STUDIES ON SOUTH AMERICAN YELLOW FEVER.*

I. The Strains of Virus in Use at the Yellow Fever Laboratory in Bahia, Brazil.

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Up to the time of writing, the middle of January, 1929, there have been five strains of yellow fever virus studied in *Macacus rhesus* monkeys at the laboratory in Bahia. One of these strains came from Rio de Janeiro, three were picked up from local cases of yellow fever, and one was first established in Africa.

In order that references to these viruses in future publications may be more comprehensible, it is our purpose to state briefly how we came into possession of the strains.

F. W. Strain.—This strain was received from Rio de Janeiro through the kindness of Dr. Aragão. The human patient, F. W., was a Hungarian, male, 16 years old. He proved to have a mild attack of the disease. Blood was taken approximately 48 hours after onset. The virus had gone through four passages before being given to us.

Early in September, 1928, Dr. J. H. Bauer brought to Bahia blood and tissues from fifth and sixth passage monkeys which he had inoculated in Rio de Janeiro. He also brought mosquitoes which had fed on these monkeys. Among the animals inoculated in Bahia after his arrival, two showed temperature curves suspiciously like those of mild yellow fever. However, direct transfers from these animals were negative; mosquitoes fed on one were definitely proved non-infective and those fed on the second were doubtfully infective.

Previous to Dr. Bauer's return to Bahia, blood and tissues had been received by airplane. At the time it was thought that the results of inoculating this material were negative. One animal showed a temperature of 104.8° on the fourth day, but after lancing a superficial abscess at the point of inoculation the fever dropped. However, at the height of the fever a batch (No. 6) of *Aedes ægypti* was fed on

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TABLE I.

Fatal Infections of Monkeys after Inoculations with Brazilian Yellow Fever Virus.

Strain	*M. rhesus* No.	Temperatures, a.m. and p.m.											Remarks
		Day of inocul.	1	2	3	4	5	6	7	8	9	10	
B. B.	6	—	103.8	103.0	103.8	104.9	101.9						Previously used animal. Inoculated with 3 cc. liver emulsion from *rhesus* No. 180
		103.2	103.4	103.6	102.3	105.3	Dead						
"	8	103.4	103.6	102.6	104.4								Previously used animal. Inoculated with 4 cc. blood mixture from *rhesus* Nos. 171, 173, 180
		103.7	102.2	103.8	101.9	Dead							
"	12	—	103.1	102.0	102.9	103.8							Previously used animal. Inoculated with 3 cc. liver emulsion from *rhesus* No. 180
		102.9	103.2	102.5	104.7	104.0	Dead						
"	14	103.0	103.1	103.7	103.0								Previously used animal. Inoculated with 4 cc. blood mixture from *rhesus* Nos. 171, 173, 180. No fever, but autopsy lesions typical
		103.4	102.6	103.9	102.3	Dead							
"	23	103.4	102.9	103.2	102.6								Previously used animal. Inoculated with 4 cc. blood mixture from *rhesus* Nos. 171, 173, 180. No fever, but autopsy lesions typical
		103.6	103.0	103.2	102.6	Dead							
"	24	—	102.4	102.7	103.9	96.8							Previously used animal. Inoculated with 3 cc. blood-liver mixture from *rhesus* Nos. 86, 188, 192. Sacrificed when moribund
		103.0	101.9	102.4	105.1	Dead							
"	47	—	104.4	103.9	104.9								Previously used animal. Inoculated with 3 cc. blood-liver mixture from *rhesus* Nos. 86, 188, 192. Sacrificed when moribund
		102.9	104.3	103.3	103.8	Dead							
"	51	—	103.7	103.4	103.9	103.9	95.7						Previously used animal. Inoculated with 3 cc. blood-liver mixture from *rhesus* No. 141. Sacrificed when moribund
		103.4	103.2	102.4	104.0	104.3	Dead						
"	72	—	103.0	104.7	105.0	103.0							Previously used animal. Inoculated with 2.5 cc. citrated blood from *rhesus* No. 166. Sacrificed on evening of 4th day, when moribund
		103.7	103.2	105.0	104.5	98.5							

