

THE SUSCEPTIBILITY OF MARMOSETS TO YELLOW FEVER VIRUS

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PLATE 16

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Previous studies at this laboratory have demonstrated a relative susceptibility to yellow fever virus in the Capuchin (1, 2), "spider," "woolly," and "squirrel" (3) monkeys of Brazil. In the present paper it is proposed to discuss transmission experiments with certain more primitive genera, the members of which are usually called "marmosets" in English and "saguins" in Portuguese. These are very small and rather delicate monkeys, not particularly suitable for laboratory experimentation. It has been our experience that the mortality is rather high among recently captured specimens, but that the survivors which become accustomed to captivity can be used after a few weeks with less likelihood of deaths from unexplained causes during the course of the experiments.

Stokes, Bauer, and Hudson (4) inoculated marmosets with yellow fever virus in Africa. Apparently, the lesions in their Marmoset 63 were similar to those found by us in *C. albicollis*. They say in regard to their experiment: "Although there was no obvious other cause of death, the lesions in this animal did not furnish sufficient evidence for the conclusion that it had died of yellow fever infection."

Genus *Callithrix*. True Marmosets. *C. albicollis* (Spix)

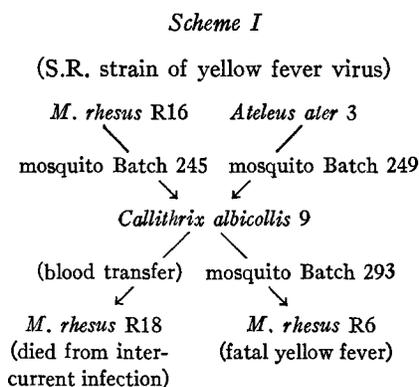
Callithrix albicollis is found in great numbers in the environs of Bahia. Twenty of this species were used in the experiments here reported.

It was found comparatively easy to pass yellow fever virus through these marmosets and back to *rhesus* monkeys, either by blood transfers

<i>Callithrix</i> 17	12/26/29	Asibi	Mosquito bites Batches 275 and 276	<i>Callithrix</i> 4 <i>Leontocebus</i> 1	— 101.9 101.8 101.9 102.5 102.9 103.7 102.9 102.6 103.5 103.2 102.0	101.6 102.0 102.2 103.0 103.5 102.7 103.4 103.0 103.5 97.9	Died on 15th day, cause unknown	
<i>Callithrix</i> 18	12/30/29	Asibi	Injection of blood	<i>Callithrix</i> 17	— 101.8 103.5 103.2 103.3 103.6 103.7 103.6 103.9 104.0 104.0	103.1 102.2 103.0 103.4 103.5 103.6 103.8 103.9 104.0 104.0	Recovered	
<i>Callithrix</i> 19	1/ 3/30	Asibi	Injection of blood	<i>Callithrix</i> 18	101.6 102.0 101.2 101.9 102.2 102.4 102.0 101.7 102.5	101.9 101.9 101.8 102.0 102.1 102.2 101.9 102.1 99.7	Killed when mori- bund. Ulcer of stomach	
<i>Callithrix</i> 20	1/ 8/30	Asibi	Injection of blood	<i>Callithrix</i> 19	101.9 102.2 102.0 104.0	102.1 102.0 105.5	94.0	Killed when mori- bund (yellow fever?)
<i>Leontocebus</i> 1	11/ 2/29	Asibi	Injection of liver emul- sion	<i>M. rhesus</i> R12	— 101.5 103.0 104.5 103.9 103.8 104.6 104.0 103.6 102.0	102.2 103.6 104.0 103.7 103.6 104.2 103.8 102.9	D	
<i>Leontocebus</i> 2	1/29/30	Asibi	Mosquito bites Batch 324	<i>M. rhesus</i> R11	— 101.9 102.7 103.9 103.9 103.8 103.7 103.6 104.0 104.0 103.7 102.9	102.1 103.5 104.4 103.6 103.6 103.4 103.9 104.8 103.9 103.5	Died on 13th day (yellow fever?)	
<i>Leontocebus</i> 3	2/ 1/30	Asibi	Injection of blood	<i>Leontocebus</i> 2	102.0 102.9 103.7 103.8 101.8	103.1 103.5 103.6 103.7	D	Died as result of bleeding?
<i>Leontocebus</i> 4	2/ 4/30	Asibi	Injection of blood	<i>Leontocebus</i> 3	— 101.9 104.6 104.7 104.1 104.2 104.5 104.0 103.6 102.7 102.1 101.9	102.8 104.8 104.4 104.5 104.0 104.2 103.9 103.1 102.2 102.4	Killed when mori- bund on 11th day (yellow fever?)	
<i>Leontocebus</i> 5	2/ 5/30	Asibi	Injection of blood	<i>Leontocebus</i> 4	— 100.9 103.0 104.2 103.9 104.0 104.2 104.1 104.0 104.1 102.2	102.2 103.9 104.0 104.1 104.0 104.0 103.9 104.2 103.9	D	Died (yellow fever?)

