THE RELATIONSHIP OF YELLOW FEVER OF THE WESTERN HEMISPHERE TO THAT OF AFRICA AND TO LEPTOSPIRAL JAUNDICE*

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(Received for publication, November 2, 1929)

In the spring of 1928 the accumulated evidence concerning the etiology of yellow fever presented seeming discrepancies which suggested that more than one independent disease had been investigated as yellow fever. To help overcome this confusion the International Health Division of the Rockefeller Foundation undertook to bring together in one laboratory the yellow fever viruses of West Africa and South America and to determine their relationship. The study was begun in June, 1928, at The Rockefeller Institute for Medical Research.

The conflicting evidence referred to may be summarized briefly.

In Cuba in 1900 and 1901 the Yellow Fever Commission of the United States Army under Major Walter Reed (1) found that the etiological agent of yellow fever was present in the circulating blood during the first three days of fever; that it was not cultivable by any of the bacteriological methods used; that it would pass a Berkefeld filter capable of holding back *Staphylococcus pyogenes aureus;* and that it could be readily transferred from a sick person to a well one with the production of infection, by the mosquito *Aëdes aegypti.* The bite of even one mosquito was sufficient to cause the disease.

From 1918 to 1924 a leptospira was isolated by Noguchi and other investigators (2) from patients in outbreaks of yellow fever in Ecuador (Guayaquil), Mexico (Mérida and Vera Cruz), Peru (Morropón), and Brazil (Palmeiras). Like the specific agent studied by the commission under Walter Reed this organism was present in the blood early in the disease, it was not cultivable in the ordinary

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation. Laboratory facilities were made available in New York through the courtesy of The Rockefeller Institute for Medical Research.

culture media for bacteria, and it was able to pass Berkefeld filters (V and N). It seemed to differ, however, in not being easily transferable by *Aëdes aegypti*. Noguchi (3) found that he could infect guinea pigs with this leptospira, which he named *Leptospira icteroides*, by means of the bites of *Aëdes aegypti* which had fed previously on a patient or on infected guinea pigs, but that transmission was obtained infrequently considering the number of mosquitoes employed. Another difficulty in the way of accepting the organism as the causative agent of yellow fever was the uncertainty of the diagnosis in the cases in which leptospirae were isolated. In the individual case, infectious jaundice, caused by *Leptospira icterohaemorrhagiae*, is ordinarily indistinguishable from yellow fever on the basis of symptoms. Elliott (4), who was associated with Noguchi in 1918 as clinician of the commission which studied yellow fever in Guayaquil, held the following opinion: "Clinically yellow fever is similar to infectious jaundice. The differences existing between the two diseases appear to be chiefly those of degree. There is more marked jaundice and less hemorrhage in yellow fever than in infectious jaundice." In the differential diagnosis between these diseases the nature of the prevailing epidemic was of necessity frequently allowed to determine the decision with regard to the individual case. To meet this difficulty Noguchi made comparative studies of *L. icteroides* and *L. icterohaemorrhagiae* and decided (2) that they were distinct morphologically and serologically, and that the evidence appeared to warrant the conclusion that *L. icteroides* is the cause of yellow fever. This view became generally accepted.

In 1925 the West African Yellow Fever Commission of the Rockefeller Foundation undertook the study of a disease in West Africa known as yellow fever and indistinguishable from the yellow fever in South America by clinical or pathological characteristics. As summarized by Stokes, Bauer, and Hudson (5), 67 cases of yellow fever were studied bacteriologically by Muller, Kligler, Sawyer, and Bauer between January, 1926, and May, 1927. Although a large number of cultures were made and many guinea pigs inoculated, no leptospira could be isolated nor any other organism which could have a relation to the disease. Yellow fever in West Africa was obviously not due to *L. icteroides*.

In 1927 it was discovered by Stokes, Bauer, and Hudson (5) that the monkey *Macacus rhesus* is susceptible to yellow fever. Using this monkey as an experimental animal they found that the specific agent of the yellow fever of Africa resembled that of the classic yellow fever studied by the army commission in Cuba in being present in the blood early in the disease, in not being cultivable, in filtrability, and in ease of transference by means of *Aëdes aegypti*.