"	86	—	102.9	103.4	105.0	103.6						Previously used animal. Inoculated with 2.5 cc. citrated blood from *rhesus* No. 166. Sacrificed on evening of 4th day, when moribund
		103.6	103.1	104.4	104.4	94.2						
"	111	—	103.6	103.5	103.2	103.0	105.0	104.8	104.5	101.7		New animal. Inoculated with 5 cc. citrated blood from *rhesus* No. 107. Sacrificed when moribund
		104.9	103.5	103.7	103.4	103.6	105.2	104.6	104.9	95.0		
"	124	—	103.4	103.8	103.4	103.6	104.5	102.9	102.0			New animal. Inoculated 3 times with a total of 20 cc. from 4 animals
		104.7	103.7	104.4	104.4	104.6	103.4	102.6	102.2	Dead		
"	126	—	102.9	103.8	104.6	106.2	99.6					New animal. Inoculated with 8 cc. citrated blood from 4 animals. Sacrificed when moribund
		—	103.9	104.8	104.9	105.6	98.4					
"	128	—	102.1	104.0	102.3	102.4	104.7	102.8				New animal. Inoculated with 9 cc. citrated blood mixture from *rhesus* Nos. 124 and 125
		102.9	103.8	102.1	105.3	104.8	105.4	Dead				
S. R.	133	102.8	104.4	103.9	103.6	104.4	93.0					New animal. Inoculated with 7 cc. blood from *rhesus* No. 116 (1st passage from S. R.). Sacrificed when moribund
		103.8	103.9	103.0	103.9	106.0						
B. B.	136	—	102.9	103.2	104.5	104.9	101.2					New animal. Inoculated with 4 cc. citrated blood from *rhesus* No. 128
		103.7	102.8	103.5	103.9	105.0	Dead					
"	144	—	103.9	103.4	103.8	103.9						New animal. Inoculated with 8 cc. liver emulsion from *rhesus* Nos. 136 and 137
		103.9	103.7	103.9	103.5	105.3	Dead					
"	155	—	103.6	104.6	105.3	105.0	102.9	102.9				New animal. Inoculated with 8 cc. blood from *rhesus* No. 144. Autopsy showed tuberculosis also
		103.1	103.4	104.2	106.0	104.2	103.9	102.0	Dead			
"	158	102.0	102.8	102.9	103.4	102.8	102.3					Previously used aninal. Inoculated with 3 cc. blood-liver mixture from *rhesus* Nos. 24, 47, 197
		102.5	102.7	103.0	102.7	105.3	102.7	Dead				
"	170	103.9	103.2	104.0	104.2	104.5	104.6					New animal. Inoculated with 10 cc. liver emulsion from *rhesus* No. 161. Liver frozen 10 days. Animal sacrificed when moribund on 5th day
		103.4	103.0	104.4	105.0	106.4	97.2					

Note: Animals 111 to 155, inclusive, were infected earlier chronologically than animals 6 to 86; apparently, the virus was more active when used in the later experiments.

Animals infected through mosquito transmission will be considered in a separate paper.

TABLE I.—*Continued.*

Strain	*M. rhesus* No.	Temperatures, a.m. and p.m.											Remarks
		Day of inocul.	1	2	3	4	5	6	7	8	9	10	
B. B.	180	—	103.6	105.4	104.6								New animal. Inoculated with 2.5 cc. citrated blood from *rhesus* No. 166. Sacrificed. Not moribund, but would not have lived until following day
		102.9	103.8	104.5	102.4								
"	188	103.6	102.9	103.4	104.2	103.9							New animal. Inoculated with 4 cc. blood mixture from *rhesus* Nos. 171, 173, 180
		102.7	102.5	104.5	105.3	103.6	Dead						
"	192	—	102.8	102.6	104.6	105.4							New animal. Inoculated with 2 cc. blood-liver mixture from *rhesus* Nos. 170, 180
		102.4	103.8	102.2	106.2	105.6	Dead						

this monkey. These mosquitoes, together with batch No. 7, fed on a sixth passage animal in Rio de Janeiro, were allowed to feed on *rhesus* No. 52 on Sept. 17. The following day the temperature of this animal reached 106.1°F. and it passed 104°F. daily for five days. On the seventh day the monkey was killed. The liver showed some fat and a thin scattering of necrotic cells. It was evident that the animal was on the road to recovery when sacrificed.

