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RIFT VALLEY FEVER VIRUS IN MICE.
I. GENERAL FEATURES OF THE INFECTION

C. A. MIMS

From the East African Virus Research Institute, Entebbe, Uganda

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RIFT Valley Fever (R.V.F.) virus was first isolated during an epizootic involving sheep and cattle in the Rift Valley, Kenya, in 1931 (Daubney and Hudson, 1931). The virus was shown to kill lambs and mice within a day or two, producing massive hepatic necrosis of which the histological picture (Daubney and Hudson, 1931; Findlay, 1933) was similar to that of yellow fever. The host range and pathology was studied, and infection in man recorded (Findlay, 1931). Subsequent workers described the neuro-adaptation of the virus (Mackenzie and Findlay, 1936; Smithburn, 1949; Kitchen, 1950), its growth in tissue culture and embryonated eggs (Mackenzie, 1933; Saddington, 1934), its oncolytic action (Takemori, 1954), and its transmission by, and isolation from, mosquitoes of the genus *Eretmapodites* (Smithburn, Haddow and Gillett, 1948; Smithburn, Haddow and Lumsden, 1949). More recently another outbreak in cattle in South Africa was reported (Gear, de Meillon, Measroch and Davis, 1951), where infections in man also occurred (Joubert, Ferguson and Gear, 1951; Mundel and Gear, 1951).

Findlay and MacCallum (1937) drew attention to similarities between R.V.F. and yellow fever viruses. Interference, but not cross-immunity (Findlay, 1931), has been demonstrated, and both viruses have been neuro-adapted and are closely similar in host range, pathology, size and clinical picture in man. R.V.F. virus however, unlike yellow fever, is viscerotropic in mice when inoculated by the intracerebral or any other route, and is so rapidly and regularly lethal that infectivity titrations are remarkably clear-cut and reproducible. This virus-host system was considered worthy of investigation because a yellow fever-like viscerotropic virus can be studied in a highly and uniformly susceptible yet comparatively cheap and plentiful laboratory animal. The system is also suited to the quantitative studies described in later papers.

MATERIALS AND METHODS

Virus.—A strain isolated in mice from pooled *Eretmapodites* mosquitoes in Bwamba County, Western Uganda (Smithburn *et al.*, 1948) was used, which had had about 90 mouse

passages. Seed virus for experiments was obtained in the form of pooled serum from sick or newly dead mice. Brains were not harvested, for not only is the titre in brain lower than in blood, but neurotropic strains of virus may thus be selected (Smithburn, 1949).

Mice.—The mice used were of Swiss stock, their ancestors originating from Carworth Farms, New York. Unless otherwise stated, mice were used when 4–6 weeks old and inoculation was intracerebral.

Infectivity titrations

Serial $10^{0.5}$ fold (or sometimes tenfold) dilutions of infective material were made and each dilution inoculated (0·03 ml.) intracerebrally to 5 or 6 mice, which were not as a rule randomised. The diluting medium was 0·5 per cent bovine plasma albumin in pH 7·4 Sørensen's buffer. Mice were observed for 5 days, and end-points then calculated from deaths by the method of Reed and Muench (1938). Deaths which occurred on the first day were not included in calculations. Organs and carcasses were made up as 10 per cent suspensions in standard diluent and the suspension considered as a 10^{-1} dilution in titrations, so that an organ titre is expressed in LD_{50} per 0·03 ml. of solid tissue. All titres are expressed in log 10 LD_{50}/0·03 ml. unless otherwise stated. Antibiotics, when used, were added so that each ml. of inoculum contained 1000 units of soluble penicillin and 1 mg. streptomycin. It was found to be important not to add too much antibiotic to the inoculum, because mice given as much as a few hundred units of penicillin or several tenths of a milligram of streptomycin alone in saline intracerebrally, died within 24 hr. This was the routine titration method used during the work described in this and the three subsequent papers of this series.

Neutralisation tests

Neutralisation tests were done intraperitoneally in adult mice, and from 10^2–10^3 intraperitoneal LD_{50} were used. Sera, including the normal mouse serum added to virus in the control titration, were inactivated for 30 min. at 56° before testing. Serum-virus mixtures remained at room temperature for 15 min. before inoculation.

