RIFT VALLEY FEVER. ISOLATION OF THE VIRUS FROM WILD MOSQUITOES.*

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Received for publication January 19, 1948.

IN their original article announcing the discovery of Rift Valley fever virus, Daubney and Hudson (1931) expressed the opinion that the virus is transmitted by mosquitoes, and brought forth a considerable amount of circumstantial evidence to support this view. Later they (Daubney and Hudson, 1933) explored this possibility further, and found that the virus could be recovered from mosquitoes several days after an infective feeding by inocualtion of a suspension of the insects into susceptible animals. However, up to this time, the virus has not been isolated from naturally infected mosquitoes, and there has been no report of the successful transmission of the disease by the bite of naturally or experimentally infected mosquitoes.

In 1944 we were engaged in co-ordinated field and laboratory investigations on the epidemiology of yellow fever, one aspect of which was an attempt to identify the vector of that disease in primeval forest uninhabited by humans. Large numbers of mosquitoes were captured (Haddow, Gillett and Highton, 1947), and suspensions of them were inoculated into mice and monkeys (Smithburn and Haddow, 1946). Over 60 species of the insects were included in the captures (Haddow, Gillett and Highton, 1947), and some grouping of species was resorted to for the preparation and inoculation of the suspensions. During the course of this study Rift Valley fever virus was isolated 6 times from mosquitoes included in these forest catches. The present communication is an account of these isolations of the virus. A report of the successful experimental transmission of the disease by one of the groups of mosquitoes from which the virus was isolated is given in a separate paper (Smithburn and Haddow, in press).

MATERIALS AND METHODS.

The methods employed in the mosquito catches (Haddow, Gillett and Highton, 1947 ; Haddow, 1945) and in the preparation of mosquitoes for inoculation of animals (Smithburn and Haddow, 1946) have been described in detail previously and need be repeated here only briefly.

Mosquito catches.

The insects were captured at ground level in forest by a squad of about 20 experienced African youths who moved singly through the forest, each provided with a supply of glass tubes, and collected the mosquitoes in the tubes as they alighted to feed. The tubes were then lightly plugged with cotton and were transported to the field laboratory, where the mosquitoes were

sorted and identified. They were then released into separate Barraud cages by groups or species, and fed on bananás and water until such time as the whole catch was sent to Entebbe. For this journey the Barraud cages were placed in ventilated wooden cases and were covered above with layers of wet cotton.

Catches were made daily on five consecutive days (Tuesday to Saturday). The mosquitoes were sent to Entebbe on Sunday and inoculations were made on Monday. All mosquitoes with which we are here concerned were caught in uninhabited forest at Mongiro, Bwamba County, Toro District, in western Uganda. The 275-mile journey to the laboratory at Entebbe did not give rise to excessive mortality in the mosquitoes.

Inoculations.

On arrival at the laboratory at Entebbe the mosquitoes were inspected and the number of dead in each species or group was recorded. In most instances these were used along with the living, but in a few instances dead insects were discarded. The living insects were killed with chloroform and were then triturated in a sterile porcelain mortar. When the number of insects was very large, sterile powdered pyrex glass was employed to facilitate the grinding. After thorough grinding, sufficient 10 per cent normal serum in physiological saline solution (hereafter referred to as diluent) was added to make a good suspension. This was spun in the angle centrifuge for 20 minutes to one hour at about 3000 r.p.m., and the supernate was removed. A small portion of this supernate was passed through a Seitz E.K. pad, and the filtrate was used for intracerebral inoculation of a group of 6 mice. Another group of mice was inoculated with unfiltered supernate, and the remainder of both the unfiltered and Seitz-filtered portions were inoculated subcutaneously into a normal rhesus monkey.

Observations of animals.

Inoculated monkeys were observed at frequent intervals, and their temperatures were taken twice daily. Whenever a monkey had a temperature of 104° F. or higher, or when there was other reason for doing so, the animal was bled and its serum was subinoculated intracerebrally into mice. Portions of pre-inoculation serum were compared with post-inoculation sera in tests for immunity whenever virus was isolated from mosquitoes with which the monkey concerned had been inoculated.

Mice were examined daily in the forenoon, and brain passages were made from any which appeared ill. Intracerebral passage of brain is neither the optimal route nor tissue for Rift Valley fever virus, but as we were engaged in yellow fever investigations the identity of the virus with which we were dealing was not immediately discovered. Tissues of some sick and dying mice were saved for histological studies, and it was by this means that the identity of the virus was first discovered.

Isolations of the Virus.

The catch which yielded the first strains of Rift Valley fever virus was made from the 18th to the 22nd of April, 1944, and included 4310 mosquitoes, of which 3860 were sent to Entebbe. The catch included large numbers of *Aëdes*

(*Aëdimorphus*) *tarsalis* Newst. group, and *A.* (*Banksinella*) *circumluteolus* Theo., which were kept as separate species. All other mosquitoes were grouped generically and so used for inoculation. Separate groups of mice were inoculated with suspensions of *Anopheles*, *Culex*, *Taeniorhynchus*, *Eretmapodites*, *Aëdes*, *A. tarsalis* and *A. circumluteolus*. Separate normal rhesus monkeys were inoculated with all these except *Culex*. Yellow fever virus was isolated from the *Aëdes* mosquitoes (Smithburn and Haddow, 1946) and Rift Valley fever virus from the *A. tarsalis* and the *Eretmapodites*, as will be seen from the following accounts.