In 1928 Aragão (6) transferred the yellow fever of South America from patients to *Macacus rhesus* monkeys during an epidemic in Rio de Janeiro. The infectious agent was found to be filtrable and was easily transmitted by *Aëdes aegypti*. A search for *L. icteroides* in 15 cases of yellow fever yielded negative results.

In the meanwhile several experimenters made observations which called in question the opinion that *L. icteroides* was the cause of the true yellow fever of

the Americas, as represented by the disease studied by the commission under Reed. Sellards (7) was unable to demonstrate by serological methods any relationship between a yellow fever outbreak in Parahyba, Brazil, and *L. icteroides* or *L. icterohaemorrhagiae*. Theiler and Sellards (8) and Schüffner and Mochtar (9) compared *L. icteroides* and *L. icterohaemorrhagiae* and obtained strong evidence of their serological identity.

Attempts to transfer *L. icteroides* by means of *Aëdes aegypti* failed in the hands of a number of investigators (Kligler (10), Gay and Sellards (11), Schüffner and Mochtar (9), and Sawyer and Bauer (12)) and they became convinced that this mosquito is not suited to act as intermediate host for *L. icteroides*.

When our experiments were undertaken there seemed to be need for further investigation of the relationships of the "yellow fever" of West Africa, the "yellow fever" of South America not due to a leptospira, the "yellow fever" of the Americas apparently caused by a leptospira, and a hypothetical "yellow fever" in which the etiological factors of the two last mentioned diseases are both present.

Collection of Strains of Yellow Fever Virus

The strains of virus used in our experiments were the French and the Asibi from West Africa and the F. W. from Brazil.

The French strain was given us by Dr. A. W. Sellards and Dr. Max Theiler of the Harvard Medical School. It had been obtained originally from a yellow fever patient in Senegal by Mathis, Sellards, and Laigret (13). During our experiments this strain was indistinguishable from the Asibi strain in its effects on monkeys.

The Asibi strain was sent us from Lagos, Nigeria, by Dr. Henry Beeuwkes, Director of the West African Yellow Fever Commission of the Rockefeller Foundation. It had been obtained in the Gold Coast from an African native during an attack of yellow fever, and had been used by Stokes, Bauer, and Hudson (5) in most of their experiments.

The establishment in our laboratory of a strain of yellow fever virus from South America proved to be difficult. We are indebted to Dr. F. L. Soper and Dr. H. Muench for obtaining for us a large number of specimens of blood from yellow fever patients in Rio de Janeiro. We wish to acknowledge also our special obligation to Dr. Clementino Fraga, Director of the National Department of Health, and to Dr. Sinval Lins, Chief of the Communicable Disease Section of Hospital São Sebastião, for their courtesy in granting Dr. Soper and Dr. Muench

free access to the patients. Specimens of blood and tissues from monkeys experimentally infected with yellow fever were received through the kindness of Dr. H. de B. Aragão of the Oswaldo Cruz Institute in Rio de Janeiro and of Dr. N. C. Davis and Dr. J. H. Bauer of the Yellow Fever Laboratory of The Rockefeller Foundation at Bahia.

The specimens of human blood from Rio de Janeiro were taken from 103 patients having yellow fever or suspected of having it. A citrated specimen from each patient was received and also dried blood from three and clotted blood from four. Among the patients supplying the blood were at least two from whom yellow fever was transmitted to monkeys in Rio de Janeiro. This was accomplished by blood inoculation by Da Cunha and Muniz (14) in the case of F.W., and through the bites of mosquitoes by Aragão (15) in the case of N.M. The blood was drawn on the first day of the disease from 11 patients, on the second from 37, on the third from 53, and on the fourth from one. In one instance the day of disease was not recorded.

