

ZIKA VIRUS

(II). PATHOGENICITY AND PHYSICAL PROPERTIES

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In this communication some studies which have been made to investigate the pathogenicity and physical properties of Zika virus (DICK et al., 1952) will be reported.

HOST RANGE

Mice.

Zika virus was first isolated from Swiss albino mice which were susceptible to intracerebral inoculations with this agent. The adaptation of the strains of virus isolated from (a) the sentinel* rhesus monkey (766), and (b) from the mice (E/1) and monkey (758) which were inoculated with suspensions of *Aedes (Stegomyia) africanus* Theobald (DICK et al., 1952) are presented graphically in Fig. 1.

With the 766 strain, 100 per cent. mortality of inoculated mice occurred by the 17th passage of 10 per cent. Seitz-filtered mouse brain suspensions. (In all estimates of the average survival time the mouse used for passage is excluded.) In the 18th passage of this line there was one sick mouse which recovered. The average survival time in the next four passages was 10.6 days, the incubation period remained at about 6 days and occasional survivors were present from the 22nd to the 59th passage. By the 60th passage the incubation period had become 5 days and between the 70th and 79th passage it was reduced to about 4 days, at which it has remained constant up to the 157th passage at the time of writing.

The passages originating from the serum of Rhesus 758 were not continued consecutively beyond the 17th passage. There were, however, more paralytic

* It will be recalled that the term sentinel monkey has been used for monkeys held captive in the canopy of trees in forests in Uganda for the purpose of indicating foci of sylvan yellow fever activity.