or by mosquito feeding. However, after the virus was carried through a series of marmosets, it appeared to decrease in virulence for *M. rhesus*. This was not the case when the virus was passed through certain other species of Brazilian monkeys. A very few of the marmosets showed a definite fever (104°F., or above). Most of them died while under observation, but the lesions found at necropsy did not resemble those of yellow fever as seen in humans, in the *rhesus* monkey, and in certain other species of monkeys.

Experiment I.—On October 29, 1929, Marmoset 4 was inoculated intraperitoneally with 2.5 cc. of citrated blood, containing Asibi strain virus, from *M. rhesus*



R1. The animal's initial temperature was 101.7°. On November 1 the temperature reached 104°, blood was transferred to *M. rhesus* R2, and mosquito Batch 275 was allowed to engorge. Marmoset 4 was found dead on November 4. There was a little dried blood about the nose and mouth. Jaundice was doubtful. The lungs showed a patchy congestion with, perhaps, a trace of bronchopneumonia. The liver had a slate-colored surface, but on section appeared yellow and unmistakably fatty; the organ was friable. The spleen was enlarged; its pulp was soft and its follicles were small. Kidney sections were opaque, with slight bile-staining and injection of the vessels. The gastro-intestinal tract was negative. Microscopically the liver showed degeneration, mainly midzonal, including a few necrotic cells. Fat was present in large amount, with the heaviest deposition in the midzones. A few leucocytes were noted. *M. rhesus* R2 died on the fifth day with yellow fever. Mosquito Batch 275 fed on *M. rhesus* R3 on November 21. This animal also died on the fifth day with typical yellow fever.

Experiment II.—On November 12, 1929, Marmoset 7 was fed upon by mosquito Batch 248, infected with Asibi strain virus. The initial temperature was 101.6°.

There was no subsequent fever and no significant rise in temperature (see table). However, on November 16, 1.5 cc. of blood was taken from the heart and injected into *M. rhesus* R4, which died on the sixth day with typical yellow fever.

Experiment III.—Marmoset 8 was fed upon November 12 by mosquito Batch 245, infected with the S.R. strain of virus. On the fourth day (November 16) blood was withdrawn for injection into *M. rhesus* R5. This monkey died on the fifth day with typical yellow fever. Marmoset 8 showed no significant rise in temperature at any time.