Virus storage.—A fresh infective serum pool was titrated, and stored in aliquots in rubber-stoppered test-tubes at − 20°. At intervals a tube was removed and the serum titrated. As shown in Table I, the titre remained steady for at least a month ; small amounts of virus were still present after eight months. Such stock serum was used for experiments as a virus seed of known titre.

TABLE I.—*Stability of Rift Valley Fever Virus at* −20°.

	Days.							
	0.	1.	3.	5.	10.	15.	33.	69.
Titre	7·8	7·9	7·7	7·7	7·4	8·0	7·4	5·0

RESULTS

Age of mice, inoculation route and susceptibility

A given stock infective serum was titrated intraperitoneally, intracerebrally and subcutaneously in 4-day, 4-week, and 8-week-old mice. There was (Table II)

TABLE II.—*The Titre of a Given Infective Serum according to the Age of the Mice used in the Titration and the Route by which they were Inoculated.*

	Mouse age.		
	4 days.	4 weeks.	8 weeks.
Intracerebral	8·5	8·3	8·3
Intraperitoneal	8·2	7·5	7·4
Subcutaneous	8·0	7·3	7·1

Titres for 4-day mice, which received only 0·01 ml., have been multiplied by three.

some fall in susceptibility to extraneural inoculation in older mice, but the intracerebral route gave titres approximately independent of age. The result was confirmed in a subsequent experiment.

Another stock serum was titrated intracerebrally and intravenously in 5-week-old mice (6 mice per 0·5 log $_{10}$ dilution) and the end-points found to be $10^{8\cdot8}$ and $10^{8\cdot9}$ respectively. Thus, adult mice are equally susceptible to intravenously and to intracerebrally injected virus, although they show a diminished susceptibility to intraperitoneal and subcutaneous injection. Moreover, when 10 mice were given 10 LD_{50} intracerebrally, and another 10 mice given the same inoculum intravenously, incubation periods in the two groups were identical. In another experiment, a very small virus inoculum was given intravenously to some mice, and the identical inoculum intracerebrally to others. The difference between blood and brain titres of individual mice which died was virtually the same (Table III) whether the inoculum had been given by the intravenous or the intracerebral route. There was thus no more virus present in the brain after an intracerebral inoculation. Calculations from the blood content of mouse brain as determined by Kaliss and Pressman (1950) show that the virus content of the brain can approximately be accounted for by the contained blood. Thus there is no evidence that viscerotropic virus grows in the brain to any appreciable extent, except in the exceptional circumstances described in Paper IV of this series. It is known (Cairns, 1950) that an intracerebral inoculation, apart from the inevitable damage to the brain, is in effect the same as an intravenous one, and inoculated material has begun to spill over into the neck veins of mice after as little as five seconds. R.V.F. virus is primarily viscerotropic in mice, and intracerebral inoculation is merely a convenient method of putting virus into the blood stream.

TABLE III.—*Brain and Blood Titres in Individual Mice Given the Same Inoculum either Intracerebrally or Intravenously.*

Route of inoculation.	Death time in hours.	Blood titre.	Brain titre.	Blood-brain titre difference.
Intracerebral	48	8·5	7·0	1·5
,,	46	>8·5	7·7	>0·8
,,	53	>8·5	6·7	>1·8
,,	86	7·8	6·3	1·5
,,	110	8·0	6·2	1·8
Intravenous	45	8·4	6·4	2·0
,,	45	>8·5	7·4	>1·1
,,	50	7·7	6·2	1·5

Titration reproducibility.—Serum from each of 5 virus-sick mice was titrated four times intracerebrally with separate sets of dilutions (6 adult mice per group, and $10^{0\cdot5}$-fold dilutions). Results in Table IV show that the maximum titre variation between mice was $10^{0\cdot7}$ and that between different titrations of a given mouse blood $10^{0\cdot5}$. Variations in titre of more than $10^{0\cdot5}$ are thus unlikely to be due to titration inaccuracies. A more accurate estimate of titration reproducibility was made when Lauffler and Miller (1944) determined the standard deviation for influenza infectivity titrations in mice, using their data, and those of Horsfall (1939). Five mice were inoculated with each tenfold dilution of virus, and it was shown that when end-points were calculated from a combination of the extent of lung consolidation and deaths, the standard deviation was $10^{0\cdot26}$. Titration

differences of $10^{0.73}$ would therefore be significant in 19 cases out of 20. Similar standard deviations ($10^{0.26}$ and $10^{0.23}$) were obtained by Knight (1944) and von Magnus (1951), when influenza virus was titrated in chick embryos, inoculating five embryos with each tenfold dilution of virus.