ADAPTATION OF ZIKA VIRUS TO MICE

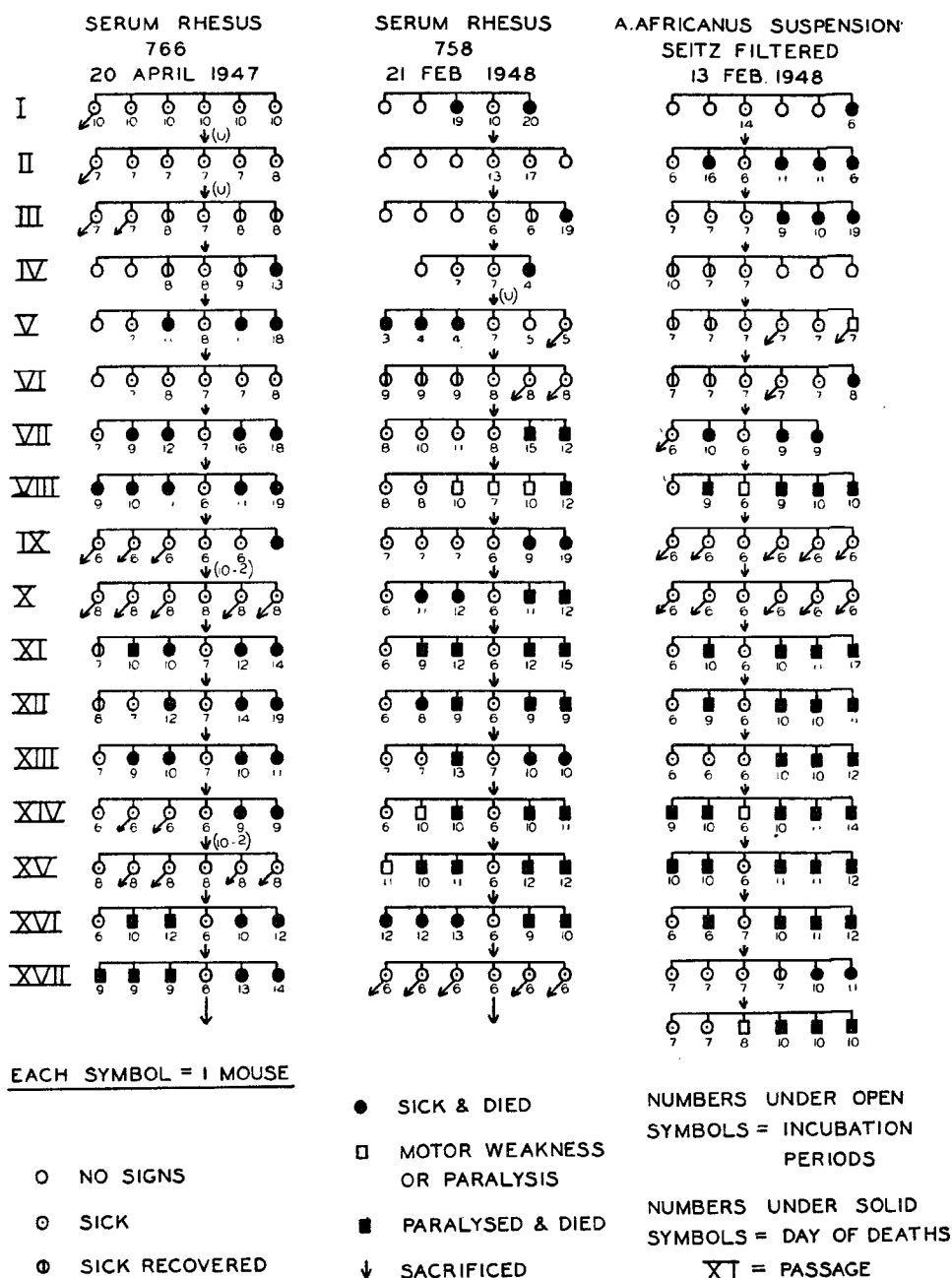


FIG. 1

mice in early passages with this strain of virus than with the 766 strain and 100 per cent. mortality of inoculated mice occurred at the 16th passage as compared with 17th passage in the 766 strain.

The behaviour of the E/1 strain of virus was similar to that of other strains in the early passages. One hundred per cent. mortality was observed by the 15th passage of Seitz-filtered 10 per cent. mouse brain suspensions at which time the incubation period was 5 days, but occasional survivors were found in subsequent passages up to the 50th passage. Between the 50th and 59th passages the incubation period became reduced to 4 days at which it has remained up to the 115th passage.

Apart from the one mouse which was found dead on the 6th day, in the group of mice inoculated intracerebrally with the Seitz-filtered suspension of *Aedes africanus* (Fig. 1.), the first signs of infection with all strains of virus were inactivity and marked roughness of the coat. In the early passages using both high and low concentrations of virus, some inoculated mice failed to exhibit any visible signs of illness, others remained rough and inactive for as long as 30 days after the onset of signs of infection. On the other hand it was not infrequent to find mice recovering after having shown signs of infection of from 1 day up to 2 weeks duration. (No virus was recovered from the brains of these chronically sick mice when tests were made 10 to 14 days after the onset of signs of illness). After a few passages (Fig. 1) some of the mice began to show evidence of motor weakness and paralysis of the limbs which was usually followed by death. Occasionally, however, mice which showed marked motor weakness subsequently recovered. As each of the strains of virus became better adapted to mice, there was increasing evidence of paralysis followed by death within 24 to 48 hours.

The fact that numbers of mice in early passages recovered from the experimental disease necessitated the use of a 50 per cent. morbidity rate in calculating the infectivity of early passage material. All mice which, whether they died or recovered, remained sick for 1 or more days after a normal incubation period were considered as sick mice for the purpose of calculating the morbidity rates and the 50 per cent. morbidity dose (MD_{50}). A comparison between the morbidity and mortality rates in a titration of 10th-passage mouse brain virus is shown in Table I. (All LD_{50} and MD_{50} are calculated by the method of REED and MUENCH, 1938.)

It was shown that with early passage virus mice of 7 days and under were susceptible to inoculations of virus by the intraperitoneal route, but that mice of 2 weeks of age and over could only rarely be infected by that route even when large doses of virus were used. Because of the irregularity of the titres obtained with early-passage virus it was, however, seldom that reproducible end points could be obtained, and the majority of experiments recorded in this paper have been taken from studies made with virus which had been through 90 or more passages in mice. After that number of passages the virus had become well adapted to mice and was much more satisfactory for reproducible experimental work.

