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SECTION MEETINGS

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Human Infection with Rift Valley Fever Virus and Immunity Twelve Years After Single Attack.*

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The virus of Rift Valley Fever (a disease of sheep and cattle in Kenya Colony, British East Africa) was first isolated in 1930 by Daubney, Hudson and Garnham,[1] who also established its pathogenicity for human beings. Since that time there have been accidental human infections in practically every laboratory in which this virus has been studied—4 cases in Kenya[1] in 1930, 4 cases in 1931 in London[2] and another in 1934,[3] in New York 1 case in 1932,[4] 3 cases in 1933[5] and 3 cases in 1934,[6] and at least 1 case in Uganda[7] in 1944—a total of 17. The purpose of the present communication is not merely to record still another instance of accidental laboratory infection, but rather to indicate (a) that a strain of virus which has had at least about 300 brain to brain passages in mice has not undergone any modification in the type of disease it can produce in human beings, and (b) that neutralizing antibodies for the virus can persist for at least 12 years after a single attack of

* This investigation was conducted with the aid of the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D.C.

1 Daubney, R., Hudson, J. R., and Garnham, P. C., *J. Path. and Bact.*, 1931, **34**, 545.

2 Findlay, G. M., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1932, **25**, 229.

3 Findlay, G. M., quoted by Kitchen.[5]

4 Schwentker, F. F., and Rivers, T. M., *J. Exp. Med.*, 1934, **59**, 305.

5 Kitchen, S. F., *Am. J. Trop. Med.*, 1934, **14**, 547.

6 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1935, **62**, 433.

7 Smithburn, K. C., personal communication concerning case at Yellow Fever Research Institute, Entebbe, Uganda, East Africa.

ACCIDENTAL HUMAN LABORATORY INFECTION WITH RIFT VALLEY FEVER VIRUS WHICH HAS UNDERGONE ABOUT 300 OR MORE INTRACEREBRAL PASSAGES IN MICE

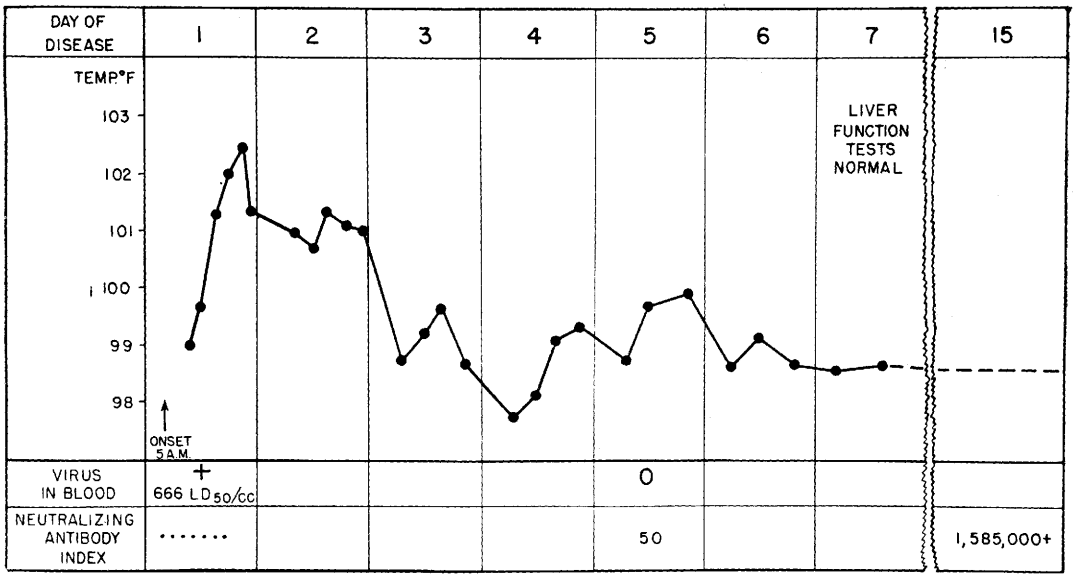

FIG. 1.

the disease without further exposure to the virus.