Strain 1.—The 2307 mosquitoes of the *A. tarsalis* group (lot 26) were used to prepare a suspension with which 6 mice and one rhesus monkey were inoculated. The mice received Seitz-filtered suspension intracerebrally, and rhesus 395 received subcutaneously 10·3 ml. of the unfiltered suspension, the total volume of which was originally 17 ml.

Two of the inoculated mice sickened, and brain passages were made from them without positive result. The other 4 mice remained well.

The rhesus, No. 395, had fever 4 days after inoculation ; it was bled in the morning and afternoon, and mice were inoculated intracerebrally with its serum on each occasion. Rhesus 509 was also inoculated subcutaneously with both specimens of serum. Both groups of subinoculated mice were well 24 hours after inoculation, but 10 of the 12 mice were dead by the morning of the second day. Subinoculations of brain were made from the 2 sick mice remaining, and two lines of virus, each readily transmissible in series, were established in mice. These were carried through 24 passage generations.

Rhesus 395 had no further fever or other signs of illness, but was subsequently found to have developed specific antibody against the virus isolated. Rhesus 509 had neither febrile nor other objective reaction to the inoculation of virus-containing serum from rhesus 395, but likewise developed antibody against the virus (Table I).

It is noteworthy that this virus strain failed to become established directly in mice following intracerebral inoculation with Seitz-filtered mosquito suspension, but did so readily after a single passage through a rhesus monkey.

Strain 2.—441 *Eretmapodites* spp. mosquitoes (lot 28) taken in the same catch as the *A. tarsalis* (lot 26) were used to prepare a suspension from the Seitz filtrate of which a group of mice was inoculated intracerebrally. Rhesus 398 received a subcutaneous inoculation of 4 ml. of unfiltered supernate representing 2/3 of the original suspension.

All the mice remained well during three days, but on the fourth day 3 were dead and 2 sick. Brain passages were made from both the latter. The second passage mice remained objectively well for two days, but were all dead on the morning of the third day. A third passage from a dead mouse was successful and gave continuity to a line of virus transmissible in series, with the survival time thereafter averaging less than 72 hours from the time of inoculation.

Rhesus 398 had no objective reaction to inoculation of the mosquito suspension. However, on the morning when the mice receiving the same inoculation first showed evidence of infection, Rhesus 398 was bled and its serum was subinoculated into mice. Two days later 2 of the mice were dead and another, which was sick, was sacrificed for subinoculation. The remaining 3 mice were dead on the third day. Subinoculation from the sick mouse gave rise to a line

of virus which was regularly transmissible in series, and which proved to be identical with the virus isolated directly in mice.

Rhesus 398 was subsequently found to have developed neutralizing antibody against this virus (Table I) following the inoculation of the *Eretmapodites* mosquitoes.

The 2 lines of this strain were carried through 19 and 17 passage generations respectively. Two lots of the virus were successfully preserved by drying while frozen ; further batches were preserved in glycerine, likewise with success.

Strain 3.—Strains 3 and 4 were isolated from mosquitoes caught between May 16 and May 20 in the same forest area as the mosquitoes from which strains 1 and 2 were isolated. No catches were made in the interval between April 22 and May 15, so that the catch which started on May 16 was the next in succession.

A suspension was prepared from 60 *Aëdes* (*Stegomyia*) *de-boeri* ssp. *de-meilloni* Edw. (lot 33) in 4·0 ml. of diluent, and intracerebral inoculations of filtered and unfiltered portions were made into adult mice, and of the Seitz filtrate into 4-day-old mice. Rhesus 396 received the remaining 2·4 ml. of unfiltered supernate subcutaneously.

Three days after these inoculations all the mice receiving unfiltered suspension were dead, though all had appeared well on the second day. Passages were made from brains of 2 dead mice, with the result that 2 lines of virus were established and carried in series through 10 generations. As with the previous strains, the survival time in mice receiving 10 per cent brain suspension intracerebrally (unfiltered or filtered) averaged less than 72 hours.

The adult mice receiving Seitz-filtered mosquito suspension remained well ; a " blind " passage made from one of them on the third day was unsuccessful. Of the infant mice receiving the Seitz filtrate intracerebrally 1 died. Two others sickened and were sacrificed for passage, but virus was not isolated from them. This failure of mice receiving the Seitz filtrate of the mosquito suspension to become sick may have been due to a low content of virus in the mosquitoes, with resultant failure of the virus to pass the filter.

Rhesus 396 had no clinical reaction as a result of the inoculation of mosquito suspension, but was subsequently found to have developed neutralizing antibody against the virus isolated in mice.