From mosquitoes fed on *rhesus* No. 52 the strain was maintained. Separate feeding and separate injection of mosquito emulsion of batches Nos. 6 and 7 caused no rise of temperature in experimental animals. However, later tests indicated that *both* batches had caused immunity.

The F. W. strain in our hands has not proved very virulent. It sometimes gives rise to a rather severe temperature reaction, but it has caused death in only three instances.

B. B. Strain.—On Sept. 2, 1928, the writers were notified of a suspected case of yellow fever in the person of B. B., a Russian Jew, 16 years of age, who had been one year in Brazil. Blood was drawn at 10.30 a.m. and inoculated into two guinea-pigs and a *rhesus* monkey. The boy was said to have been taken sick at 10 a.m. on Aug. 30, although there was an indefinite history of indisposition since the evening of the 29th. At the time of our visit he lay hunched up in bed, rather dull, with pulse of 90 and temperature of about 103°F. The eyes were congested and slightly icteric. The tongue was pointed, had red margins, and was furred white on the dorsum. The lips were cracked and excoriated. He had complained of headache previously. There had been vomiting, but no black vomit. Urine was said to contain casts and 1 gm. of albumen per liter. Later he developed bleeding gums, and black vomitus appeared. It was clinically a frank case of yellow fever. On Sept. 4 he became semicomatose and died in the evening. No autopsy was obtainable.

On the seventh day after inoculation one guinea-pig developed a fever of 105.2°, but transfer to another animal was negative and blood cultures in semi-solid medium gave no growth. On the eleventh day the *rhesus* monkey (No. 31) had a temperature of 104.3° and transfer was made to another animal (No. 45). The latter showed fever on the seventh day. Third and fourth passage animals both had high fever on the third day and were sacrificed to obtain tissues for Dr. Bauer to take to New York. A fifth passage animal (No. 63) died, but with a complicating peritonitis. After the seventh passage direct transfers lost in virulence for a time, owing, probably, to shortage of animals and the necessity of using partially immune monkeys. The strain was established again in virulent form through mosquitoes. The batch fed on *rhesus* No. 63 caused a fatal infection when allowed to feed on *rhesus* No. 98. From this point the virus has been carried by a combination of direct transfers and mosquito transmission through a total of at least 14 passages. To the end of December, 1928, 135 animals had been inoculated with this strain, of which 26 had either died or been sacrificed when moribund. However, this small percentage of deaths does not give a true picture of the present virulence, since the total (135) includes all those animals used in building up the virulence, many

negative or partially successful mosquito transmissions, and a large number of animals inoculated with other material and later tested for immunity with the B. B. virus. Autopsies on fatal cases have shown the classical picture of yellow fever. (See reports by Hudson on African studies.[1])

S. R. Strain.—On Oct. 26, 1928, Dr. Eduardo de Araujo notified us that he had heard of a suspicious case of fever. However, the attending physician had not considered it sufficiently suspicious to report to the Health Department.

We found the patient, S. R., to be a Spanish woman, married, 18 years of age. She had become ill on the afternoon of Oct. 23, *i.e.*, approximately 66 hours before our visit. Fever had risen to 104°F., but was down to 100.8°, axillary. The eyes were not injected nor icteric. The tongue was slightly coated, but was not pointed, nor did it have the red margin frequently seen in yellow fever. There had been a little bilious vomiting, but no black vomit. The attending physician reported the urine to have albumen in considerable amount. The patient appeared to be in no pain, was bright and attentive, but not anxious. On the fifth day she was frankly convalescent, with normal pulse and temperature, without ever having shown jaundice or black vomit. The only suspicious signs had been the albumen in the urine and a rather high temperature.