As soon as received, the specimens were injected into *M. rhesus* monkeys, in varying amounts, usually intraperitoneally, rarely subcutaneously. Of the 110 specimens, including duplicates, 16 were injected into separate animals. The remaining 94 were divided into 14 groups and pooled, and part of each of the 14 mixtures was injected into one or two monkeys. In some instances animals which showed no symptoms were inoculated later with another specimen as the number of available monkeys was limited. Test injections with a known virus were not given at the end of the observation period to determine whether immunity to yellow fever had been established as we had no yellow fever virus during the early part of the work. The rectal temperature of the monkeys under observation was taken twice each day.

The results from the inoculation of the specimens of human blood sent from Rio de Janeiro were uniformly negative. Most of the monkeys developed no fever. Six had isolated rises of temperature which seemed of no significance. We concluded that it was not practicable to isolate strains of yellow fever from the blood of patients in South America while working at so great a distance from the source of material.

Attempts to bring in a strain of yellow fever already established in monkeys finally met with success. From Rio de Janeiro Dr. Aragão sent us material from monkeys infected with several strains (F.W., N.M., N.M. and J.K., N.M. and D.A., J.K. and R.M.). We received citrated, glycerinated, and dried monkey blood (one specimen of each) under refrigeration; an emulsion of mosquitoes which had fed on an infected monkey; and monkey liver which had been refrigerated (5 specimens), frozen (3), glycerinated (3), dried (2), and one kept at room temperature. These eighteen specimens were prepared and injected into animals

intraperitoneally or subcutaneously. Six of the animals died from causes other than yellow fever. The other animals remained normal except one.

A monkey which had been inoculated with a specimen of frozen liver, F.W. strain, tenth passage in monkeys, showed fever on the seventh day and recovered. After an observation period of 28 days from the date of inoculation this monkey was given a test injection of African yellow fever virus, Asibi strain, and was found to be immune. The strain obtained by bleeding this monkey at the beginning of its fever was carried through many passages in this laboratory, and the F.W. virus used in our experiments was derived from this succession.

Like the African strains, the F.W. strain thus procured could withstand long storage under suitable conditions. At one time the strain was apparently lost, but was reestablished by inoculating a monkey with stored liver tissue. The specimen of liver had been kept continually frozen for 67 days. Between experiments this strain was successfully preserved in storage for varying intervals up to 92 days in monkey blood dried in the frozen state by the methods used by Sawyer, Lloyd, and Kitchen (16) in preserving African strains of yellow fever virus.* We have not yet tested older dried blood specimens of the F.W. strain by inoculation of monkeys.

The material from Bahia, Brazil, consisted of 28 specimens of blood and liver from 20 monkeys experimentally infected with yellow fever of the B.B. strain (27 specimens) and the S.R. strain (1 specimen). These strains had been obtained from yellow fever patients in Bahia, Brazil, by Davis and Burke (17). This material was injected into monkeys and the B.B. strain was established in our laboratory, but the virulence of this strain for monkeys was so low that it seemed unsuitable for our projected cross-immunity experiments.

In the hands of Davis and Burke (17) the B.B. strain has shown a higher degree of virulence. Immunity against African virus was possessed by four of eight animals that developed fever after inoculation by us with the B.B. strain specimens. This adds to the evidence supporting Davis (18) in his conclusion that this strain is immunologically the same as African yellow fever virus.

Bacteriological Examinations

The specimens of citrated human blood received from Rio de Janeiro were tested for bacterial contamination by inoculation of broth or agar slants. Only three specimens produced visible growths and they were caused by three different organisms. A more critical bacterial examination was made by Frobisher (19) of 30 of these specimens of human blood and also of the blood of 16 monkeys infected

* A specimen of African yellow fever virus (French strain) in monkey blood preserved in this way has been tested after one year in storage and found to be highly virulent for *rhesus* monkeys.

with African or South American yellow fever virus and of the liver tissue of 10 monkeys infected with African virus. Cultures were made in a wide variety of media, including the Noguchi lepto-medium, and guinea pigs were inoculated. The results were essentially negative.