TABLE IV.—*The Reproducibility of R.V.F. Virus Intracerebral Titrations in Mice.*

Mouse.*	Log $_{10}$ LD_{50}/0·03 ml.			
1	8·4	8·2	8·4	8·4
2	8·8	8·7	8·6	8·7
3	8·5	8·7	8·2	8·6
4	8·2	8·7	8·5	8·5
5	8·8	8·9	8·7	8·6

* Each mouse had received an identical inoculum.

The distribution of virus in the mouse

Blood.—Pooled blood was obtained from 3 mice sick after R.V.F. virus inoculation and titrated. It contained $10^{9.3}$ LD_{50}/0·03 ml. The plasma was separated and titrated ($10^{9.7}$) and the cells washed four times in normal saline and then titrated ($10^{8.3}$). The greater part of virus in the blood is thus associated with the plasma, although the cells seem to contain more than can be accounted for by contaminating plasma. It was then shown that the age of the mouse does not affect the peak blood titre attained. Mice 2, 4, 6, 8 and 10 weeks old were given the same inoculum and when sick the blood of each mouse was titrated. Since titres were low (Table V), " incomplete "virus had no doubt been produced and it is shown in Paper IV that mice may vary considerably in peak infective titre when this happens. In spite of this, the scatter in the mean titres between age groups is small, suggesting that the final blood titre is about the same for mice between the ages of 2 and 10 weeks.

TABLE V.—*Peak Blood Virus Titres in Mice of Varying Age, following Identical Inocula.*

	Age of mice (weeks).				
	2.	4.	6.	8.	10.
Titres in individual mice	7·9	7·0	7·2	7·2	7·3
	7·9	7·5	7·6	7·2	7·6
	7·4	6·8		7·5	7·2
Mean titre	7·7	7·1	7·4	7·3	7·4

Organs.—In several experiments it was discovered that neither kidney, liver, brain, spleen nor lung had higher titres than the blood. Liver titres were almost as high as serum titres, and it is shown in Paper IV of this series that under exceptional circumstances they may even be higher than blood titres, but brain titres, for instance, were 10–100-fold lower. Moreover titres in the liver, kidney and spleen were even lower when, after mice had been heparinised and sacrificed, warm saline was flushed through the portal vein or aorta until the emerging fluid was clear. It would be extremely difficult to wash out all the blood from an organ, or even to remove the same amount each time. The efficiency of removal would

inevitably vary on different occasions, and very small traces of such high titre blood left behind might appreciably affect the organ titre. How, then, could one distinguish between the virus content of an organ and the virus content of the blood in the organ ? It was concluded that growth curves for virus in different organs might be very difficult to construct and interpret, and, instead, a study was made of the occurrence of virus in various body secretions and excretions, and in embryos.