The "K" strain of virus was obtained from Dr. N. Ishii of Japan in 1945 and was alleged to be a strain of mouse-adapted dengue virus. However, studies, which will be reported at a later date, established that the "K" virus was a strain of Rift Valley Fever virus. The history of the virus prior to the time it came to be called "dengue" is not known, although it is known that Rift Valley Fever virus was brought into Japan from France for laboratory investigations. It was stated that the "K" virus had undergone 293 brain to brain passages in mice since 1942 and it had had 5 additional intracerebral passages in mice in Cincinnati, when the accidental human infection to be reported here had occurred. One of us (A.B.S.) worked continuously with this virus from October 26, 1945 until early January, 1946, without becoming infected or developing neutralizing antibodies for the virus. He resumed work with this virus on October 31, 1946 when he ground a number of infected mouse brains in a mortar and performed an intra-abdominal neutralization test in mice, at which time contamination of the skin or aspiration of droplets of virus might have occurred. Between October 31 and the onset of illness on November 6, the only other activity related to this virus consisted of the daily examination of the inoculated mice.

A.B.S., who had been in good health, was awakened from his sleep at 5 a. m. on November 6 by a very severe frontal headache. Acetylsalicylic acid and codeine were taken at that time and since they brought no relief, no more was taken during the course of the illness so that the temperature curve (rectal temperatures shown in Fig. 1) was not subsequently affected by antipyretics. The temperature did not rise until the late afternoon and evening. Severe aching in the muscles, bones, and joints, and anorexia were the only other symptoms which developed during the course of the day and persisted for about 48 hours. The temperature returned to normal on the 3rd day and only mild muscle aching and weakness remained. He was out of bed on the 4th day and back in the laboratory on the 5th day. The low-grade fever up to 99.9° on the 5th day was associated with a feeling of feverishness and slight malaise, but recovery was complete and uneventful thereafter. It may be of interest

TABLE I.
Immunological Identity of Virus Recovered from Patient "A.B.S." and "K" Strain of Rift Valley Fever Virus.

Dilution of virus in mixture	Intra-abdominal Neutralization Test in Mice. Mortality in mixture with: Normal rabbit serum	"K" hyperimmune rabbit serum
10-1	—	0, 0, 0, 0
10-2	1+*, 1+, 1+, 1+	0, 0, 0, 0
10-3	1+, 1+, 1+, 1+	0, 0, 0, 0
10-4	2, 2, 2, 3	0, 0, 0, 0
10-5	2, 2, 2, 2	0, 0, 0
10-6	2, 2, 0, 0	0, 0, 0, 0
10-7	3, 0, 0, 0	

* Each digit represents day of death of each mouse used in the test; 1+ = more than 24 but less than 36 hours; 0 = survival.

to note that the following tests, for which we are indebted to Dr. S. Rapoport, carried out on the blood 6 days after onset revealed no abnormality in liver function: Total bilirubin —0.3 mg %; cephalin flocculation negative; phosphorus—3.2 mg % and phosphatase—3.2 Bodansky units; total protein—7.6 g % with an albumin-globulin ratio of 2.8.

Approximately 10 hours after the first appearance of headache, blood was drawn and inoculated in mice—0.5 cc of defibrinated blood intra-abdominally into each of 5 mice and 0.03 cc of the serum intracerebrally into each of 5 additional mice. All of these mice succumbed within 48 hours, and 3 successful serial passages by the intra-abdominal or intracerebral routes were carried out. Extensive hepatic necrosis and acidophilic intranuclear inclusions were present in the liver cells of the first passage mice. The immunological identity of the virus recovered from the blood of A.B.S. and the "K" strain of Rift Valley Fever virus was established by the results of the intra-abdominal neutralization test shown in Table I. Varying dilutions of mouse brain suspension containing the 3rd passage of the "A.B.S." virus were mixed with equal parts of "K" hyperimmune rabbit serum and for control a similar set was prepared with normal rabbit serum. After incubation of the mixtures in a water bath at 37°C for about one hour, 0.1 cc was injected intra-abdominally into approximately 4-week-old Swiss mice. Some of this

TABLE II.
Early Development and Long Persistence of Neutralizing Antibodies for Rift Valley Fever Virus ("K" Strain) After a Single Attack of the Disease in Human Beings.