TABLE I. Showing a comparison of the morbidity and mortality rates of mice inoculated with unfiltered or Seitz-filtered 10th-passage Zika virus.

Virus dilution	Morbidity ratio*		Mortality ratio*	
	Unfiltered	Seitz	Unfiltered	Seitz
10 ⁻²	6/6	5/6	4/6	4/6
10 ⁻³	6/6	5/6	6/6	4/6
10 ⁻⁴	4/5	4/6	4/5	3/6
10 ⁻⁵	5/6	4/6	2/6	4/6
10 ⁻⁶	0/6	0/6	0/6	0/6
LD or MD* ₅₀	5.3	4.6	4.4	4.0

* The denominator = number of inoculated mice.

The numerator of the morbidity ratio = number of mice showing signs of sickness followed by death or by recovery (see text) ; the numerator of the mortality ratio = number which died.

There was no significant difference in the infectivity of late-passage virus inoculated intracerebrally to unweaned mice as compared with mice 5 to 6 weeks old. Mice over 6 weeks of age were, however, slightly less susceptible to inoculation by that route than were younger mice. The relationship between age and susceptibility to intraperitoneal inoculation is clearly shown in the experiment recorded in Table II in which titrations of the lightly spun supernate of 99th mouse passage virus were made in mice of various ages.

TABLE II. Showing the susceptibility of mice of 7 to 35 — 42 days of age to intraperitoneal inoculations of 99th passage Zika virus.

Age in days	LD ₅₀ per 0.06 ml.
7	6.0
14	1.5 or <
21	1.4 or <
28	1.2 or <
35-42	1.0 or <

Each dilution of virus was tested in six mice, except in the case of mice under 21 days where two litters each of four mice were used. A comparative intracerebral titration which was made at the same time in 35 to 42-day-old mice gave an LD₅₀ of 6.8 per 0.03 ml.

It may be seen from this experiment that there is very little difference in the susceptibility of 7-day-old mice to intraperitoneal inoculation as compared with adult mice to inoculation by the intracerebral route. By the end of the second week mice are only slightly susceptible to intraperitoneal inoculations, and in this respect there is little difference in mice of 2 weeks of age as compared with those of 5 to 6 weeks of age.

Adult mice can be infected occasionally by intranasal inoculations of mouse

brain virus and their susceptibility to inoculation by this route appears to decrease with increasing age, but infection by this route is uncertain and irregular except in mice of 1 to 2 weeks of age.

Distribution of the virus in viscera.

In order to determine if virus was present in tissues other than central nervous system of infected mice, the following experiment was done: the kidneys, lungs, livers, spleens and brains of three 20th-passages mice (sacrificed on the 1st day of illness) were made into 10 per cent. suspensions, and groups of normal mice were inoculated intracerebrally with lightly centrifuged supernates of the suspension of each tissue. None showed any signs of infection except those which had been inoculated with the brain suspension. Apparently at the onset of illness no organ other than the brain contains demonstrable quantities of the virus.

Multiplication of virus in brain.

The following experiment was done to find out when the maximum concentration of virus was present in the brains of mice and to determine if there was any circulation of virus in the blood of mice after intracerebral inoculation. Forty-two mice were inoculated intracerebrally with a 10 per cent. Seitz-filtered suspension of 26th mouse brain passage virus. Each day, for the next 10 days, four mice were bled from the heart, exsanguinated and then their brains were removed. The blood specimens and the brains from each group of four mice were separately pooled. The sera and suspensions of the brains were titrated daily in groups of mice. Virus was present in the serum only on the 1st day after inoculation, but just as a trace, since only two of the mice showed signs of sickness after inoculation with undiluted serum. There was then no evidence of any viraemia and the virus in the blood stream was probably a spill over of the intracerebral inoculum. On the other hand, the virus content of the brain gradually rose and on the 5th day a titre of 5.5 per 0.03 ml. was obtained. On the following day, which was the 1st day on which the mice showed signs of illness, the titre of brain tissue was 6.2. The titres of virus in the pooled brains of groups of four mice which were found dead or were sacrificed as sick mice from the 7th to 10th day were:

Day	7th	8th	9th	10th
LD ₅₀	5.8	3.6	2.5	0.8 or <

The maximum virus titre was present therefore on the 1st day of signs of illness and gradually fell thereafter.