Of the specimens of citrated human blood sent us from Rio de Janeiro, 66 were examined also by H. R. Muller and E. B. Tilden (20) at The Rockefeller Institute for Medical Research. Two of these specimens yielded cultures of a spiral organism agreeing with the cultures of *L. icteroides* isolated in yellow fever epidemics by Dr. Noguchi. Both of the specimens containing leptospirae came from severe cases with the symptoms of yellow fever; one of the cases was fatal. Both specimens were among the first 36 examined, which group was included in the 103 specimens inoculated by us into monkeys.

Tests of Monkeys Immunized with American Yellow Fever Virus for Immunity to African Virus

In order to show the relationship of American yellow fever to the African, monkeys were immunized with American yellow fever virus and afterward tested for susceptibility to African virus, and *vice versa*.

In Table I are given the results of the tests of eleven monkeys which had exhibited fever after intraperitoneal inoculation with the F.W. strain of American yellow fever virus. Monkeys which had not shown fever after inoculation with this strain were sometimes found to be immune to African virus and sometimes not. They were all excluded from this experiment as some had probably not been infected.

Three of the monkeys were inoculated a second time with the F.W. strain from 5 to 7 weeks after the first inoculation, in order to make certain that they had become immune to American yellow fever virus. To obtain additional evidence regarding the immunity produced and a better idea of its degree, blood was taken from six of the animals between the 16th and 41st days after the last injection of American virus, and the serum was tested for protective power against African virus. All eleven of the monkeys were tested by intraperitoneal injection of African virus when at least 30 days had elapsed after their last inoculation with American virus, and the high virulence of the African virus used was shown by its effect on control animals. Except when otherwise stated in the table, the period

of observation after the test inoculation was 30 days or longer. Only temperatures of 40°C. or over were accepted as fever unless otherwise stated.

All of the eleven monkeys immunized against American yellow fever virus survived inoculation with a highly virulent strain of African yellow fever virus, and only one showed fever within the period of

TABLE I

Immunity to African Yellow Fever Virus in Monkeys after Infection with American Yellow Fever Virus

Monkey	First inoculation with American virus		Second inoculation with American virus		Protection test of monkey's serum against African virus†	Test of monkey for immunity by inoculation with African virus (Asibi)
	Strain	Febrile period*	Strain	Febrile period*		
B	F.W.	5.5 to 12.0	F.W.	11.0 to 13.0	Protected‡	Immune
C	F.W.	2.5 to 15.0	F.W.	None	Protected	Immune
D	F.W.	2.5 to 9.0	Not inoculated		Protected	Immune
E	F.W.	7.0 & 12.0	Not inoculated		Protected‡	Immune
F	F.W.	7.5 to 9.5§	F.W.	None	Protected	Immune
G	F.W.	3.0§	Not inoculated		Not tested	Immune
H	F.W.	2.0 to 8.5	Not inoculated		Protected‡	Immune
I	F.W.	6.0 to 7.5	Not inoculated		Not tested	Immune‖
J	F.W.	3.0 to 4.5	Not inoculated		Not tested	Immune¶
K	F.W.	4.0§	Not inoculated		Not tested	Immune
L	F.W.	4.0 to 12.0	Not inoculated		Not tested	Immune

* Expressed in days after inoculation.

† Performed with Experiment IV; same methods, same virus (Asibi), regular amount of serum, and same controls (Table IV).

‡ Had one elevation of temperature; Monkey B on 14th day after inoculation, Monkey E on 7th, Monkey H on 8th.

§ Maximum temperature 39.7°C. (Monkey G) or 39.9°C. (Monkey K).

‖ Observed for 24 days only.

¶ Fever 4.0 to 6.0 day. Observed for 12 days only.

observation. The sera of six of these monkeys taken before the test inoculation were tested for power to protect monkeys against the same African strain. All protected against death, although three permitted fever.

