5		6.4	20
		19	27	0/5	0/4	0/5		5.5-?	160+
Shanghai	C	Rabbit control		5/5	5/5	3/5	0/5	8.2	—
		1	19	0/5	0/5	0/5		5.5-?	500+
		2	30	0/5	0/5	0/5		5.5-?	500+
		3	51	0/3	0/3	0/3		5.5-?	500+
		4	38	0/5	0/3	0/5		5.5-?	500+
		5	30	0/5	0/5	0/5		5.5-?	500+
	D	Rabbit control		5/5	5/5	1/5	0/4	7.6	—
		6	32	0/4	0/3	0/5		5.5-?	130+
		7	49	0/4	0/5	0/5		5.5-?	130+
		8	16	0/5	0/5	0/5		5.5-?	130+
		9	27	0/5	0/5	0/5		5.5-?	130+
		10	31	3/5	0/4	0/3		6.2-?	25+?
		11	18	0/5	0/5	0/5		5.5-?	130+
		12	20	0/3	0/5	0/5		5.5-?	130+
		13	32	4/5	0/3	0/5		6.4-?	16+?

a seasonal increase previously noted by others,[2,3] the total numbers are, nevertheless, small. While not all of the summer cases are necessarily caused by the virus of Japanese B encephalitis, it is probable that a good many of them may be. Since the majority of human beings obviously escape with inapparent infection, one should at least consider the possibility that the unknown factors which predispose to clinically apparent infection may be found less frequently among the Chinese than among the Japanese.

Summary. Complement fixation tests on acute and convalescent sera established the virus of Japanese B encephalitis as the cause of acute encephalitis in a Chinese physician in Shanghai during July, 1946. Evidence of extensive, *inapparent* dissemination of this virus among the populations of Shanghai and Tientsin, where no epidemics of encephalitis have been observed, was obtained when neutralizing antibodies for the Japanese B encephalitis virus were found in 85% of 13 Chinese residents of Shanghai, aged 16 to 51, and in 89% of 19 life-long, Chinese residents of Tientsin, aged 17 to 57, all without history of encephalitis. Since the great majority of human beings escape with inap-

TABLE III.
Cases of Acute Encephalitis Admitted to Central Hospital, Peiping (1942-1945).

	June, July, August, September				Other 8 months			
Year	No. admitted	No. diagnosis ?	No. died	Ages of patients yr	No. admitted	No. diagnosis ?	No. died	Ages of patients yr
1942	6	0	2	20, 18, 17, 26, 16, 19	4	1	2	?, 19, 10, 17
1943	6	1	3	42, 29, 23, 13, 21, 11	5	1	3	10, 36, 16, 29, 36
1944	12	3	5	16, 45, 65, 24, 8, 9, 25, 37, 47, 4, 22, 15	6	3	3	1, 40, 5, 3, 40, 2
1945	6	1	2	13, 50, 48, 14, 24, 15	2	1	0	13, 19

Data supplied by Dr. William H. L. Chung, Medical Director of Central Hospital.

parent infection even in Japan, where severe epidemics have occurred, the question is raised whether the unknown factors, which predispose to clinically apparent infection, may be found less frequently among the Chinese than among the Japanese.

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Electrophoretic Studies on the Leukocytosis-Promoting Factor of Exudates.*

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The studies of one of us (V.M.) have demonstrated the presence of a factor in inflammatory exudates of dogs capable of inducing both a growth in the bone marrow and a discharge into the circulation of polymorphonuclear leukocytes.[1] This canine material is active in human beings, suggesting its possible clinical application.[2] Salting out studies have indicated its close association with the pseudoglobulin fraction of exudates.[3] Recent studies at Temple University by one of us (V.M.) indicate that the active component of the leukocytosis-promoting factor (abbreviated as the LPF) appears to be a polypeptide. This has been shown by aging the LPF. The material splits as an active soluble polypeptide component from the rest of the molecule.[4] The present studies represent work performed in the past at Duke University by means of the Tiselius electrophoretic apparatus which indicates that the leukocytosis-promoting factor of exudates seems to be associated with the α_1 and α_2 globulins of exudates.

Materials and methods. The leukocytosis-promoting factor was obtained from exudate of dogs essentially as described by one of us.[2] Electrophoresis was carried out as previously described at 1°C in barbital buffer at pH 8.6 and 0.1 M ionic strength.[5,6] The patterns representing the migrating boundaries were recorded by the method of crossed slits described by Svensson.[7]

The serum was prepared for electrophoresis

* This paper is No. 39 of a series entitled "Studies on Inflammation."

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1 Menkin, Valy, *Am. J. Path.*, 1940, **16**, 13; *Am. J. Path.*, 1943, **19**, 1021.

2 Menkin, Valy, *Arch. Path.*, 1946, **41**, 376.

3 Menkin, Valy, *Arch. Path.*, 1940, **30**, 363.

4 Menkin, Valy, in press, 1947.

5 Cooper, G. R., Craig, H. W., and Beard, J. W., *Am. J. Syph., Gonor., and Ven. Dis.*, 1946, **30**, 555.

6 Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1942, **142**, 193.

7 Svensson, H., *Kolloid Z.*, 1940, **90**, 141.