Cotton-rats (*Sigmodon hispidus hispidus*).

Mouse brain virus of the 123rd-passage was titrated intracerebrally from 10^{-2} to 10^{-6} in groups each of four cotton-rats and of six mice. The log titre of

virus of the preparation in mice was 5.1, the rats were thus inoculated with from less than 1 to 1,250 mouse intracerebral M.L.D. None of the cotton-rats showed any sign of infection. They developed no clinical illness when they were subsequently challenged intracerebrally with more than 100,000 mouse intracerebral M.L.D. of Zika mouse brain virus.

Guineapigs.

Two guineapigs (319 and 320) inoculated intracerebrally with 0.12 ml. of the lightly spun supernate of a 10 per cent. brain suspension of 3rd mouse passage virus were found dead on the 6th day after inoculation. Neither had shown any pyrexia nor other signs of infection and no virus was recovered in mice from the brains of these animals. It was not possible to repeat this experiment using 3rd-passage virus but in experiments in which guineapigs have been inoculated intracerebrally with 10th, 98th and 115th-passage virus, none of the inoculated guineapigs showed either pyrexia or other signs of infection during observation periods of up to 30 days. (No tests were made for the presence of antibody in any of these animals.) Therefore, while it is possible that before Zika virus is well adapted to mice it produces a clinical disease in guineapigs, there is no evidence of their developing any illness after inoculation with virus which has been passed intracerebrally in mice from 10 to 115 times.

Rabbits.

Four rabbits inoculated intracerebrally with 15×10^6 mouse intracerebral LD_{50} showed no signs of infection, but their sera taken 21 days after inoculation contained antibody to Zika virus.

Monkeys.

As has been mentioned (DICK et al., 1952), none of the monkeys which were employed in the isolations of Zika virus showed any physical signs of infection except Rhesus 766 which exhibited a slight pyrexia. Virus was present in the serum of the latter monkey on the 3rd day of fever (which was the only day on which a test was made). Circulating virus was demonstrated in the serum of Rhesus 758 on the 8th and 9th days after inoculation with an infected lot of *A. africanus*. (No tests of the serum of this latter monkey were made before the 8th day, and virus was not present in a sample taken on the 10th day.)

No physical signs and no pyrexia have been observed in rhesus monkeys inoculated subcutaneously with mouse brain virus but circulating virus has been present in the serum in all those which have been tested for from 4 to 5 days after inoculation (Table III).

As in the case of the monkeys employed in the isolation of Zika virus, all monkeys inoculated subcutaneously with mouse brain virus have developed neutralizing antibody within 2 to 3 weeks after infection.

TABLE III. Showing titre of virus circulating in blood of monkeys after subcutaneous inoculation with mouse brain passage Zika virus.

Species	Number	Pass No.	Inoculum Mouse i.c. LD ₅₀	Mouse i.c. LD ₅₀ of circulating virus, on day						
				1	2	3	4	5	6	7
Grivet ⁽¹⁾	860	97	>67	TR ⁽³⁾	1.0 or >	2.2	1.8	0.6	TR	0
Rhesus ⁽²⁾	961	113	10,500	TR	1.5 or >	2.6	0.5	0	0	0
Grivet ⁽¹⁾	1045	113	10,500	TR	1.5 or >	1.5 or >	TR	0	0	0

(1) *Cercopithecus aethiops centralis* Neumann.(2) *Macaca mulatta* Zimmerman.

(3) Trace.

TABLE IV. Summarising the intracerebral inoculations of monkeys with Zika mouse brain virus.