Strain 4.—942 *Eretmapodites* spp. (lot 52) of the catch of May 16 to 20 were used to prepare a suspension, filtered and unfiltered portions of which were inoculated intracerebrally into mice. No inoculation of this suspension was made into a monkey.

All the mice which received the unfiltered suspension of mosquitoes were dead 24 hours after inoculation. These deaths were believed to be due to bacterial contamination, and no passage was made.

Of the 6 mice which received the Seitz filtrate of this lot of *Eretmapodites*, 1 was dead on the first day, 3 on the third day and 1 on the sixth day. Passage was made on the third day with the brain of a mouse which appeared well. This was successful, and the virus was thereafter easily transmissible with either unfiltered brain suspension or Seitz filtrate. It was transmitted in series through 10 passages, the mean survival time from the second passage onward being less than three days.

Strain 5.—Strains 5 and 6 were isolated from mosquitoes caught May 23 to 27, 1944, in the same area as the mosquitoes which gave rise to strains 1 to 4.

This catch included 2326 *Aëdes tarsalis* group mosquitoes (lot 60). Unfiltered supernate of a suspension of these mosquitoes in 20 ml. of diluent was inoculated intracerebrally into a group of adult mice, and the Seitz filtrate of the same suspension was inoculated intracerebrally into a group of adult and a group of 3-day-old mice. The remainder of the suspension, 16 ml., was inoculated subcutaneously into Rhesus 535.

All mice receiving the unfiltered suspension were dead 24 hours after inoculation. The deaths were believed due to bacterial contamination, and no passage was made. The adult and infant mice which received the filtered suspension remained well.

Rhesus 535 had fever on the third and fourth days after inoculation with the unfiltered suspension of *Aëdes tarsalis* group, lot 60, and died on the fifth day. Death was caused, at least in part, by a huge abscess at the site of inoculation. Specimens of serum taken at the time the animal had fever on the third and fourth days were inoculated intracerebrally into groups of mice. A Seitz-filtered suspension of the liver of Rhesus 535 was inoculated intracerebrally into mice, and subcutaneously into Rhesus 542. Ten of the 12 mice inoculated with the serum of Rhesus 535 were dead within 48 hours of inoculation. Another mouse which was sick on the second day was sacrificed for passage. The twelfth mouse was dead at 96 hours. Passage from the sick mouse on the second day gave rise to a line of virus which was readily transmissible in series, the average survival time being three days or less in each of the 38 serial passages made.

Inoculation of mice with filtered and unfiltered suspension of liver taken from Rhesus 535 at autopsy also resulted in isolation of the virus from that tissue. This line of virus was not passed in series, but in pathological studies the lesions induced by it were found to be identical with those induced by the virus isolated from the serum of Rhesus 535.

Rhesus 542 had no clinical reaction as a result of inoculation with the filtered suspension of liver of Rhesus 535, but it developed neutralizing antibody in its serum, and thus confirmed the recovery of virus in mice from the serum and liver of Rhesus 535.

The failure of mice receiving the Seitz-filtered suspension of *Aëdes tarsalis* (lot 60) to sicken may again indicate a low content of virus in the mosquitoes. Had Rhesus 535 not had a large subcutaneous abscess, believed largely responsible for its fever, illness and death, this strain of virus might not have been isolated.

Strain 6.—The *Eretmapodites* spp. (lot 71) used for inoculation on May 29 included 347 mosquitoes. Unfiltered and filtered portions of the suspension of these mosquitoes were inoculated intracerebrally into mice, but no inoculation was made into a monkey. Adult mice which received the unfiltered suspension appeared well on the second day, but all were dead on the third day. Passages from the brains of two of the dead mice were successful, and the line of virus so established was carried through 44 passage generations.

The 6 adult mice which received Seitz-filtered mosquito suspension remained well. However, each of a group of three-day-old mice inoculated intracerebrally with the Seitz filtrate sickened and died, and passage from the brain of a sick mouse of this group was successful. This line was likewise transferred in series through 44 mouse generations; the mean survival time in groups receiving either of these lines of virus was never more than 2·1 days. Passage of Seitz-filtered brain from infected mice was repeatedly and invariably successful.

There were no survivors in any of the groups of mice receiving inoculations in either series.

The numbers of mosquitoes used for inoculation from the three productive catches and the animals into which they were injected are shown in Table III.

Identification of the Virus.

Histological studies.

Tissues of a sick mouse of the second passage group receiving Strain 1 virus intracerebrally were fixed in 10 per cent formol-saline, and stained sections were prepared. Despite the fact that the mice were inoculated intracerebrally, sections of the brain of this mouse showed no lesions attributable to a virus disease. The heart and lungs appeared normal, both in the gross and microscopically. The kidneys, spleen and liver exhibited moderate congestion. The liver was brownish-red in colour, normal in size and slightly less firm than normal liver. Microscopically the principal lesions involved the liver. These consisted of massive pan-lobular necrosis of hepatic cells, congestion and haemorrhage, infiltration of sinusoids with polymorphonuclears and mononuclears, " hyaline " acidophilic degeneration of portions of the cytoplasm of degenerating hepatic cells, and the appearance of acidophilic inclusion bodies in the nuclei of some degenerating parenchymal cells.