Blood taken at the time of our visit (66 hours after onset) was inoculated into two guinea-pigs and two *rhesus* monkeys (Nos. 116 and 117). On the seventh day *rhesus* No. 116 showed a rise of temperature to 104.2°F. and blood transfer was made to *rhesus* No. 133. The latter had a fever on the day after inoculation but not again until the fourth day, when the temperature rose to 106.2°F. The animal died on the fifth day with typical gross and microscopic lesions of yellow fever. Mosquito batch No. 53 was allowed to feed on this monkey on both the first and fourth days. Blood transfer from the same monkey to *rhesus* No. 142 was made on the fourth day, and liver from the autopsy was emulsified and inoculated into *rhesus* No. 143. The two animals inoculated had no obvious reaction. On Dec. 1 only two stegomyias remained alive in batch No. 53. These engorged on *rhesus* No. 168 and the latter developed a fever of 104.4° on the fourth day, but recovered. Blood from this monkey (No. 168) was injected into *M. rhesus* No. 186, which died from other causes before showing any signs of yellow fever. The surviving two mosquitoes of batch No. 53, together with seventeen others whose remains were fished from the wet cotton and the pan of honey in the cage, were emulsified and inoculated into *rhesus* No. 177, without result. Mosquito batch No. 72 which had engorged on *rhesus* No. 168 was allowed to feed on *rhesus* No. 217. On the next day the temperature of this animal reached 104.0° and in the second day rose to 104.8°. Blood transfer to *rhesus* No. 228 proved positive.

The following animals which received S. R. virus have been proved immune to B. B. strain virus, having given no temperature reaction following the inoculation

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[1] Hudson, N. Paul, *Am. J. Path.*, 1928, iv, 395.

of virulent material: No. 117 (one of the original blood inoculations), No. 143 (inoculated with emulsified liver), No. 168 (fed upon by mosquitoes of batch No. 53) and No. 177 (inoculated with emulsified mosquitoes of batch No. 53). *M. rhesus* No. 116, of the first passage of the S. R. strain, proved absolutely resistant to the African strain of virus.

J. V. O. Strain.—On Sept. 14, 1928, Dr. Barros Barreto came to tell us that a suspected case had been admitted to the Isolation Hospital. Later in the evening the patient, J. V. O., a young Spaniard, was seen by Dr. Bauer and the writers. Although only at the end of the third day of the disease, he was already comatose and had a normal to subnormal temperature. There was noted muscular twitching and bleeding from nose and rectum. It was considered useless to take blood for inoculation. He died at 5 a.m., Sept. 15, and the autopsy was started at about 9:30 a.m. by Dr. Eduardo de Araujo. Icterus was very marked in the dead body. Autopsy findings were typical of yellow fever, including sub-epicardial hemorrhages, bleeding into stomach and intestines, box-wood liver, and intensely injured kidneys.

Liver, spleen, and kidney tissues taken at autopsy were emulsified separately and three animals were inoculated. *Rhesus* No. 48 received spleen emulsion. A severe necrosis of the abdominal wall developed and the monkey was sacrificed on the fifth day. There was no evidence of yellow fever. *Rhesus* No. 47 received liver emulsion. A small abscess appeared and there was a slight fever for two days. Blood transfer to a normal animal gave no results, and No. 47 recovered. On Dec. 10 this monkey was inoculated with virulent B. B. strain material and died on the fourth day with typical yellow fever. No immunity had developed.

M. rhesus No. 46 received kidney emulsion. Here also an abscess resulted. For four days following injection there was a fever, which upon one occasion reached 105.6°. On the second day blood transfer was made to *rhesus* No. 22, but culture of this blood made by Dr. Bauer, yielded a gram negative organism thought to be *B. coli*. *M. rhesus* No. 22 reacted with a high fever, but no further transfers were made, because we thought that the infection was bacterial; the animal recovered. The temperature of *rhesus* No. 46 again reached or passed 104°F. on the afternoons of the twelfth, fourteenth, and sixteenth days after inoculation. Suddenly on the morning of the twentieth day the temperature went up to 105°F. and the monkey was sacrificed. Blood culture at this time yielded a pure growth of streptococci. Both blood and liver were transferred to *rhesus* No. 44. The latter animal had previously been used for experimentation but was considered to be non-immune. Sections of the liver of *rhesus* No. 46 showed the deposition of considerable fat, but apparently no necrosis.

