evidence of inapparent infection was obtained by neutralization tests with the homologous mouse-adapted virus. None of 6 chimpanzees, whose serum was tested, had any antibodies for the dengue virus before inoculation and all developed them in high titre after inoculation.

16432

Accidental Laboratory Infection with Human Dengue Virus.*

J. L. MELNICK, E. C. CURNEN, AND A. B. SABIN.

From the Section of Preventive Medicine, Yale University School of Medicine, New Haven, and the Children's Hospital Research Foundation and Department of Pediatrics, University of Cincinnati College of Medicine.

Accidental laboratory infections with a wide variety of different viruses including viscerotropic yellow fever virus and Rift Valley fever[1] virus have frequently occurred. No such infection with dengue virus, however has previously been recorded. It is the purpose of this paper to present observations upon a patient with dengue who acquired this disease by accident in the laboratory.

The patient, a 23-year-old male negro laboratory assistant was exposed to infection under the following circumstances. In July, 1947 he participated in one of the experiments (Experiment No. 2) which were described in the previous paper on the transmission of dengue to chimpanzees.[2] It will be recalled that a pooled sample of infected human serum obtained in 1944 in Hawaii from patients acutely ill with dengue and preserved in the frozen state was separated into two fractions designated "top" and "bottom" following ultracentrifugation at 33,000 R.P.M. for 90 minutes. On 31 July each fraction was injected into chimpanzees and both fractions were subsequently found to contain dengue virus. During the efforts to restrain and inoculate the chimpanzees a syringe containing the "bottom" fraction suddenly separated from the needle, and some of the serum which squirted out struck the patient in the eye. His eye was immediately washed out with water, and he resumed his work. The subsequent developments to be described are charted in Fig. 1.

The patient remained well during the next week. On 7 August he noted a macular rash distributed over the bearded area of his face. On 9 August after a good breakfast he became tired and weak on his way to work. During the morning he developed aches in the calf muscles and behind his knees as well as throbbing frontal headache. He returned home at noon, ate an egg and vomited promptly. His temperature at noon was 101°F, at 3:30 P.M., 103.6°, at 8:00 P.M., 102.6°. During the afternoon he had repeated chills. On 10 August he complained of headache, anorexia, nausea and vomiting. His eyes burned and the muscles of his legs ached. He had no symptoms referable to the respiratory tract. His wife and two children were well and had not recently been ill. On physical examination he appeared sluggish but not particularly ill. His temperature was 102.2°. The pulse and respiratory rates were not accelerated. A macular rash was present on the face as well as a few indistinct macules on the right shoulder and back. Tenderness over the eyeballs and slight injection of the conjunctivae and pharynx were noted. Small cervical and axillary lymph nodes were palp-

*This observation occurred in the course of investigations conducted by the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of The Surgeon General, U. S. Army, Washington, D. C.

1 Sabin, A. B., and Blumberg, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 385.

2 Paul, J. R., Melnick, J. L., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 193.

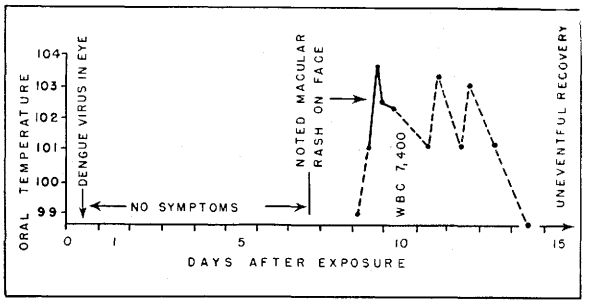

FIG. 1.

able and somewhat tender. The lungs were clear and the cardiac findings normal. The total leucocyte count was 7,400. The possibility of infection with dengue virus was considered, and blood was drawn for serological studies.

The patient remained at home and continued to have temperature elevations ranging between 101 and 103°F with aches in his head, eyes and legs. He was seen again on 13 August. The lymph nodes in the neck, axillae and both inguinal regions were larger and more tender than on the previous examination. The spleen was still not palpable and the rash had disappeared. On 14 August he felt much better and his temperature had returned to normal. The enlarged lymph nodes previously noted were still somewhat tender. His subsequent course was uneventful.

Specimens of serum were obtained at intervals 1, 13, 25, and 45 days after the onset of illness on 9 August. Neutralization tests in mice were carried out with these sera by one of us (A.B.S.) according to the technique developed by Sabin and Schlesinger which was cited in the preceding paper.[2] The dengue virus used in the neutralization tests was the 59th mouse brain passage of the Hawaii strain. The results of these tests are shown in Table I.