Urine.—In preliminary experiments urine obtained from virus-sick mice by suprapubic puncture contained virus, killing all inoculated mice in 2–5 days. There were no deaths in mice inoculated with normal urine. Subsequently, in case the needle puncture had enabled virus from blood to enter the urine, urine was expressed by abdominal pressure and, after the addition of antibiotics, inoculated into mice. There were no deaths in two of these experiments with pooled urine obtained with no-needle puncture, and in the remaining three never more than an occasional mouse died. It was concluded that there was little if any virus in the urine. This was surprising, since normal urine probably contains a few red cells and a little protein and, with such enormous blood virus titres, some spill-over into the urine seemed inevitable. A little infective mouse serum together with antibiotics was therefore added to normal mouse urine and the same amount of the same serum to Sørensen-buffered bovine albumin. After 1½ hr. incubation at 37·5° it was found that the virus in urine had a titre 10,000-fold lower than the control. Virus is thus unstable in mouse urine at body temperature. Urea is known to inactivate yellow fever (Höring, 1939), as well as other viruses (Tyrrell and Horsfall, 1954 ; Lauffler, Wheatley and Robinson, 1949) and in addition stability is influenced by pH. An experiment was therefore carried out, in which the same amount of an infective serum was added to the same volume of various Sørensen-buffers, and to pH 7·0 Sørensen-buffered bovine albumin, saline, and 0·3 and 3 per cent urea solutions. After incubations for 3 hr. at 37·5°, each was titrated. It seems (Table VI) that the virus is unstable when the pH is less than 7, and at pH 6 the titre falls even after 30 min. Virus, however, was equally stable for 3 hr. in Sørensen's buffer alone, or in bovine albumin, saline and even urea as long as the pH was between 7 and 8. Numerous determinations with paper indicators (B.D.H.) showed that normal mouse urine usually has a pH of from 4 to 6, and is only rarely as high as 7. Any virus escaping into such urine would therefore be inactivated fairly rapidly.

TABLE VI.—*Effect of Urea and pH on Virus Stability at* 37·5°.

					pH 7·0 buffered.			
pH 6·0 (½ hr.).	pH 6·0 (3 hr.).	pH 6·5.	pH 7·2.	pH 8·0.	0·75 per cent NaCl.	0·5 per cent bovine albumin.	0·3 per cent urea.	3·0 per cent urea.
4·3	<0*	3·8	4·8	5·0	5·4	5·4	5·4	5·5

* *i.e.*, 0·03 ml. of undiluted liquid did not kill inoculated mice.

Milk.—Two newly-delivered mothers were inoculated with virus and became sick 37 hr. later ; several of their infants' stomachs were then emulsified and lightly centrifuged to obtain milk, and the supernatant inoculated with antibiotics into mice. A few of these infants were placed with new mothers but they did not die,

nor did any mice inoculated with stomach contents. Since infants had been observed to take milk shortly before or just after their mothers became sick it was concluded that little if any virus appears in the milk.

Nasal mucosa.—The nasal mucosa was tested because Francis and Magill (1935) isolated R.V.F. virus from the nasal mucosa of infected laboratory workers. Nasal washings were taken from four newly dead infected mice by introducing into the nostrils 1·0 ml. of 0·5 per cent bovine plasma albumin in saline, and collecting the fluid as it came into the mouth. The washings were in each case quite clear, and, after centrifugation, the deposit was examined for red blood corpuscles. The supernatant, after the addition of antibiotics, was titrated. Nasal washings from three of the mice tested contained an occasional lymphocyte and epithelial cell, but no red blood corpuscles ; the titres of these washings were $10^{2\cdot6}$, $10^{1\cdot8}$ and $10^{3\cdot0}$. Washings from the fourth mouse contained a few red blood corpuscles and had a titre of $10^{5\cdot0}$.

Epistaxis is common in R.V.F.-infected mice, but it appears that appreciable amounts of virus are present on the nasal mucosa when there is no microscopical evidence of haemorrhage. In the one case where a small haemorrhage had occurred titres were higher still.

Embryos.—In preliminary experiments where pregnant mice 3–4 days from term were inoculated with virus, embryos removed at the time of sickness contained virus. These embryos were not washed, and, with such high blood titres, it was thought that they might become contaminated with virus as they were removed through the uterine incisions. In subsequent experiments, two or three embryos were pooled, washed thoroughly (6 or 8 times) in normal saline before being emulsified, and mothers' blood was titrated at the time of sacrifice. Results (Table VII) show that virus is present irregularly, and in any case in low titres, in embryos. Abortions were not observed to occur. To ensure that virus was capable

TABLE VII.—*The Presence of R.V.F. Virus in the Embryos of Sick Infected Pregnant Mice.*