M. rhesus No. 44 developed an abscess at the point of inoculation and the temperature passed 104°F. on the first and third days. On the fourth day there was a rise to 105.9° and mosquito batch No. 29 was allowed to feed. No blood transfer was made because it was thought that the fever arose from bacterial infection. *M. rhesus* No. 44 proceeded to recover, with only one more marked rise in temper-

ature on the tenth day. On Dec. 8, *rhesus* No. 44 was inoculated subcutaneously with 3 cc. of liver emulsion from *rhesus* No. 180, B. B. strain. Not the slightest temperature reaction resulted, although control *rhesus* No. 188 died with typical yellow fever on the fifth day.

On Nov. 1 mosquito batch No. 29, which had fed on *rhesus* No. 44, was allowed to feed on *rhesus* No. 132. Four days later the whole batch (58 remaining mosquitoes) was killed, ground up, and inoculated into the same animal. On Nov. 11 there appeared a fever of 104.6°F. and blood transfer was made to *rhesus* No. 154. The latter had a fever on the second and third days. Blood transfer was made to *rhesus* No. 158, and mosquito batch No. 61 was fed. *M. rhesus* No. 154 later developed a more or less continuous fever and it was sacrificed; early tuberculosis was present. *M. rhesus* No. 158 had a slight fever, beginning on the seventeenth day after inoculation and appearing on three days. However, this was apparently not yellow fever, because ten days after the last febrile access the animal was inoculated with B. B. strain virus and succumbed on the sixth day.

On Dec. 1 mosquito batch No. 61, which had fed on *rhesus* No. 154, was allowed to feed on *rhesus* No. 174, and this monkey had a fever of 104°F. on the second day. Eleven days after the infective feed, virulent B. B. strain material (the same as used for No. 158) was inoculated but gave no reaction. This J. V. O. strain, carried in mosquitoes, has been dropped, since it appeared to be avirulent and of no use for experimental purpose.

On Sept. 15, the day of the death of J. V. O., the writers and their assistants captured 59 female *Aedes ægypti* in the bedroom where the patient had slept during his illness until removed to the Isolation Hospital. Upon three occasions, the first time eight days after capture, these mosquitoes were allowed to feed upon *rhesus* No. 60. Five days after the first feeding and three days after the second, this monkey's temperature reached 103.9°F. There was then a drop until two days later when 104.4° was reached. Blood transferred at this point gave no reaction. The mosquitoes which fed on No. 60 were allowed to engorge on one normal monkey without result, but were destroyed through an error before further experimentation could be carried out. The original mosquitoes caught in the bedroom of J. V. O. were eventually ground up and inoculated into two monkeys. These showed no fever and were later proved susceptible to B. B. strain virus. Evidently the virus, if present, was not sufficient either to infect or to immunize two animals. However, *rhesus* No. 60, which had shown a temperature reaction following bites, appeared to be resistant upon further inoculation.

African Strain.—This is the Asibi strain, which has been in use for many months at the laboratory of the West African Yellow Fever Commission of the Rockefeller Foundation in Lagos, Nigeria. It was later established by Dr. Sawyer in New York, sent from there to Rio de Janeiro in November, 1928, established at the Oswaldo Cruz Institute, and sent from Rio to us through the kindness of Dr. Aragão. In our hands it has gone through five passages and has killed eleven monkeys.

TABLE II.

Fatal Infections of Monkeys after Inoculation with African Yellow Fever Virus.