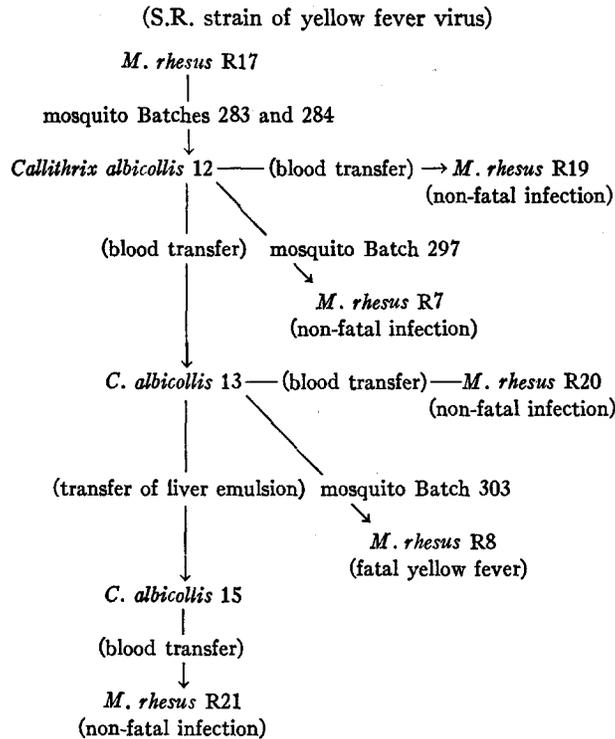
Experiment IV.—(Scheme I.) On November 22, 1929, Marmoset 9 was fed upon by mosquito Batches 245 and 249, infected with the S.R. strain of virus. The initial temperature was 101.7°. On November 26 the temperature in the afternoon was 102.8°. The animal was bled, and mosquito Batch 293 was allowed to engorge. On the following morning more blood was taken, and the monkey died soon thereafter. At necropsy the centers of the liver lobules showed as red dots surrounded by zones of an orange-yellow color; the organ was friable. The gastric mucosa showed a few dark streaks which appeared to be changed blood. The other organs revealed nothing of note. Microscopically, the lobules of the liver were seen to have a certain amount of midzonal degeneration, with deposition of fat, and perhaps a few necrotic cells. There was congestion, and many leucocytes were observed (mainly polymorphonuclear, occurring in small nests). The kidneys showed congestion and a slight cloudy swelling. On December 16 mosquito Batch 293 fed on *M. rhesus* R6, which died on the fifth day with typical yellow fever.

Experiment V.—(Scheme II.) This experiment consisted in the infection of Marmoset 12 by mosquitoes carrying the S.R. strain of virus, passage of the virus to Marmoset 13 by the injection of blood taken from Marmoset 12, and to Marmoset 15 by injection of emulsion of liver from Marmoset 13. Blood transfers to *rhesus* monkeys from Marmosets 12, 13, and 15 all produced definite infection, with fever and subsequent immunity to reinoculation, but no deaths. Mosquito transfer from Marmoset 12 to *M. rhesus* R7 was also non-fatal. However, mosquito Batch 303, infected on Marmoset 13, induced by its bites a fatal infection in *M. rhesus* R8. None of the marmosets had a definite fever (see table), and at necropsy none of them showed lesions suggestive of yellow fever.

Experiment VI.—(Scheme III.) In this experiment virus was passed to Marmoset 17 by mosquitoes, themselves infected on marmosets, and from Marmoset 17 through Marmosets 18 and 19 to Marmoset 20 by direct transfer of blood, taken in each case on the fourth day following inoculation. Blood from Marmoset 17 and the bites of mosquitoes fed on this animal caused fatal infections in *rhesus* monkeys. Blood virus from Marmoset 18 produced a fatal infection in *M. rhesus* R9. Blood from Marmosets 19 and 20 caused non-fatal infections in *M. rhesus* 10 and 11, and the bites of mosquito Batch 326, fed on Marmoset 20, produced non-fatal infections in two *M. rhesus*. Apparently the virus became attenuated for the *rhesus* monkeys by repeated passage through the marmosets. The last animal

in the series, Marmoset 20, had a clinical course suggestive of yellow fever. The initial temperature on January 8, 1930, was about 102°. On January 10 the temperature suddenly rose to 105.5°, at which time mosquitoes were fed and transfer of blood was made. On the afternoon of January 11 the temperature dropped to 94°, and the animal was killed. At necropsy there was a suggestion of icterus in the tarsal plates. The liver was a pale yellow; a small roundworm was found coiled up in one lobe. The other organs were essentially negative.

Scheme II



Microscopical examination showed the liver to be nearly one half necrotic. The necrosis was primarily central, but extended into the midzones. There was a heavy leucocytic invasion. The kidneys showed a very slight cloudy swelling.