Pregnant mice.	Maternal blood titre.	Mice inoculated with pooled embryo suspensions.
Two at 3–4 days from term	—	SSSSSS
	—	SSSSSS
One, 16 days pregnant	—	335SSS
Four at 3–4 days from term	>8·0	123SSS
	>8·0	222333
	7·0	3SSSSS
	7·6	5SSSSS
Three at 3–4 days from term	7·4	SSSSSSSSSSS
	7·5	3SSSSSSSSSS
	6·6	33SSSSSSSSS
Two, 12 days pregnant	>8·0	22333
	7·1	335SS
Two at 3–4 days from term	8·5	Embryo titre 2·2
	7·7	,, ,, >2·0

Each S or numeral indicates survival or the day of death respectively of an inoculated mouse.

of growing in embryos, laparotomies were performed under ether anaesthesia on two pregnant mice 3 or 4 days from term, and in each case two embryos given small virus inocula through the uterine wall. Twenty-nine hr. later the mothers were sacrificed and their blood as well as the inoculated embryos (washed and emulsified as before) titrated. Maternal titres were $10^{2\cdot2}$ and $10^{4\cdot8}$ $LD_{50}/0\cdot03$ ml., but both embryo titres were greater than $10^{5\cdot0}$. It is concluded that virus had multiplied in the embryos, and the maternal titre is accounted for by multiplication in the mother following the inevitable small leakage of virus from the embryos along inoculation tracks.

Bile.—Since normal mouse bile kills intracerebrally, even when diluted 1 in 6, the pooled bile from 6 virus-sick mice was diluted tenfold and inoculated intravenously (0·1–0·2 ml.) to 6 mice. Three died virus-deaths, and seitz-filtrates of liver suspensions killed inoculated mice in 2–3 days ; the rest survived. Thus only small amounts of virus are present in bile, such as might even have come from the blood through the puncture hole made in the gall bladder wall. To see whether bile inactivates virus, the same amount of virus was added to normal mouse bile and to the usual diluent, and incubated at 37·5° for $1\frac{1}{4}$ hr. The virus in bile was then found to have a titre one-hundredth as high as the control.

Sickness and death in mice.

Virus-sick mice adopt a hunched position, do not move much and may be hyperirritable, squeaking readily and giving small vertical hops when disturbed. They sit apart, instead of bunched together as usually. Breathing becomes more easily visible, and perhaps laboured, but stays at the usual rate of from 150–250 a minute. Later, mice frequently become hyperactive, and leap about in the box for a minute or two before the convulsive movements and the few gasping breaths which end in death. The interval between the onset of sickness and death is always very short. It never lasts more than 2 or 3 hr., and when very large inocula are given mice die after less than 30 min. sickness (see Paper III).

The relation between the virus dose and the survival time is described and analysed in Paper III of this series. Most deaths occur within 2–3 days of inoculation.

In two experiments it was shown that the response to inoculation is either death or survival with no immunity. Many mice were given 3–4 limiting dilution inoculations of stock infective sera intracerebrally at 10–20-day intervals. None of the few survivors, when bled a month after the last inoculation, had neutralising antibodies. There would therefore appear to be no intermediate responses where infected mice survive and become immune. The result of the experiment was the same, whether low or high titre serum was used for the inoculations, although low titre infective sera are shown in Paper IV to contain " incomplete " virus. In Paper IV it is also shown that a very exceptional type of inoculum may occasionally result in survival with immunity.

Rectal temperatures were determined for 34 normal mice under light ether anaesthesia, and in addition hourly determinations made on 5 mice during the course of the daylight hours. The mean value was 35·1° with a scatter of from 34–37°, and there were no particular diurnal fluctuations. Temperatures were then taken after virus inoculation, and it was noted not only that sick mice do not develop pyrexia, but that there may even be a fall to temperatures as low as

32–33°, as mice become sick. The same thermometer was used for all the temperature measurements.

Hypopyrexia in mice following the inoculation of bacterial antigens has been described by Anderson and Broderson (1948), and by Zahl and Hunter (1944). It may be that this is the usual response to antigens, but in R.V.F. infection, the fall in body temperature is at least partly a result of general hypoactivity, and the tendency of mice to sit apart from one another when sick.

Clinical pathology

There was no change in haemoglobin (Sahli) in sick mice, and pooled urine from 10 sick or newly dead mice contained some albumin (as determined by boiling), but no visible blood, no bilirubin (iodine test) and no bile salts (Hay's test). There was an amorphous sediment with a few epithelial cells but no blood cells.