The titer (50% mortality end point) of dengue virus combined with normal rabbit control serum was $10^{-5.7}$. The serum from the patient obtained one day after the onset of illness contained no neutralizing antibody against this virus as indicated by the fact that the titer observed in mice inoculated with the mixture of virus and this serum was similar to that of the control. The specimens of serum obtained during convalescence, however, all appeared to contain antibody as they neutralized from approximately 8,000 to 25,000 LD_{50} of virus.

Comment. The available evidence strongly suggests that the laboratory worker had dengue as a result of the accidental introduction of virus into his eye. The fact that the eye was probably the portal of entry in this case parallels the finding that dengue can be produced in human volunteers by instilling large amounts of human virus into the con-

TABLE I.
Results of Neutralization Tests with Dengue Virus (Hawaii Strain).

Source	Serum: Days between onset and bleeding	Fate of mice following intracerebral injection of serum-virus mixture*: Dilution of virus 10-1	10-2	10-3	10-4	10-5	10-6	Titer of Virus† (LD_{50})	Neutralization Index
Normal rabbit	—	—	—	5/5	5/5	3/4	2/5	5.7	—
Patient	1	—	5/5	5/5	5/5	4/5	—	5.4+?	2—?
,,	13	—	2/5	0/5	0/5	0/5	—	1.8—?	8,000+?
,,	25	5/5	1/5	0/5	0/5	—	—	1.6	13,000
,,	45	3/5	1/5	0/5	0/5	—	—	1.3	25,000

* Indicated by a fraction in which the denominator indicates the number of mice injected with each mixture and the numerator the number which died.

† Log of dilution.

junctival sac.[3] It seems quite clear from the experiments with chimpanzees that the serum which splashed into the patient's eye actually contained dengue virus. As New Haven is not in an area in which dengue is endemic there appears to have been no other likely source of infection and none of the patient's family or associates were concurrently ill. The interval of 9 days between the accident and the development of symptoms is not too long for the incubation period of dengue and the clinical course of the illness was compatible with that of the naturally occurring disease. The serological finding that neutralizing antibodies against the mouse-adapted Hawaiian strain of dengue virus developed in the patient's serum in high titer within two weeks after the onset of illness is, of course, strong evidence in support of the diagnosis. The fact that the patient developed an acute febrile illness under the circumstances which have been presented may be regarded as additional evidence that the virus which produced inapparent infection in chimpanzees[2] as described in the preceding paper had retained its pathogenicity for man.

Summary. In the course of experiments with chimpanzees, human dengue virus was accidentally introduced on to the conjunctival surface of the eye of a laboratory worker. This was followed 9 days later by a febrile illness, clinically compatible with dengue. Neutralizing antibodies for the homologous mouse-adapted strain of dengue virus were absent in the patient's serum taken one day after onset of fever, but were present in high titer in specimens of serum obtained 13, 25, and 45 days after onset.

[3] Sabin, A. B., Unpublished studies.

16433

Serum Treatment of Western Equine Encephalitis in Mice Determined by the Course of Viral Infection.

PETER K. OLITSKY AND ARTURO C. SAENZ.*†

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Certain features of serotherapy of experimental infections with neurotropic viruses are still under deliberation. A group of investigators maintain that animals prostrated by the infection can still be completely revived and restored to a normal-seeming state while other workers hold that once the virus multiplies actively or the host exhibits a definite syndrome reflecting the involvement of the central nervous system, *i.e.*, a destruction of tissue or neurons, then serotherapy is often ineffective. There is general agreement, however, on the successful prevention or treatment of the experimental disease if potent antiserum in sufficient amount is given early enough in its course, or, generally, before definite physical signs of infection of the central nervous system are manifest.

The problem was to determine at what stage during the course of an experimental infection antiserum was or was not effective, especially in relation to the a) time after exposure to the virus, b) first appearance of physical signs, and c) degree of multiplication of the virus in the brain. By means of the intracerebral route, 100% rate of lethal infection could be obtained with a sufficient dose of virus; and by the use of the Kelser strain of Western equine encephalitis virus which is only moderately active or invasive, the incubation period could be conveniently prolonged for serum treatment at different intervals after exposure to virus. Mice were

* Dr. Saenz held a Fellowship of the International Health Division of the Rockefeller Foundation; present address, Buenos Aires, Argentina.

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