The examination of tissues of mice receiving other strains of the virns yielded similar results. Moreover, the liver of Rhesus 535 exhibited the same qualitative abnormalities, although the hepatic lesions in this animal were less intense than those in the mice examined.

The histological studies in the second passage mouse receiving Strain 1 virus gave the first information of the identity of the virus. The lesions observed very closely resembled those induced in liver tissue by Rift Valley fever virus as described by Daubney and Hudson (1931) and Findlay (1933). Immunological studies were undertaken to determine the correctness of this tentative identification.

Immunological studies.

Early in the study of these new strains of virus it was found that they are highly pathogenic for mice by intraperitoneal inoculation. It was then found that an effective mouse protection (virus-neutralization) test could be performed by the intraperitoneal injection of 0·06 ml. per mouse of a mixture of 0·5 ml. of the serum to be tested and 0·25 ml. of a 10 per cent or weaker dilution of brain or liver of an infected mouse. As the histological studies had indicated the probability that the virus was that of Rift Valley fever, a supply of specific bovine antiserum against this virus was procured.* This serum protected mice completely against virus Strain 6 in a preliminary exploratory experiment. We also had in hand the convalescent serum of a laboratory technician who suffered an attack of Rift Valley fever in New York (Dr. S. F. Kitchen's (1934) Case No. 1). By this time, " convalescent " sera were available from 5 monkeys inoculated either with mosquito suspensions or with serum or liver suspension of monkeys which received mosquito suspensions from which strains of virus were

* From J. R. Hudson, of the Veterinary Research Laboratory at Kabete, Kenya, whose generosity in supplying this is gratefully acknowledged.

isolated. A protection test was set up to test these sera, together with suitable controls, against Strain 2 virus (from *Eretmapodites*). The results of this test are shown in Table I.

TABLE I.—*Summary of Results of an Intraperitoneal Protection Test Showing that Virus Strain 2 is Rift Valley Fever Virus and that Convalescent Sera of Inoculated Monkeys Neutralize the Virus.*

Serum.	Virus dilution, log.	Fate of mice. Died.	Fate of mice. Lived.	Titre of virus.	$L.D._{50}$ of virus.
Pooled preinoculation sera of Rhesus 396 and 398	3	6	0	6,600,000	6600
	4	6	0		660
	5	4	2		66
	6	5	1		6·6
	7	4	2		0·6
	8	0	6		..
Rhesus 398, preinoculation	2	6	0		66,000
,, ,, convalescent	2	0	6	<100	,,
Rhesus 265, immune yellow fever and Semliki Forest virus*	2	6	0	..	,,
Rhesus 509, convalescent	2	0	6	<100	,,
Normal Ox 1	2	6	0	..	,,
,, ,, 2	2	6	0	..	,,
Rift Valley fever immune ox	2	0	6	<100	,,
Non-immune human	2	6	0	..	,,
Human immune Rift Valley fever	2	0	6	<100	,,

* Rhesus 265 was immune to both yellow fever and Semliki Forest (Smithburn and Haddow, 1944) viruses as a result of experimental procedures.

This experiment showed not only that convalescent sera of rhesus monkeys 398 and 509 contained specific neutralizing antibody, but, more important, that Rift Valley fever immune ox serum and the serum of a human convalescent from the disease gave complete neutralization of no less than 66,000 $L.D._{50}$ of virus. The titre of virus was determined by the method of Reed and Muench (1938), and the end-point dilution is regarded as containing one $L.D._{50}$. The identity of Strain 2 was thus proved. An experiment was then undertaken to determine whether the 6 strains are immunologically identical. Pre-inoculation and convalescent sera of monkeys receiving suspensions of mosquitoes from which Strains 1 and 2 were isolated or subinoculation of serum from a monkey infected with strain 1 were tested against 1 per cent suspensions of each of the 6 strains of virus. The results of the test are summarized in Table II.

In the foregoing cross-neutralization test no titrations of virus were done. However, in other experiments being done at this time the titres were consistently over 1 in 100,000, so it is reasonable to suppose that the test dose of each virus in this experiment was at least 1000 M.L.D. The results show that the pre-inoculation sera contained no protective antibody against any of the strains, that convalescent sera which contained neutralizing antibody against the homologous strains (1 and 2) neutralized the other 4 strains as well, and therefore that the 6 strains are identical.