Test	Serum	Interval between onset and specimen	Neutralization Tests by Intra-abdominal Route in Mice. Mortality at indicated dilutions of virus in mixtures: 10-1	10-2	10-3	10-4	10-5	10-6	10-7	LD_{50}	Neutralization index
A	Normal rabbit control	—	—	—	—	2,2,3,3†	2,2,2,2	2,2,2,5	6,0,0,0	10-6.7	—
	Patient A.B.S.—1946	4 days	2,2,2,2	2,2,2,2	2,2,2,2	2,2,2,2	2,3,0,0	0,0,0,0	—	10-5.0	50
	Patient A.B.S.—1946	14 ,,	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	—	10-0.5—?	1,585,000+?
	R.W.B.—1946	Specimen 1	2,2,2,2	2,2,2,2	2,2,2,2	2,2,2,2	2,2,2,2	2,3,3,5	—	10-6.5+?	2 — ?
	worked with virus	Specimen 2, 13 days later	2,2,2,2	2,2,2,2	2,2,2,2	2,2,2,2	2,2,2,0	2,2,0,0	—	10-5.8+?	8 — ?
B	Normal rabbit control	—	—	—	2,2,2,2	2,2,2,4	2,2,2,2	2,2,2,2	0,0,0,0	10-6.5	—
	Patient K.C.S.—1944	16 mo	—	—	5,8*,9,0	7,0,0,0	0,0,0,0	0,0,0,0	—	10-3.5	1,000
	Patient T.F.—1934	12 yrs	6,8‡,10,0	4,6,6,0	8*,10,0,0	7,10,0,0	0,0,0,0	0,0,0,0	—	10-3.0	3,160

† Each digit represents day of death of each mouse used in the tests. 0 = survival.
* Rift Valley Fever virus shown to be cause of death by passage of liver.
‡ No virus recovered by passage of liver.

same preparation of "K" hyperimmune rabbit serum was sent to Dr. Kenneth Smithburn at the Yellow Fever Research Institute in East Africa who reported that it neutralized their viscerotropic Rift Valley Fever virus.

In order to determine how much virus was present in the blood at the beginning of the human infection, some of the serum obtained 10 hours after onset and kept frozen in a CO_2 box was titrated by intracerebral inoculation of mice. It was rather surprising to find only 666 LD_{50} of virus per cc of serum (the titer was $10^{-1.3}$ for 0.03 cc), in view of the demonstration by Kitchen[5] that the blood of sick mice contained at least 100 billion LD_{50} of virus per cc. Another specimen of the patient's serum taken on the 5th day, coincident with the slight elevation in temperature, contained no virus but a definite, though small, amount of neutralizing antibody was already present (Table II).

R.W.B. first began working with the "K" virus on October 30, 1946 and because he felt feverish (temperature not taken) and had a moderately severe headache on the night of November 5, his blood taken on November 6 was inoculated in mice in the same manner and at the same time as that of A.B.S. However, no virus was recovered. The results of the neutralization tests against the "K" virus with the sera obtained from both A.B.S. and R.W.B., shown in Table II, indicate that only A.B.S. developed neutralizing antibodies and that the titer in his case had risen from 50 in the serum taken 4 days after onset to at least 1,585,000 in the serum taken 10 days later. The results of test B (Table II) are of especial interest because they suggest that after an initial high peak, the neutralizing antibody drops to a titer of about 1,000 and can then persist for at least 12 years without any further exposure to the virus in the interim. Both K.C.S.† and T.F.‡ suffered from accidental laboratory infections, and T.F. has had no further exposure to Rift Valley Fever virus since he stopped working with it in 1934.