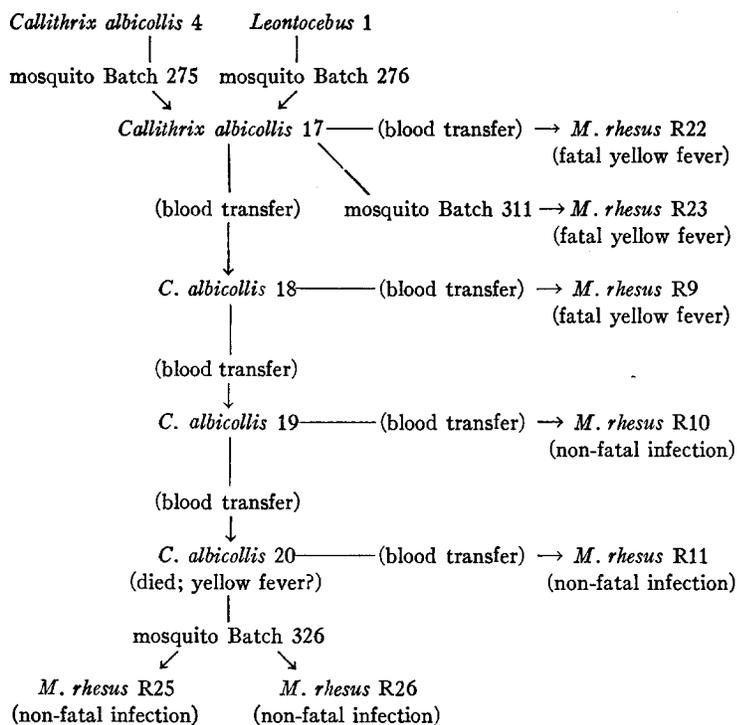
Genus *Leontocebus*. Tamarins. *Leontocebus ursulus* (Humboldt)

The little black tamarin, or marmoset *Leontocebus ursulus*, comes from Para. Elliot (5) calls it *Cercopithecus ursulus*. We were able

to secure five specimens for experimental work. These animals proved to be more susceptible to yellow fever virus than *C. albicollis*. Four of the five developed fever; all five died, two of them showing marked liver necrosis (Fig. 1), and two others the most intense jaundice that we have ever seen in monkeys.

Scheme III

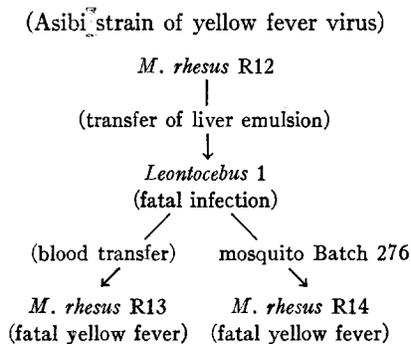
(Asibi strain of yellow fever virus)



Experiment VII.—(Scheme IV.) On November 2, 1929, *L. ursulus* 1 was inoculated subcutaneously with 1.5 cc. of liver emulsion, containing Asibi strain virus, from *M. rhesus* R12. The initial temperature was 101.5°. On November 4 the temperature rose to 104.5°, at which time blood was transferred to *M. rhesus* R13 and mosquito Batch 276 was allowed to engorge. *M. rhesus* R13 died on the sixth day with typical yellow fever. Mosquito Batch 276 later induced a fatal attack of yellow fever in *M. rhesus* R14. *L. ursulus* 1 was found dead on No-

ember 11. Icterus was marked in the tarsal plates and in the intima of the aorta. A small abscess was found in the anterior abdominal wall at the point of injection of liver emulsion. The liver was rather friable, with sharp edges; the peritoneal surface was reddish brown, with purple discolorations in places. The cut surface was also reddish brown, with indistinct lobular markings. The spleen was swollen, with rounded edges, and was congested and purplish in color. The other organs did not appear abnormal in the gross. Microscopically, the liver showed a severe, but irregular, necrosis. In the midzones frequent islands and strands of cells were left intact. Necrosis sometimes extended to the central veins. Near the capsule several areas were noted where necrosis was complete and digestion advanced.