TABLE II.—*Intraperitoneal Cross-protection Tests with the 6 Strains of Virus, each Tested against Preinoculation and Convalescent Sera of Monkeys Inoculated with Strains 1 and 2.*

Serum of Rhesus.			Tested against 1 per cent virus, strain.	Fate of mice.	
Number.	Time taken.	Inoculated with Strain.		Died.	Lived.
395	Pre-inoculation	1	1	6	0
398	,,	2		6	0
509	,,	1		6	0
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6
395	Pre-inoculation	1	2	6	0
398	,,	2		4	2
509	,,	1		5	1
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6
395	Pre-inoculation	1	3	6	0
398	,,	2		6	0
509	,,	1		6	0
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6
395	Pre-inoculation	1	4	6	0
398	,,	2		6	0
509	,,	1		6	0
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6
395	Pre-inoculation	1	5	6	0
398	,,	2		6	0
509	,,	1		6	0
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6
395	Pre-inoculation	1	6	6	0
398	,,	2		6	0
509	,,	1		6	0
395	Convalescent	1		0	6
398	,,	2		0	6
509	,,	1		0	6

Other studies carried out at this time, but which need not be described in detail, showed that the virus is not neutralized by immune sera against the Semliki Forest (Smithburn and Haddow, 1944), Bunyamwera (Smithburn, Haddow and Mahaffy, 1946), yellow fever or eastern or western equine encephalomyelitis viruses.

Epidemiological Investigations.

This being the first occasion on which Rift Valley fever virus was isolated from naturally infected mosquitoes, it was deemed advisable to attempt to discover—

1. Whether other mosquitoes were involved in the cycle of infection.
2. Whether humans became infected.
3. Whether wild animals were attacked by the virus.

Investigations of these points were undertaken. The results, although largely negative, nevertheless shed some light on this outbreak of Rift Valley fever.

The role of other mosquitoes.

Mosquito catches were in progress at Mongiro, Bwamba, from January to July 8, 1944. Series of catches were made on 5 consecutive days in January, February and March. Two 5-day catches were made from April 4 to 8 and 18 to 22. From May 16 until May 27 catches were made on 5 days of each week. Ten 24-hour catches were made between May 29 and July 8. No virus was isolated from the 12,557 mosquitoes caught prior to April 18, nor from the 3042 taken and used for inoculation after May 27. Each of the three catches made between April 18 and May 27 yielded strains of Rift Valley fever virus, so it can be stated with certainty that Mongiro was a focus of virus activity during that period.

Many species and genera were included in the 18,884 mosquitoes of the productive catches, and during the period of virus activity suspensions of 50 lots were inoculated into 26 different non-immune rhesus monkeys and into mice, or, in the case of 9 lots, into mice only. The numbers of each genus or species of mosquitoes from each of the productive catches, suspensions of which were inoculated into experimental animals, are shown in Table III, together with the animals into which they were inoculated.

Three of the rhesus monkeys used in these experiments died, one (No. 421) of yellow fever, one (No. 535) with sepsis and Rift Valley fever, and one (No. 525) from haemopericardium following cardiac puncture. Monkeys 395, 396, 398 and 509 became immune as a result of inoculations made in conjunction with the aforementioned isolations of virus. Sera of the other 19 monkeys inoculated with suspensions of mosquitoes during the period of virus activity at Mongiro were all tested for antibody against Rift Valley fever, the samples of blood being taken at least 35 days after the May 29 inoculations. None of the 19 monkeys had protective antibody in its serum. Further, although each generic or species lot was inoculated into mice, Rift Valley fever virus was not isolated from any of the lots other than those mentioned. It may, therefore, be concluded that no species included in the catches contained virus in demonstrable quantities other than those from which virus was isolated.

Search for immunity in humans.

Twenty-five African youths who had participated in the field work as mosquito catchers at Mongiro were bled and their sera were tested against virus strain 2. None of their sera contained neutralizing antibody.

As previously stated, Mongiro, the locality in which the virus-containing mosquitoes were captured, is an uninhabited area on the northern boundary of

TABLE III.—*Mosquitoes of the Three Productive Catches, showing Groupings used for Inoculations and Animals into which they were Injected.*

Species or generic grouping.	Catch April 18–22; inoculations April 24.		Catch May 16–20; inoculations May 22.		Catch May 23–27; inoculations May 29.	
	Number of mosquitoes.	Rhesus Number.	Number of Mosquitoes.	Rhesus Number.	Number of Mosquitoes.	Rhesus Number.
Anopheles spp.	567	441	1802	Mice only	267	Mice only
Culex spp.	209	Mice only	423	,, ,,	186	,, ,,
Eretmapodites spp.	441*	398†	942*	,, ,,	347*	,, ,,
Taeniorhynchus spp.	20	396	145	,, ,,	42	,, ,,
Aëdes (*Finlaya*) *ingrami* Edw.	..	..	24	440	14	541
A. (*Stegomyia*) *aegypti* L.	..	..	6	440	..	..
A. (*S.*) *apicoargenteus* Theo.	3	421	93	395	22	533
A. (*S.*) *fraseri* Edw.	..	..	4	440	..	..
A. (*S.*) *de-boeri* ssp. *de-meilloni* Edw.	12	421	60*	396†	33	531
A. (*S.*) *africanus* Theo.	3	421‡	133	398	19	532
A. (*S.*) *luteocephalus* Newst.	..	..	2	440	..	..
A. (*Aëdimorphus*) *capensis* Edw.	..	..	..	..	3	541
A. (*A.*) *haworthi* Edw.	1	421	16	441	9	536
A. (*A.*) *argenteopunctatus* Theo.	4	421	1	509§	7	530
A. (*A.*) *mutilus* Edw.	1	421	13	519	..	..
A. (*A.*) *domesticus* Theo.	..	..	5	440	5	541
A. (*A.*) *tarsalis* Newst. group	2307*	395†	6647	520	2326*	535‖
A. (*A.*) *abnormalis* Theo. group	9	421	..	..	..	..
A. (*A.*) *lamborni* Edw.	3	421	5	522	2	538
A. (*A.*) *cumminsi* Theo.	13	421	26	524	16	537
A. (*A.*) *natronius* Edw.	11	421	228	525	58	539
A. (*Banksinella*) *circumluteolus* Theo.	236	440	408	440	608	541
A. (*B.*) *taeniarostris* Theo.	9	421	14	527	4	540
A. (*B.*) *palpalis* Newst.	11	421	28	526	15	534
A. (*Dunnius*) sp.n.	..	..	11	440	5	541