Discussion. The clinical features and the duration of the febrile phase of the illness reported in this communication are no different from the human cases which resulted from exposure to lamb or early mouse-passage virus. Kitchen[5] quotes a personal communication from G. M. Findlay regarding a human laboratory infection with virus which had undergone more than 400 successive passages in mice, the route and tissue used for passage not being indicated however; this case probably had a more protracted course than usual since it is stated that the virus was recovered from the blood on the 6th day of illness. The absence of any appreciable modification in the virulence of this virus for human beings by approximately 300 or more intracerebral passages in mice is to be contrasted with the rapid modification which occurs when dengue virus is passaged by the route in mice.[8] The chances for modification or mutation would appear to be much less for a virus, such as that of Rift Valley Fever, which, without requiring any preliminary adaptation, multiplies readily in mice, than for a virus which, like that of dengue, can be made to propagate in mice only with the greatest difficulty. The disease produced by the virus of Rift Valley Fever has previously been compared to dengue,[1] sandfly fever[1] and influenza.[4,5] However, one of us (A.B.S.) having spent several years studying both dengue and sandfly (pappataci) fever, finds that human infection with Rift Valley Fever virus is clinically indistinguishable from sandfly fever, but unlike the type of disease produced in the majority of human beings as a result of primary infection with various types of dengue virus. It has indeed been suggested[1] that what has in the past been diagnosed as 3-day fever or sandfly fever in certain parts of East Africa may very well be examples of Rift Valley Fever in man. Fortunately the differentiation is

† We are indebted to Dr. J. E. Smadel of the Army Medical School for this specimen of serum and to Dr. Kenneth C. Smithburn of the Rockefeller Foundation for the details of the case.

‡ We are indebted to Dr. Thomas Francis, Jr., of the University of Michigan for the serum on this case (described in reference 6).

8 Sabin, A. B., and Schlesinger, R. W., *Science*, 1945, **101**, 640, and unpublished observations.

in the laboratory since the blood of patients, with sandfly fever or dengue, inoculated intra-abdominally in mice, is without effect, while in the case of Rift Valley Fever the mice die in about 2 to 3 days.

Findlay[9] has previously reported the persistence of neutralizing antibodies for the virus of Rift Valley Fever for 4 to 5 years in laboratory workers, who, however, might have had further exposures to the virus. The present demonstration of the persistence of the antibodies for a period of 12 years, is an unusual example of the duration of immunity after a single attack without further exposure to the virus during the intervening years.

Summary. 1. Accidental human laboratory infection with a strain of Rift Valley Fever virus which had undergone at least about 300 intracerebral passages in mice, indicated that no modification in its pathogenicity for man resulted from this mode of passage.

2. The virus was recovered from the blood 10 hours after the first appearance of headache but titration revealed that the virus content was very low—only 666 LD_{50} per cc of serum. Four days after onset, no virus was detected in the blood, but neutralizing antibodies (index of 50) were already present and in the next 10 days increased to yield an index of at least 1,585,000.

3. Neutralizing antibodies for Rift Valley Fever virus (index of 3,160) were found 12 years after a single attack in the serum of an individual who had no further exposure to the virus during the intervening years.

[9] Findlay, G. M., *Brit. J. Exp. Path.*, 1936, **17**, 89.

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Streptomycin in the Therapy of Granuloma Inguinale.*

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The use of antimonial preparations in the therapy of granuloma inguinale is well established. Its limitations, particularly in chronic cases, have long been known. Recurrences of the disease have occurred with a great degree of frequency even after apparent healing was attained. Other forms of treatment have been employed alone or in conjunction with the antimonials and have ranged from the local application of escharotic agents to surgery and x-radiation therapy. Although cure may be attained by adhering to one or another of these procedures, much is to be desired in the management of this minor venereal disease.

With the advent of the antibiotics it was hoped that a more adequate and specific therapy for granuloma inguinale might be evolved. Preliminary observation with penicillin and tyrothricin showed that these drugs were of little value. One to 4 million units of penicillin administered for the expressed purpose of treating granuloma inguinale failed to control the granulomatous lesions. Tyrothricin was also without effect when it was applied locally in the treatment of granuloma inguinale.

Streptomycin, however, has proved so strikingly effective in the therapy of granuloma inguinale that a preliminary report is warranted at this time. A total of 23 patients has received streptomycin for granuloma inguinale. The diagnosis of the disease was established in all cases by demonstrating the presence of Donovan bodies in either spreads made from the lesion or by

* This study was aided by a grant from the United States Public Health Service.