Blood sugar.—If hypoglycaemia occurs in sick mice it is not a primary or only cause of sickness and death, for intravenous glucose did not noticeably affect sick mice, who died over the same time interval as did controls.

Clotting time.—While sick mice were being bled during routine virus harvesting it was noticed that blood did not clot readily, and a more detailed study of the phenomenon was therefore made. The clotting times of blood from ten normal mice were determined by taking blood from the tail into a capillary tube, and breaking off lengths of tube until a stringy clot was seen between the broken ends. The normal tail blood clotting times were all found to be 4 min. or less when this method was used. Times were then determined for 6 virus-sick mice. Values were very much greater than normal, ranging from 10 to more than 70 min., and in one case blood never clotted. It was thought possible that a large amount of virus in the blood might by its very presence interfere with blood coagulation. Therefore 2 mice were given $10^{9\cdot8}$ LD_{50} of virus intravenously, so that their blood titres were straight away greater than $10^{8\cdot0}$ $LD_{50}/0{\cdot}03$ ml., but clotting times remained normal during 6 hr. of observation. Abnormalities in blood coagulation thus do not appear except in association with virus growth, and determinations were made on a number of mice as they became sick 50–80 hr. after the inoculation of virus. The increase in clotting time did not occur until just before or just after sickness was first detectable. There was normal clotting when normal mouse blood was mixed with three times the volume of blood from virus-sick mice. Thus the defect is caused by a shortage of something rather than by the presence of a coagulation inhibitor. Further investigations into the nature of this clotting defect are in progress.

Pathology

Important features were described by Daubney and Hudson (1931), and Findlay (1931, 1933), and a few further observations on haemorrhagic phenomena are recorded here. These phenomena were of variable occurrence and usually took place late in infection when mice were already sick. Epistaxis, which was often agonal, might occur in 4 of 6 mice in a single box, or not at all in 50 boxes. Haemorrhages into the gut occurred occasionally although they were never seen above the jejunum, and bright red blood was sometimes passed rectally. Haemoperitoneum also occurred with no obvious site of bleeding. Haemorrhagic areas in the lungs were common, and of 50 mice which had died virus-deaths, 18 had haemorrhagic

spots and blotches visible on the lung surfaces, commonly at the bases. Five mice had lungs of normal appearance, but the lungs of all the others, including those with haemorrhages, were congested with a surface appearance varying from a fine mottled pink to a deep red.

DISCUSSION

Mice dying of R.V.F. virus infection have extremely high blood titres. Levels of $10^{8 \cdot 5}$ LD_{50}/0·03 ml. (or about 10^{10} LD_{50}/ml.) are among the highest attained in tissues infected with any plant or animal virus. Nevertheless little or no virus appears in the urine, milk or bile and only to a small extent in the embryo. It is possible that there is a true excretion of virus on to the nasal mucosa, although it is difficult to be quite sure that a minute haemorrhage had not occurred, in spite of the absence of red blood corpuscles from the nasal washings.

The virtual absence of virus from urine and bile is explained by the inactivation of any virus which reaches these fluids. The action of bile is probably due to contained bile salts. Smith (1939) made a study of the susceptibility of viruses to bile salts ; it seems probable (from more recent work) that although arthropod-borne viruses are susceptible, viruses which infect by the gastro-intestinal route are relatively resistant to the action of bile.

Virus isolated from embryos may well have resulted from minute blood leakages, since haemorrhagic phenomena are common in infected mice. Haemorrhages do not occur until infection is well advanced in the mother, and blood titres are high. A small leakage of virus across the placental barrier would be adsorbed by susceptible embryo cells in the usual way (see Paper II), but before any noticeable multiplication of virus in the embryo the mother would be dead. Findlay (1931) showed that washed mouse embryos from sick mothers contained virus, in that the embryo emulsion killed all of four mice inoculated, but the matter was not pursued further. Particular attention has been paid here to embryos, because abortions in sheep and cattle were a marked feature of the original outbreak in the Rift Valley. Although aborted foetuses were not then tested for the presence of virus, virus has on occasions been isolated from the organs of aborted bovine foetuses by workers at the Kabete Veterinary Research Laboratory, Kenya, during the course of routine diagnosis, but no titrations were made (Dr. Piercy, personal communication).