* Rift Valley fever virus isolated from these lots.
† Monkey became immune to Rift Valley fever as result of inoculation.
‡ Yellow fever virus isolated in Rhesus 421, and this mosquito is the most probable vector.
§ Immune to Rift Valley fever at time of this injection as result of previous inoculation with virus-containing serum of rhesus 395.
‖ Animal died. Subinoculation to Rhesus 542 resulted in the latter becoming immune to Rift Valley fever.

the Semliki Forest. Adjoining the forest on the north are the Semliki Plains, which are populated. A serum-collecting programme was carried out in the plains area in August, 1944, for the purpose of a yellow fever post-vaccination immunity survey. The residues of these sera being available, 20 from adults and 20 from children under 15 years of age were tested against virus strain 2. Three of these, all from adults, gave inconclusive results, and could not be retested owing to insufficiency of serum. The other 37 contained no protective antibody.

Eighty-nine sera from as many humans residing in other localities in Bwamba, south and east of the forest, were also tested. Forty-four of these were from adults and 45 from children under 15. Two adults residing in different localities, the nearest 12 miles from Mongiro, had protective antibody in their sera, while all the others were non-immune. For comparison with these, sera of 40 adults residing at Old Entebbe were also tested, with the result that the serum of 1 was fully protective, while the others contained no antibody against Rift Valley fever virus.

From these results it may be concluded that humans were not actively, if at all, involved in the outbreak of the disease at Mongiro in 1944. This is not surprising, as the only persons much exposed to infection there were those engaged in the mosquito catches. The absence of immunity in the latter may indicate that the insect vector operative in this outbreak does not feed actively on humans when its natural hosts are present, as must have been the case at Mongiro.

Tests for immunity in wild animals.

Sera of 72 wild monkeys belonging to 9 species taken in Bwamba, 30 before, 1 during and 41 after the period of activity of the virus at Mongiro, were tested for capacity to neutralize the new strains of virus. None of these contained neutralizing antibody, although 15 of the monkeys came from the Mongiro area and 10 of these were taken during or after the virus isolations. It therefore seems likely that monkeys were little, if at all, involved in this or any recent cycles of infection with Rift Valley fever virus in the Bwamba lowlands.

Two non-immune rhesus monkeys were stationed at Mongiro as yellow fever sentinels during April and May, 1944. Neither acquired immunity to Rift Valley fever virus during this period.

Sera from a red forest buffalo (*Syncerus nanus* (Boddaert)) and a waterbuck (*Kobus defassa* ? ssp.) shot in 1946 near Mongiro were tested for immunity to Rift Valley fever. Both animals were adults and quite old enough to have been alive at the time of the virus activity in 1944, yet both sera were non-protective.

It is perhaps worthy of note that a dead buffalo was encountered in the middle of the mosquito-catching area during the first of the three catches from which virus was isolated. A sick buffalo calf was also seen daily on the road nearby for a period of about ten days, after which it disappeared. It is possible that these animals were infected with Rift Valley fever, but by the time the virus was isolated it was impossible to obtain any information on the point.

Entomological Note on Mosquitoes from which Virus was Isolated.

Rift Valley fever virus was isolated from a lot of 2307 *Aëdes* (*Aëdimorphus*) *tarsalis* Newst. group, and subsequently from another lot of 2326 of the same group. At Mongiro this group consists of roughly equal numbers of *A.* (*A.*)

tarsalis Newst. and *A.* (*A.*) *albocephalus* Theo., the females of which cannot be distinguished reliably in life. There is therefore no possibility of telling from which of these species the virus was isolated, or whether both may have been involved.