Scheme IV

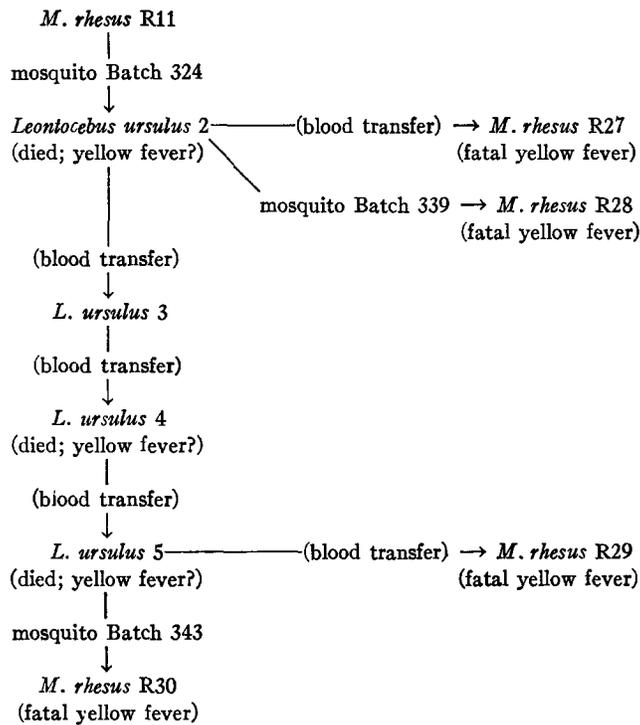


Experiment VIII.—(Scheme V.) On January 29, 1930, mosquito Batch 324, infected with Asibi strain virus, fed upon *L. ursulus* 2. The strain was passed in succession through *L. ursulus* 2, 3, 4, and 5 and back to *M. rhesus* from the last marmoset by blood transfer and by mosquito bites, producing fatal infections. Transfers from *L. ursulus* 2 also induced fatal infections in *rhesus* monkeys; no transmission to *M. rhesus* was attempted from *L. ursulus* 3 and 4. The temperature reactions are given in the table. Although intense icterus was noted at autopsy in *L. ursulus* 4 and 5, the most remarkable lesions were found in *L. ursulus* 2. This animal had fever on February 1 and again on February 5 and 6. It died on February 11. Marked icterus was noted in the tarsal plates, in the intima of the aorta, and even in the skin. The liver was friable and of a curiously mottled yellow, orange, and brown (in general, lighter than normal). The spleen appeared to be swollen. The kidneys were pale and bile-stained. The stomach contained food mixed with a considerable amount of changed blood. Microscopically, the liver showed over 80 per cent necrosis, essentially midzonal, with rings of cells, relatively unaffected (except for fat), around the vessels. Occasional nests of intact cells were also found in the midzones. The necrotic cells showed very

eosinophilic cytoplasm and nuclei in various stages of degeneration and fragmentation. There was congestion of vessels, generalized pigmentation, and deposition of fat (especially in cells around the central veins). Round-cell infiltration and hyperplasia of epithelium of the bile-ducts was noted in the portal spaces. The kidneys showed slight cloudy swelling and injection of the glomeruli.

Scheme V

(Asibi strain of yellow fever virus)



DISCUSSION

The experiments here presented show that yellow fever virus can be introduced into the marmosets, *Callithrix albicollis* and *Leontocebus ursulus*, by mosquito bites, that it can be passed through at least four animals and can be taken out again by mosquitoes which subsequently are able to transmit the infection to *rhesus* monkeys. There is some evidence that in such a series of passages through *C. albicollis*

the virus becomes somewhat attenuated for *M. rhesus*. Apparently no change in the virus takes place in passage through *L. ursulus*.

From five *C. albicollis*, numbers 3, 5, 7, 8, and 18, convalescent serum was used in protection tests against Asibi strain virus. Protection was secured in every instance with these convalescent sera, but not with serum from a normal marmoset. Numbers 7, 8, and 18 are mentioned in the protocols. Numbers 3 and 5 were inoculated with blood containing virus but neither showed a febrile reaction; no attempt was made to transfer the virus from them to other monkeys.

The lesions found at necropsy in *C. albicollis* did not closely resemble those found in human beings or *rhesus* monkeys that had died of yellow fever. On the other hand, the liver lesions in two of the five *L. ursulus* were very extensive and comparable to the usual midzonal necrosis of susceptible species.

The fact that the virus of yellow fever can be passed through marmosets with comparative ease may have some importance in the epidemiology of the disease in nature. These little monkeys are frequently kept as household pets, and even the wild ones are often found on uncleared land within urban limits. It is conceivable that they might pick up yellow fever from domestic sources, carry it to outlying districts, and aid in its wide dissemination. Such a course of events seems the more possible since we know that various "wild" species of mosquitoes are fairly efficient vectors of the virus.