It is conceivable that virus could enter and infect the embryo and thereby produce an abortion before the mother died, but it is unlikely in view of the results obtained here with mice. It seems more likely that a severe febrile illness together with haemorrhagic phenomena in a cow or ewe is enough to produce an abortion of a non-specific nature.

Blood virus titres of lethal magnitude are produced straightaway after massive intravenous injections of fresh infective blood or serum, but mice are unaffected for many hours (see also Paper III). There is thus no evidence that virus particles are themselves toxic, or that toxic substances are produced in the blood. It is tempting to attribute sickness and death to some consequence of the extensive liver damage which occurs in infected mice. Following experimental hepatectomy, hypoglycaemia is the cause of death, but if R.V.F.-sick mice are hypoglycaemic their condition is unaffected by intravenous glucose. Hypoglycaemia, if it occurs in mice, is therefore not a sole or primary cause of sickness. The comparison with a

hepatectomised animal is in any case not strictly justified, because the damaged liver remains *in situ* in infected mice. Yellow fever-sick monkeys have been shown to have low blood sugars in the terminal stages of infection (Wakeman and Morrell, 1931), but here, too, the administration of glucose intravenously did not affect their condition. Moreover, there were no clinical effects when equally low blood sugars were produced by insulin in a normal monkey.

The coagulation defect in infected mice is the probable cause of the haemorrhagic phenomena. The defect and the haemorrhages occur when mice are sick, and tissue and organ damage as a result of virus growth is maximal. The extensively damaged liver might result in a lowered prothrombin or fibrinogen concentration. This is known to occur in man following acute liver injury, and, indeed, defects in blood coagulation have been reported in men sick with yellow fever (Vellard and Vianne, 1929): the more severe the defect the commoner were the haemorrhagic phenomena. Wakeman and Morrell (1932) reported coagulation defects in yellow fever-sick monkeys, together with lowered plasma fibrinogen levels. Smithburn (1949) noticed prolonged clotting times in yellow fever-infected galagos. Further work is in progress on the relation between the haemorrhagic events and sickness and death.

It has been shown that adult mice are susceptible to extraneurally as well as to intracerebrally inoculated R.V.F. virus, and that viraemia and death rapidly ensue. Blood titres are higher than organ titres, and there is no evidence that this viscerotropic strain of virus grows in the brain to any appreciable extent, even after intracerebral inoculation. These features, together with the clotting defect, the haemorrhagic events, the histopathology in infected livers, and the general similarities between the two viruses, all suggest that the R.V.F.-infected mouse may be used as a convenient laboratory model for the yellow fever-infected man or monkey.

SUMMARY

A titration method for Rift Valley Fever virus in mice is described and its reproducibility estimated. Blood virus titres as high as 10^{10} LD_{50}/ml. occur in virus-sick mice, and the titre of the parenchyma of vascular organs is thus difficult to determine. Adult mice are as susceptible to intravenous as they are to intracerebral virus, and brain titres are lower than blood titres. The evidence indicates that this virus is viscerotropic in mice, even when inoculated intracerebrally.

Little or no virus is detectable in urine, perhaps because it is inactivated at the pH of normal mouse urine. Only small amounts of virus were detected in bile, and bile inactivates virus. Virus is virtually absent from the milk of infected mice. It is possible that there is a true excretion of virus on to the nasal mucosa. The embryos of infected pregnant mice contain irregular but small quantities of virus, which could have passed the placental barrier at the site of a small haemorrhage.

Sickness in infected mice is described. Not only is there no fever, but there may be a fall in body temperature. Deaths occur within 2–3 days, except when very small inocula are given. The interval between the onset of sickness and death is at the most 2–3 hr., and is much shorter than this when very large inocula are given. Inoculated mice either die, or survive and remain susceptible to infection.

The clotting time is greatly prolonged as mice become sick. This clotting defect is associated with virus multiplication, and is a probable cause of the haemorrhagic phenomena.

It is suggested that the R.V.F.-infected mouse can be used as a model for the yellow fever-infected man or monkey.

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