Virus was also isolated from a lot of 60 *Stegomyia*, belonging to a species which at the moment we must refer to as *A.* (*S.*) *de-boeri* ssp. *de-meilloni* Edw., though final determination is still impossible, for the following reasons: In 1942 we found in Bwamba a *Stegomyia* sp., which was referable either to *A.* (*S.*) *de-boeri* ssp. *de-meilloni* or to *A.* (*S.*) *dendrophilus* Edw. The larvae resembled those of *dendrophilus*, but the male terminalia resembled those of *de-meilloni*. This species was found simultaneously, by Dr. P. C. C. Garnham and Mr. J. O. Harper, in Kenya. The material was studied by Mrs. van Someren (1946), who described the specimens in order to record their considerable range of individual variation. She concluded that perhaps we were dealing with a new racial form of *de-boeri*, but considered it inadvisable, at that stage, to form a final conclusion. Subsequently she obtained in Kenya further specimens of *de-meilloni*, in which the larva was typical (personal communication). The conclusion was thus that we were dealing with a new race or subspecies of *de-boeri*. In the meantime, however, we had examined the terminalia of the type male of *A. dendrophilus* at the British Museum. We found that Edwards had overlooked an important point—the presence in this species of two small spines on the basal plaque, previously believed to be diagnostic of *A. de-boeri* and *A. de-boeri* ssp. *de-meilloni*. The Bwamba mosquito thus resembles *A. dendrophilus* in the larval stage, and the male terminalia are exceedingly similar. The remaining differences are in adult coloration, and may be bridged by the great range of individual variation known to exist. Further, the type locality for *dendrophilus* is Oblogo, Gold Coast, so that we may be dealing with a new subspecies of *A. dendrophilus*. It is not possible to settle this matter finally, but it is here pointed out that it may subsequently be necessary to assign all Bwamba material so far recorded as *A. de-boeri* ssp. *de-meilloni* to *A. dendrophilus*, *A. dendrophilus* new subspecies, or even perhaps to a completely new species.

Virus was isolated three times from mixed lots of *Eretmapodites* ssp., as follows:

(*a*) From 441 mosquitoes, including 324 *E. chrysogaster* Graham group, 93 *E. inornatus* Newst. group, 22 *E. ferox* sp. n. (Haddow, 1946) and 2 *E. leucopus* ssp. *productus* Edw.

(*b*) From 942 mosquitoes, including 713 *E. chrysogaster* group, 106 *E. inornatus* group, 121 *E. ferox* and 2 *E. leucopus* ssp. *productus*.

(*c*) From 347 mosquitoes. The original lot totalled 482, including 367 *E. chrysogaster* group, 58 *E. inornatus* group, and 57 *E. ferox*, but 135 which were found dead on arrival at the laboratory were discarded without being reclassified.

It is necessary to discuss the two groupings used here. In Bwamba the *E. inornatus* group consists of approximately equal numbers of *E. inornatus* Newst. and *E. penicillatus* Edw. We have found (Haddow, 1936) that the only reliable method of distinguishing the females of these species is by examining the fore-claws under a high power of the microscope—a method which cannot be used while the mosquitoes are alive. The *E. chrysogaster* group is more complex.

We have found that the three common species in Bwamba are *E. chrysogaster* Graham, *E. semisimplicipes* Edw., and *E. grahami* Edw., and that the females cannot be reliably distinguished (Haddow, 1936). More recent studies by Mrs. van Someren have, however, revealed the presence of *E. intermedius* Edw. and four hitherto unknown species—easily identifiable by study of the male terminalia, but without reliable characters for the differentiation of females. Some, indeed, are still known only as males (van Someren, 1948). We are thus unable to say how many of the eight Bwamba species belonging to this group were included in the infected lots, nor can we hope to separate these species in future field or laboratory work of the same nature. In the case of the *E. inornatus* group we may safely assume that both species were present in all the infected lots, though we are unable to give the exact numbers of each.

The fact that the virus was isolated from relatively small lots of *Eretmapodites* spp. in three successive catches, that virus isolated from these mosquitoes had a short incubation in the initial passages in animals, and that it was readily recovered from Seitz filtrates of the mosquito suspensions, seems to indicate that these mosquitoes contained an abundance of virus and that, if the disease is insect-borne, this genus probably includes at least one vector. Though twice isolated from the *A. tarsalis* group, the virus was absent from the 6647 mosquitoes of this species in the second catch. Moreover, the failure to recover virus from Seitz-filtered suspensions of *A. tarsalis* group seems to indicate that these mosquitoes contained relatively little virus. The occurrence of the virus in *A. tarsalis* group mosquitoes may well have been casual, as they occur in vast numbers at Mongiro, forming by far the commonest group in the area. The failure of Seitz-filtered suspension of *A. de-boeri* ssp. *de-meilloni* lot 33 to yield virus indicates that these mosquitoes also probably contained little virus. Further, this species was taken in all three catches, yet only those of the second catch yielded virus. It therefore appears that this species may also be of less importance as a vector than one or other of the *Eretmapodites* spp.

Catches made at ground level and in the forest canopy at Mongiro and elsewhere, subsequent to the isolations of Rift Valley fever virus, indicate that each of the species concerned bites almost exclusively at ground level. This fact probably accounts for the absence of immunity among the wild monkeys of Bwamba.

Discussion.