Tests of Monkeys Immunized with African Yellow Fever Virus for Immunity to American Virus

As it was a rare exception for a monkey to survive inoculation with fresh virus of the African strains which we used (French and Asibi), it was necessary in our cross immunity tests to use monkeys which

TABLE II

Immunity to American Yellow Fever Virus in Monkeys after Infection with African Yellow Fever Virus

Monkey	First inoculation with African virus		Second inoculation with African virus		Protection test of monkey's serum against American virus (F.W.)	Test of monkey for immunity by inoculation with American virus (F.W.)
	Strain	Febrile period	Strain	Febrile period		
M	Asibi	None	Asibi	None	Not tested	Immune*‖
N	Asibi	None†	Asibi	1.5	Not tested	Immune*
O	French	None†	Asibi	None	Not tested	Immune
P	French	None†	Asibi	None	Not tested	Immune
Q	French	12.5 to 15.0	Asibi	None	Not tested	Immune‖
R	French	6.0 to 7.0	Asibi	4.5	Not tested	Immune
S	Asibi	5.0	French	None	Not tested	Immune
T	Asibi	3.0, 3.5	French	None	Not tested	Immune
U	French	1.5 to 5.0	Not inoculated		Doubtful‡	Immune
Control: C§						Febrile period 2.5 to 15.0

* Had received an injection of the B. B. strain of American virus but, as the control monkey as well as the animal tested showed no reaction, the monkey was reinoculated 14 days later with the F. W. strain.

† Protected by an injection of African immune serum from recovered African natives (Monkeys N, and P) or from immunized Monkey A, Table IV (Monkey O).

‡ Had irregular unexplained rises of temperature from day of inoculation.

§ Monkey C was control for the examination of the sera of Monkeys M, N, O, P, Q, R. The other tests were made with the controls shown in Table V.

‖ Observation period only 20 days (Monkey M) or 21 days (Monkey Q).

had been protected against death by injections of African immune serum or which had received virus attenuated by some method of preservation or by long storage. Nine animals immunized against African virus were available for testing. Particulars with regard to the tests are shown in Table II.

Eight of the monkeys tested had been given a second injection of the African virus from 20 to 48 days after the first as a test of immunity to that virus. The monkeys were tested by inoculation with American virus (F.W. strain) after an interval of between 31 and 39 days from the last injection of African virus, except in one instance in which the interval was 143 days. They were kept under observation for 30 days or more, except when otherwise stated in the table.

Blood was taken from one of the animals before the test inoculation and the serum was tested for protective power against American virus. As the monkey receiving this serum with the virus had irregular fevers not due to the yellow fever virus, it was impossible to know whether an attack of fever was caused by the virus and no conclusion could be drawn, as death does not ordinarily result from the F.W. virus in this laboratory.

All nine of the monkeys immunized against African yellow fever were free from fever following inoculation with the F.W. strain of American virus.

In the cross immunity tests, monkeys immunized against American yellow fever virus resisted African yellow fever virus and, conversely, those immunized with the African virus resisted the American virus.

Protective Power of American Yellow Fever Sera against African Virus and against Leptospirae

A. Sera Taken Soon after Undoubted Yellow Fever

An exceptional opportunity to investigate the relationship of American yellow fever to African yellow fever and to leptospiral jaundice was presented by an epidemic of yellow fever in Rio de Janeiro. Through the courtesy of Dr. H. de B. Aragão and Dr. F. L. Soper we received 15 specimens of serum from 14 persons who had recovered recently from yellow fever in that city. They are listed in Table III under the heading "Recent epidemic," and our findings with regard to these sera are summarized there.

In all the 14 cases a definite diagnosis of yellow fever had been made on clinical grounds. In one case (N.M.) the diagnosis had been proven, for Aragão (15) had transferred the infection to a monkey by means of mosquitoes. In eight cases the attack was described as severe; in six as mild.