Monkey Species ⁽¹⁾	Number	Mouse Passage	Inoculum		Site of inoculation	Tests for circulating virus on days after inoculation						
			LD ₅₀	Vol. ml.		1	2	3	4	5	6	7
Grivet	733	34	1.5 x 10 ⁶	0.5	Frontal lobe	+(2)	+	+	+	+	neg ⁽³⁾	neg
Grivet	1019	97	3 x 10 ⁶ or >	0.8	Both thalami	neg	neg	neg	neg	neg ⁽⁵⁾	neg	neg
Redtail ⁽⁴⁾	1044	97	"	0.8	"	+	+	+	+	+	+	+
Rhesus	853	107	4 x 10 ⁶	1.0	Right thalamus	+	+	+	+	+	+	+
Rhesus	970	107	"	1.0	"	+	+	+	+	+	+	+

(1) See Table III.

(2) Circulating virus present.

(3) No circulating virus demonstrated.

(4) *Cercopithecus ascanius schmidti* Matschie.

(5) No test made.

Since Zika virus had been shown to be highly neurotropic in mice, its pathogenicity for monkeys by the intracerebral route was tested by the inoculations summarised in Table IV.

The sera of all the monkeys recorded in Table IV were tested for antibody before inoculation. In none except Grivet 1019 was demonstrable antibody present. The pre-inoculation serum of Grivet 1019 neutralized more than 2.5 log units of mouse brain virus. This monkey had been trapped in Uganda and had not been subjected to any previous experimental procedures.

Grivet 733 showed a slight elevation of temperature on the 1st to 4th days after inoculation, otherwise none of the monkeys recorded in Table IV showed any physical signs of infection. Grivet 1019 (the naturally immune grey monkey) showed no circulating virus as compared with two other monkeys (Grivet 733 and Redtail 1044) whose sera were tested daily after inoculation.

Antibody was present by the 14th day after inoculation in all monkeys inoculated intracerebrally.

Following the identification of the strain of virus isolated from the batch of *A. africanus* (DICK et al., 1952), the sera of all the rhesus monkeys which were being employed as sentinel monkeys at Zika were tested for Zika antibody by Dr. S. F. KITCHEN. In the tests made by KITCHEN, the serum of one of six sentinel rhesus monkeys (No. 801) was found to contain antibody. Further neutralization tests were made in 1950 with the sera of nine other rhesus monkeys which had been stationed at Zika during 1947-48. The serum of one of these (No. 777) was found to neutralize 1,000 or more LD₅₀ of virus.

In addition to these tests, neutralization tests were made with the sera of 13 grivet and 2 redtail monkeys, only the serum of one (Grivet 1019), as has been mentioned, contained antibody. The majority of the grivet monkeys were trapped on the Sese Islands and none of them was from the Zika area.

Human antibody surveys.

Since antibody had been demonstrated in the sera of captive monkeys and of a wild monkey in Uganda and since a strain of Zika virus had been isolated from *A. africanus*, it was decided to test some human sera, primarily collected for yellow fever studies, for the presence of Zika virus antibody. The results of tests of these sera are presented in Table V.

TABLE V. Showing the results of tests of human sera for Zika antibody.

Locality	Adults		Children		Total % positive
	Tested	Positive	Tested	Positive	
Zika	25	0	11	0	0
Kampala	18	0	0	—	0
Bwamba	7	2	13	2	20.0
West Nile	21	2	4	0	9.5
Totals	71	4	28	2	6.1

Only those sera which were found to neutralize 100 or more LD_{50} have been considered as positive. This may be too high a level and may be modified when further information has been collected on human immunity to this virus. However, the presence of antibody in that titre is suggestive evidence of previous experience with Zika virus or with some virus very closely related to it.

Filterability.

Suspensions of Zika mouse brain virus can be passed through Berkefeld filters of grades V, N or W with but slight loss of virus.