Strain	*M. rhesus* No.	Temperatures, a.m. and p.m.											Remarks
		Day of inocul.	1	2	3	4	5	6	7	8	9	10	
Asibi	175	—	104.7	103.9	103.1	106.5	104.9						Inoculated with monkey liver sent from Rio de Janeiro. Developed abscess
		103.4	104.1	103.9	103.9	106.0	103.3	Dead					
"	185	—	103.7	106.0									Inoculated with 2 cc. blood from No. 175 (taken on 4th day)
		103.9	104.9	104.0	Dead								
"	190	—	102.7	102.9	103.6								Inoculated with 2.5 cc. citrated blood from No. 185. Sacrificed when moribund on evening of 3rd day
		102.7	102.2	104.6	100.4								
"	118	—	102.0	103.9	103.4	104.0	104.2	101.7					Previously used animal probably partially immune. Inoculated with 2.5 cc. citrated blood from No. 185
		101.8	102.3	103.7	103.2	103.6	105.0	102.0	Dead				
"	201	—	103.1	103.9	104.4								Inoculated with 2 cc. blood-liver mixture from No. 190
		103.8	103.6	105.1	104.4	Dead							
"	216	102.9	103.0	103.6	102.9	103.8	103.9	99.1					Inoculated with 10 cc. blood-liver mixture from No. 201. Blood 19 days old, liver 17 days old (frozen most of time). Sacrificed when moribund
		102.7	103.4	103.5	103.0	103.6	104.8						
"	229	—	102.7	104.4	105.0	102.4							Inoculated with 2 cc. citrated blood from No. 209 (the latter infected by mosquitoes). Sacrificed when moribund
		103.0	103.8	105.7	104.9								
"	204	103.1	103.5	103.4	104.2	104.0	99.0						Previously used animal. Inoculated with 2 cc. citrated blood from No. 209
		103.3	103.8	103.6	104.4	104.2	Dead						
"	189	—	102.5	103.6	104.8	104.6	105.8	102.0					Previously used animal. Inoculated with 2 cc. citrated blood from No. 209
		102.0	103.1	103.8	105.4	104.8	105.2	Dead					

DISCUSSION AND CONCLUSION.

It would appear that inoculation of *rhesus* monkeys served a diagnostic purpose in one of our cases, that of S. R. The disease was so mild that in the absence of an epidemic no clinician would have made the diagnosis without this laboratory procedure.

It should be pointed out that following inoculation of liver emulsion, and sometimes even of blood, it is impossible to tell whether an early fever resulting is due to a protein reaction, to bacterial infection, or to true yellow fever. If plenty of animals are available the only safe plan is to take blood for subinoculation and for cultures, and to feed mosquitoes. Blood may be kept frozen for some time before inoculation if it is found to be necessary, or if it is desired to await the outcome of the disease in the animal bled.

A combined blood and liver transfer showed the presence of virus in *rhesus* No. 46 twenty days after the original inoculation with kidney from the autopsy in the case of J. V. O. The same animal had had an infected body wall and a bacteriemia.

On the basis of later experience we can see some of our mistakes. In the second S. R. passage (*rhesus* No. 133) blood transfer should have been made at the time of initial fever, the day following inoculation. In the case of *rhesus* No. 60, mosquito infection from J. V. O_t, transfer probably should have been made when the temperature reached 103.9°F. on the fifth day. Two days later, with a fever of 104.4°F., the blood appeared to be non-infective. A temperature of 104°F. in monkeys is usually a safe borderline between fever and no fever, but seemingly not always.

Our experience with the South American viruses so far indicates that it takes much care, patience, and an abundance of monkeys to build up and maintain a high degree of virulence.

For invaluable help in securing these yellow fever strains, and for much assistance and advice in establishing the laboratory we gratefully acknowledge our indebtedness to Dr. J. H. Bauer of the West African Yellow Fever Commission of the Rockefeller Foundation, to Dr. Henrique de Beaurepaire Aragão, of the Oswaldo Cruz Institute, Rio de Janeiro, to Dr. Antonio Luiz C. A. de Barros Barreto, Director of the Department of Health, State of Bahia, and to Dr. Eduardo de Araujo, Director of the Oswaldo Cruz Institute, São Salvador, State of Bahia.