Six of the sera were divided in New York and a portion of each was sent to the

TABLE III

Protection by Sera of Persons Recovered from American Yellow Fever against African Yellow Fever Virus and Leptospira icteroides or L. icterohaemorrhagiae

Serum	Time after attack	Protection of monkey against yellow fever virus*		Protection of guinea pig against leptospirae*			Experiment	
		Prevented fever	Prevented death	Pfeiffer phenomenon	Prevented fever	Prevented death	Tests with virus	Tests with leptospira
Recent epidemic:								
N. M.	31 days	+	+	−	−	−	I	A
C. F.	39 days	+	+	−	−	+‡	I	A
D. A.	26 days	−(1.5)†	−(3.5)†	−	−	−	I	A
M. A.	16 days	+	+	−	−	‖	I	A
(1st specimen)								
F. C.	10 days	+	+	−	−	−	I	A
J. R. S.	85 days	+	+	−	−	−	I	A
G. V.	15 days	−¶	+	−	−	−	II	A
M. R.	49 days	+	+	−	−	−	II	B
M. A.	40 days	+	+	−	−	−	II	B
(2nd specimen)								
J. M.	20 days	+	+	−	−	+‡	II	B
M. M.	144 days	−(3.5)§	+	−	−	−	III	C
M. J. S.	153 days	−(2.0)	−(4.5)	+	+	+	III	C
R. B. S.	156 days	**	−(4.0)	−	−	−	III	C
J. M.	153 days	−(4.0)§	+	−	††	††	III	C
C. W. G.	47 days	−(2.0)	−(4.5)	+	+	+	IV	C

* The yellow fever virus was of the strains Asibi in Experiments I and IV and French in Experiments II and III. The strains of leptospira used were *Leptospira icteroides*, Brazil 49, in Experiments A and B, and *Leptospira icterohaemorrhagiae*, Rat I, in Experiment C.

† The figure in parenthesis is the number of days from inoculation to the first observation of fever in column "Prevented Fever," and from inoculation to death in column "Prevented Death."

‡ Guinea pig had fever but survived and was found to be immune to a second injection of *L. icteroides*.

§ Temperature rose as high as 40°C. only once.

‖ Guinea pig had fever, but died on second day after inoculation while being bled; too early for characteristic lesions.

¶ Had unexplained occasional single elevations of temperature from the 1st to the 58th day after inoculation.

** No fever observed.

†† Guinea pig died from unknown cause on day following inoculation.

TABLE III—*Concluded*

Serum	Time after attack	Protection of monkey against yellow fever virus*		Protection of guinea pig against leptospirae*			Experiment	
		Prevented fever	Prevented death	Pfeiffer phenomenon	Prevented fever	Prevented death	Tests with virus	Tests with leptospira
Doubtful cases:								
Car. F.	brief	−(1.5)	−(4.0)	−	**	−	III	C
M. D.	brief	−(1.5)	−(4.5)	−	−	−	III	C
H. D.	brief	+	+	−	**	−	III	C
Dj. A.	brief	+	+	‡‡			III	
Former epidemics:								
G. E.	9 years	−(5.0)	+	−	−	−	II	B
C. H. H.	5 years	−(2.0)	−(5.0)	−	−	−	II	B
D. O. L. (1st test)	23 years	−(2.0)	−(9.0)	−	−	−	II	B
D. O. L., double amount		−(4.0)	+				IV	
I. J. K., 0.4 of amount	8½ years	+	+	‡‡			III	
P. S. R., double amount	30 years	−(2.0)	−(4.5)	‡‡			IV	

‡‡ No serum remained for test.

West African Yellow Fever Commission of the Rockefeller Foundation in Lagos. The results of the tests made by the Commission in Africa were reported by Hudson, Philip, and Davis (21). They found that the sera of N.M., C.F., M.R., M.A., and J.M. protected monkeys against injection of African yellow fever virus (Cases 16, 15, 12, 14, and 17 in their series). One of the sera, however, that of D.A. (Case 13 in their series) failed to protect either of two monkeys against the injection of the virus. Four of these sera, those of N.M., C.F., M.A., and J.M., were tested in guinea pigs against *L. icterohaemorrhagiae*, and in every case the Pfeiffer phenomenon was absent and the animal died of leptospirosis. The results of the protection tests in monkeys were in agreement with those of the tests we performed independently in New York with the same sera, and their Pfeiffer tests gave results consistent with those we obtained using different strains of leptospira.