Using Elford's technique (ELFORD et al., 1935), the particle size of the virus is estimated to be in the region of 30 to 45 $m\mu$. This is concluded from the observation that active virus could be recovered from infected mouse brain suspensions passed through collodion filters of average pore diameter of 90 $m\mu$ and greater, but not from filtrates passed through filters of 70 $m\mu$ and less.

Stability.

Suspensions of mouse brain virus in whole normal serum have been preserved for periods up to 30 months by desiccation on a Flosdorf-Mudd manifold followed by storage at 0 to 4°C.

Virus has been demonstrated in infected mouse brains which have been stored in 50 per cent. glycerol at 0 to 4°C. for periods up to 6 months.

In order to test the susceptibility of the virus to chemicals, aliquots of the lightly spun supernate of a 20 per cent. suspension of 124th-passage virus were mixed with equal quantities of ether, with 1 per cent. potassium permanganate, 20 per cent. ethyl alcohol or 1 per cent. phenol in distilled water, and with distilled water alone as a control. These mixtures were allowed to stand at room temperature (68-78°F.) and samples were taken from each mixture after a contact time of 2, 5 and 24 hours. Each sample was further diluted 1 : 10 and inoculated intracerebrally into mice with the following results :

Mixture of 10% virus in final concentration of	Mortality ratios of mice inoculated with 10^{-1} dilutions of mixtures, after contact of hours,		
	2	5	24
50% ether	7/7	6/6	0/5
0.5% pot. permang.	0/6	0/6	0/6
10% ethyl alcohol	6/6	6/6	4/6
0.5% phenol	6/6	6/6	6/6
Distilled water (control)	6/6	6/6	6/6

It may be seen that virus (as a 10 per cent. mouse brain suspension) is destroyed rapidly by potassium permanganate in a concentration of 0.5 per cent., and that contact with ether destroys the virus within 24 hours. Active virus was

found 24 hours after contact with both 10 per cent. ethyl alcohol and 0.5 per cent. phenol.

Suspensions of mouse brain virus in buffered saline solution are rapidly inactivated at pH of under 6.2 and over 7.8 ; buffered saline suspensions of virus are most stable at pH of 6.8 to 7.4.

Thermal death point.

To determine the thermal death point, the lightly spun supernate of a 10 per cent. weight/volume suspension of infected mouse brain was distributed in 1.0 ml. quantities in thin glass ampoules. The ampoules were held at the temperatures for the times indicated in the following table, which gives the cumulative results of several experiments :

Time of exposure mins.	Mortality ratio of mice inoculated with suspensions heated at, °C.				
	50	54	58	60	70
15	11/11	12/12	1/12	0/11	0/11
30	20/21	6/12	0/12	0/22	—

It may be concluded therefore that the virus in 10 per cent. mouse brain suspensions is destroyed when infected mouse brain virus suspensions are exposed to a temperature of 58°C. for 30 minutes or 60°C. for 15 minutes.

Histopathology.

The pathological changes in mice sacrificed on the 1st day of signs of infection have been confined to the central nervous system. Various stages of infiltration and degeneration including widespread softening have been observed in the brains of some mice ; neuronal degeneration and cellular infiltration have also been found in the cords. There have been minimal inflammatory changes of the membranes or of the ependyma.

Inclusion bodies of Cowdry type A have been observed in damaged nerve cells. These intranuclear inclusions have been more obvious in pathological material from baby mice than in adult mouse brains. Some of the intranuclear eosinophilic inclusions were agglomerated into larger bodies and in general the inclusion bodies were very variable in shape and size. No inclusion bodies were seen in the brains of mice which have been sick for several days. In some of the chronically ill animals there has been very extensive round cell infiltration of the brain, and in some of them degenerative changes in the viscera have been seen. These latter changes are not considered to be virus specific changes. It should be noted that the histological picture tends further to differentiate Zika virus from some other neurotropic viruses with which it has not been compared serologically.

A more detailed report of the pathological lesions produced by Zika virus will be presented elsewhere (DICK, in preparation).