In their studies on the transmission of Rift Valley fever, Daubney and Hudson (1933) noted that a lamb inoculated with a suspension of five wild-caught *Taeniorhynchus fuscopennatus* was immune to this disease after the inoculation. The virus was not isolated, but the inference is that the mosquitoes were infected. These authors also showed that sheep which are protected from mosquito bites are likewise prevented from contracting the disease under natural conditions, and that protection from mosquitoes at night alone reduced the hazard of infection significantly.

The six isolations of virus here reported from mosquitoes caught in a relatively circumscribed area over a period of 39 days is evidence enough of virus activity of epidemic proportions. However, the outbreak with which we are here concerned is very different from outbreaks studied by the workers in Kenya. Whereas the outbreaks in Kenya involved humans, sheep and cattle, the outbreak in

the Semliki Forest at Mongiro could not have involved any of these, as the area is uninhabited. It must have involved wild animals, yet the only suggestion of evidence obtained on this point is the observance of a dead buffalo and a sick buffalo calf in the catching area at the time. Immunity surveys of the mosquito catchers and of residents of the nearby Semliki Plains were negative, as were surveys carried out in wild monkeys of the Bwamba lowlands. Daubney and Hudson (1933) found the buffalo to be susceptible to experimental inoculation with Rift Valley fever virus. These animals are numerous in the Mongiro area, as are buck of several species, giant forest hogs and Uganda red hogs, elephants and other wild animals. It seems probable that we were dealing with the mosquito phase of an epizootic " in the wild " involving animals of species which remain unknown.

The isolation of a virus from mosquitoes is not positive proof that those insects are actively involved in cycles of transmission. Indeed, in the experience at Mongiro, it seems unlikely that *Aëdes de-boeri* and *Aëdes tarsalis* were vectors, since neither of these species was infected on every occasion, and the evidence indicates that neither contained virus in large quantity. The situation was rather different, however, with reference to the *Eretmapodites* spp. These mosquitoes contained Rift Valley fever virus in each of three successive 5-day catches made during the 39-day period, and the indications were that they contained the virus in relatively large amounts in each instance. We assumed, therefore, that if the mosquitoes implicated included a vector species, it must be one of the genus *Eretmapodites* included in the catches. The soundness of this assumption was borne out by the successful experimental transmission of the infection with *E. chrysogaster* group, as will be shown in a separate communication (Smithburn and Haddow, in the press).

SUMMARY.

A filterable virus with hepatotropic affinities, shown by immunological methods to be Rift Valley fever virus, was isolated from six different lots of mosquitoes caught in the uninhabited Semliki Forest in western Uganda in 1944. The mosquitoes involved included at least six species of the genus *Eretmapodites* and three of the genus *Aëdes*. Indications were that the latter may have been only casually infected, and that the local vector, if any was included in the species implicated, was one of the genus *Eretmapodites*.

Epidemiological studies pertaining to the infection are reported from which it is concluded that the outbreak did not involve humans, but probably affected some unidentified species of wild animals.

The studies and observations on which this paper is based were conducted with the support and under the auspices of the Medical Department of the Uganda Protectorate and the International Health Division of The Rockefeller Foundation.

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BODILY REACTIONS TO TRAUMA. THE EFFECT OF ISCHAEMIA ON MUSCLE PROTEIN.

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Received for publication January 20, 1948.

OUR previous work on the effect of ischaemia on the thromboplastic activity of extracts prepared from muscle (Stoner and Green, 1947) led us to consider the possible changes in the muscle proteins which might be produced by that form of trauma. In the previous paper we discussed the possibility that the increased thromboplastic activity of the ischaemic muscle extracts might be due to the liberation of the active material from an intracellular store of inactive "combined thromboplastin" possibly as a result of the action of proteolytic enzymes. Cullumbine and Rydon (1946) have suggested a similar mechanism for the liberation of leukotaxine. Subsequent work on the thromboplastic activity of muscle extracts has provided little, if any, evidence in favour of such an hypothesis, but led us to a study of proteolysis in ischaemic muscle. Special attention has been paid to possible differences between ischaemic and autolytic muscle since previous work (Stoner and Green, 1947) has shown that their behaviour is not always the same.

It was first necessary to find the solubility of the muscle proteins in salt solution, and the method described by Deuticke (1930, 1932) was used. In confirmation of the results of Erdós (1943), the decrease in the solubility of muscle proteins after a period of 5 hours autolysis was found to be very slight. The same period of ischaemia produced a similar slight change in solubility.

Maver, Johnson and Voegtlin (1935) have shown that under conditions of low oxygen tension and acid reaction, i.e. under conditions which will be present in ischaemic and autolytic muscle, proteolysis occurs. Our first step in investigating this possibility was to determine the acid soluble nitrogen content of the muscle after periods of autolysis and ischaemia, since Bradley (1938) considers this test to be the best indication of primary cleavage of protein. However, no changes were found during the short periods studied, and it was felt that this method was too crude for our purpose. Accordingly we determined the amino acid content of the muscle with the results to be described.