SUMMARY AND CONCLUSIONS

1. It has been possible to introduce yellow fever virus into the small Brazilian monkeys, *Callithrix albicollis* and *Leontocebus ursulus*, by the bites of infected mosquitoes and to carry the virus through a series of four passages in each species and back to *rhesus* monkeys by the bites of *Stegomyia* mosquitoes fed on the last marmoset of each series.

2. Five specimens of *L. ursulus* were used. Four developed fever, and all died during the experiments. At least two showed liver necroses comparable to those found in human beings and *rhesus* monkeys that died of yellow fever.

3. Twenty specimens of *C. albicollis* were used. Very few showed a

temperature reaction following the introduction of virus. Of those that died, none had lesions typical of yellow fever as seen in certain other species of monkeys and in humans.

4. The convalescent serum from each of five *C. albicollis* protected a *rhesus* monkey against yellow fever virus, but the serum from a normal marmoset of the same species was found to be non-protective.

REFERENCES

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2. Davis, N. C., *Amer. Jour. Hygiene*, 1930, **11**, 321.
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EXPLANATION OF PLATE 16

FIG. 1. Section of the liver of a black marmoset which died of yellow fever. The tissue shows marked necrosis. ($\times 320$.)

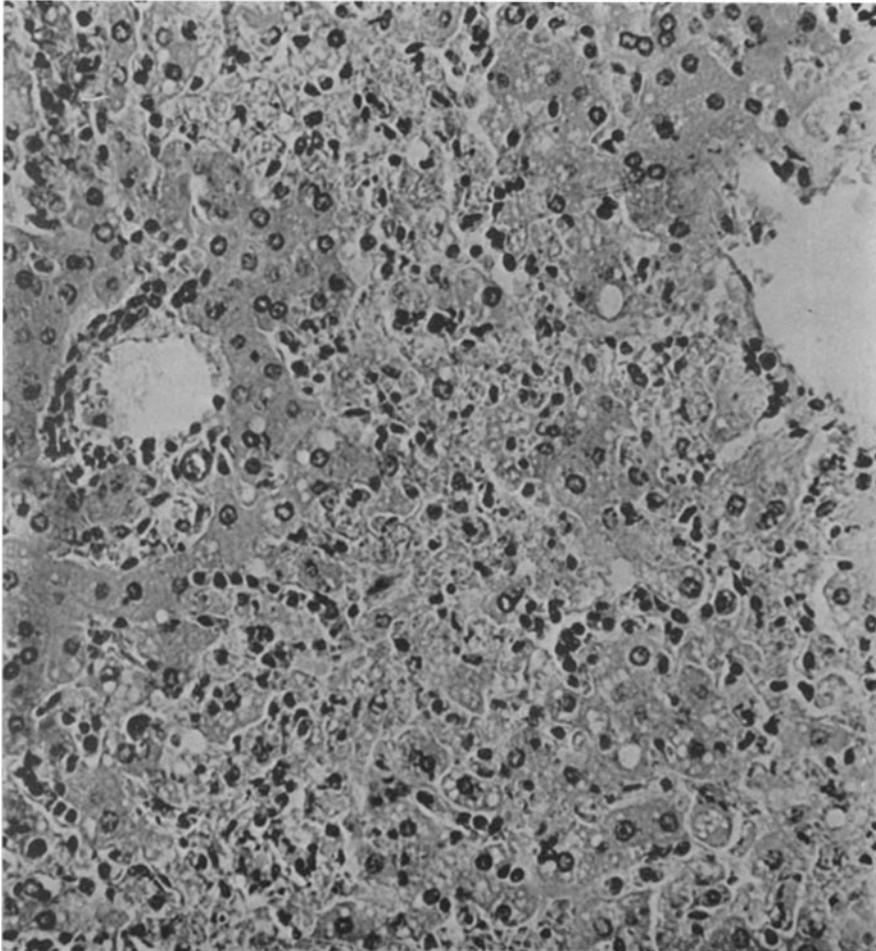


FIG. 1

(Davis: Susceptibility to yellow fever virus)