DISCUSSION

Zika virus was encountered in the course of studies which were designed to discover the vector responsible for the non-human cycle of sylvan yellow fever in Uganda (HADDOW, SMITHBURN, DICK et al., 1948 ; SMITHBURN, HADDOW and LUMSDEN, 1949). The procedure employed was to capture mosquitoes in selected localities and to inoculate suspensions of these mosquitoes into mice and rhesus monkeys. Since it seemed a waste of effort to make large scale catches in areas in which there was no evidence that yellow fever virus was present at the time the catches were being made, rhesus monkeys were established as virus sentinels. These monkeys were placed on platforms in the canopy and understory of the Semliki Forest and of the forest at Zika. At the beginning, the sentinel monkeys were caged, but when it had been shown that *A. africanus* did not readily enter the type of cage which was being used to confine the monkeys (HADDOW, SMITHBURN, DICK et al., 1948), they were secured uncaged on the tree platforms.

The temperatures of these sentinel monkeys were taken daily and blood samples were taken from any monkey showing a rise in temperature above the normal variation. These blood samples were inoculated into mice and monkeys. From one of these sentinel monkeys (Rhesus 766), the first isolation of Zika virus was made on 20th April, 1947. Rhesus 766 occupied platform No. 5, which was about one-fifth of a mile from platform No. 3. It was on this platform, No. 3, that the mosquito catch was made on 12th to 13th January, 1948 which yielded a second strain of Zika virus isolated by Dr. S. F. KITCHEN. It was also on platform No. 3 that Rhesus 801 had been secured. This monkey, it will be recalled, was one of the two sentinel monkeys which developed antibody to Zika virus while it was stationed in Zika forest. The other sentinel monkey (777) which was found to have antibody had been stationed at various sites in the forest which included platform No. 3. Both Rhesus 801 and 777 had several bouts of pyrexia while at Zika, but no virus was isolated from either of them and no signs of infection other than pyrexia were recorded. The evidence is thus suggestive that Zika virus persisted in the forest strip at Zika between April, 1947, and January, 1948. During that period the major focus of infection apparently shifted a distance of less than 300 to 400 yards, whether through the agency of indigenous monkeys or mosquitoes, it is not possible to say.

Of the 27 species of biting insects taken during the January 1948 catches (and some were represented by generous samples) (HADDOW, unpublished), only *A. africanus* was found to be infected with Zika virus. It is not known if this mosquito acts as a vector of the virus or whether the virus found in these mosquitos was being carried mechanically. Perhaps against the likelihood of *A. africanus* being the vector are the observations that (a) this mosquito does not readily enter

the type of cage which was being used to confine the sentinel monkeys (HADDOW et al., 1948) at the time when Rhesus 766 was infected, and (b) no sentinel rhesus monkey has become infected with yellow fever while confined in a cage. It is well established that *A. africanus* is the sylvan vector of yellow fever in Bwamba, Uganda, and if *A. africanus* failed to enter monkey cages and infect the monkeys with yellow fever, it seems unlikely that a caged monkey would be infected by *A. africanus* carrying Zika virus. While Rhesus 766 became infected at a time when sentinel monkeys were caged, Rhesus 801 and 777 became immunized after all the sentinel monkeys had been removed from cages and were secured by run-wires on the tree platforms. The part, if any, which *A. africanus* may play in the epidemiology of infection with Zika virus requires further investigation.

It is not known whether Zika virus produces a clinical disease or a latent infection in humans. However, it seems probable that man has contact with the virus as evidenced by the demonstration of specific antibody in high titre in 6.1 per cent. of the sera which have been tested. The absence of antibody in the samples of sera taken from the residents of Zika and from patients in Kampala as compared with the presence of antibody in samples taken from Bwamba and West Nile natives, may be due to the fact that the residents of the latter areas have more contact with the forest in which the reservoir of Zika virus may be present.

The absence of the recognition of a disease in humans caused by Zika virus does not necessarily mean that the disease is either rare or unimportant. In this respect, it may be recalled that while yellow fever is endemic among the human population of Bwamba, Uganda, only one case has ever been diagnosed there during the acute phase. (This case was only found after a careful and prolonged search (MAHAFFY, SMITHBURN, JACOBS et al., 1942).) The available information suggests that perhaps not only yellow fever but also Zika virus and some of the other viruses isolated in Uganda are at the moment well adapted to the human host.

So far only very limited immunity studies with monkey sera have been possible. The grey monkey which was found in nature to have neutralizing antibody in its serum was found to be immune to the intracerebral inoculation of Zika mouse brain virus as evidenced by its failure to circulate virus after inoculation. Zika virus would appear to be yet another example of a virus infection in which both man and monkey may be involved in the natural history of the disease. The best known and most completely studied of these viruses involving man and monkeys is yellow fever, others are Semliki Forest, Bunyamwera, Bwamba fever, Uganda S (DICK and HADDOW, to be published) and Ilhéus virus.

The available evidence indicates that Zika virus is not identical with any known virus. The limited host range, the difficulty of adaptation of this virus to mice and the inapparent infection in monkeys after inoculation with mouse-brain virus suggest a similarity to dengue virus. No serological relationship between

Zika and the Hawaii strain of dengue virus has been found. A further differentiation from dengue virus is the presence of inclusion bodies which, as far as is known, have not been described in dengue-infected mice.

The marked neurotropism of Zika virus in mice is in contrast with its lack of neurotropism in monkeys, cotton-rats, guineapigs and rabbits. The production of inapparent infection in monkeys inoculated subcutaneously with mouse brain virus as compared with the pyrexia exhibited by the naturally infected rhesus monkey (766) suggest that perhaps the unadapted virus was more pathogenic for monkeys than mouse brain passage virus.

From the physical properties of Zika virus which have been described it will be noted that the virus is a relatively unstable agent when in suspensions.

Finally it should be emphasized that the studies presented in this and the preceding paper are preliminary field studies which were carried out in Uganda. It is believed, however, that they are sufficient to establish that Zika virus is a hitherto undescribed virus probably infecting man.

SUMMARY

(1) A description is given of the adaptation to mice of two strains of Zika virus. Zika is the name of a forest area near Entebbe, Uganda, where both strains of virus were isolated. One of the strains was isolated from a pyrexial rhesus monkey which was being employed as a yellow fever sentinel and the other was obtained from a batch of *A. africanus*.

(2) The signs of infection in mice are described. While mice of all ages tested are susceptible to intracerebral inoculations with Zika mouse brain virus, mice of 2 weeks of age and over can rarely be infected by the intraperitoneal route. Mice younger than 2 weeks are highly susceptible to intraperitoneal inoculation of the virus.

(3) Zika virus is highly neurotropic in mice and no virus has been recovered from tissues other than the brains of infected mice.

(4) Cotton-rats, guineapigs and rabbits show no clinical signs of infection after intracerebral inoculation of late passage mouse brain virus.

(5) Monkeys develop an inapparent infection after subcutaneous inoculation with mouse brain virus. After intracerebral inoculation one of five monkeys showed a mild pyrexia, the others showed no signs of infection. Viraemia during the first week after inoculation has been found in all monkeys tested and antibody has been demonstrated by the 14th day after inoculation.

(6) Of 99 human sera tested, 6 (6.1 per cent.) have neutralized more than

100 LD₅₀ of virus. Antibody has also been found in the serum of one of 15 wild monkeys tested.

(7) The size of Zika virus is estimated to be in the region of 30 to 45 m μ in diameter. The virus may be preserved up to 6 months in 50 per cent. glycerol and up to 30 months after drying. It is susceptible to anaesthetic ether and the thermal death point is 58°C. for 30 minutes.

(8) Neuronal degeneration, cellular infiltration and areas of softening are present in infected mouse brains. Cowdry type A inclusion bodies have been found, particularly in the brains of young mice showing extensive lesions.

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The Society is indebted to The Rockefeller Foundation for generously defraying the cost of publishing these two papers on Zika virus.