Explanation of Tables III and IV. The results of tests with the sera of persons who had recovered from American yellow fever for protective power against African yellow fever virus and against *L. icteroides* or *L. icterohaemorrhagiae* are shown in Tables III and IV. The results of the tests of the sera themselves are in Table III and those of the corresponding control tests are in Table IV. The tests for protection against yellow fever virus were made in four separate experiments

TABLE IV

Control Tests in the Experiments Recorded in Table III

Serum	Time after attack	Protection of monkey against yellow fever virus		Protection of guinea pig against leptospira			Experiment	
		Prevented fever	Prevented death	Pfeiffer phenomenon	Prevented fever	Prevented death	Tests with virus	Tests with leptospira
Recovered African native, K. S.	10 mos.	+	+	−	−	−	I	B
Recovered African native, K. Owe.	9 mos.	−(6.5)	+	−	−	+*	II	B
Recovered African native, K. Ot.	22 mos.			−	†	−		C
Monkey A, immunized to African virus		+	+	−	−	−	II	B
Normal *rhesus* monkey, double amount		−(2.5)**	−(4.5)	−	−	−	IV	B
Anti-icteroides, double amount		−(2.5)	−(6.5)	+	‡	‡	I	A
Anti-icteroides, 1:10 dilution				+	+	+		C
Anti-icteroides, 1:100 dilution				+	§	§		B
Normal horse				−	−	+*		A
Normal human, double amount		−(1.5)	−(4.5)	−	−	−	I	A
Normal human, double amount		−(1.5)	−(4.0)	−	−	−	II	C
Normal human, double amount		−(1.5)	−(3.5)				III	
Normal human, double amount		‖	−(6.0)				IV	

* Guinea pig had definite febrile attack but survived and was found to be immune to a second injection of *L. icteroides*.

† No fever observed. Death from leptospiral jaundice 5 days after inoculation.

** Temperature rose only to 39.9°C.

‡ Guinea pig had no fever for 17 days, then developed fever and died 6 days later of pneumonia and peritonitis, but without lesions suggesting leptospiral jaundice.

§ Guinea pig had fever and died of peritonitis on the seventh day. No lesions suggestive of leptospiral jaundice.

‖ No fever was observed.

TABLE IV—*Concluded*

Serum	Time after attack	Protection of monkey against yellow fever virus		Protection of guinea pig against leptospira			Experiment	
		Prevented fever	Prevented death	Pfeiffer phenomenon	Prevented fever	Prevented death	Tests with virus	Tests with leptospira
No serum, full amount of virus		−(1.5)	−(5.0)				I	
No serum, 0.1 amount of virus		−(2.0)	−(4.5)				I	
No serum, 0.01 amount of virus		−(10.0)	−(11.0)				I	
No serum, full amount of virus		−(6.0)	+¶				II	
No serum, 0.1 amount of virus		−(2.0)	−(4.0)				II	
No serum, 0.01 amount of virus		‖	−(8.5)				II	
No serum, 0.1 amount of virus		−(3.0)**	−(5.0)				III	
No serum, full amount of virus		−(2.5)††	−(4.5)				IV	
No serum, 0.01 amount of virus		−(2.5)	−(4.0)				IV	
Salt solution				−	−	−		A
Salt solution				−	−	−		B
Salt solution				−		‡‡		C

¶ Monkey survived, although it had received no serum.

†† Temperature rose only to 39.8°C.

‡‡ Guinea pig died on night following inoculation.

designated by the Roman numerals I, II, III, and IV, and the tests for protection against leptospirae were made in three experiments, A, B, and C. In order to show which controls belong to each experiment the numbers and letters identifying them are given in the column headed "Experiment."

The sera tested were injected intraperitoneally into *M. rhesus* monkeys in amounts of 1.5 cc. per kilogram of body weight except where otherwise stated in the column headed "Serum." The expression "double amount" means that 3.0 cc. per kilogram was given, and "0.4 amount" means 0.6 cc. per kilogram. These variations in amount of serum do not apply to the tests with leptospirae. The "time after attack" was measured from the onset of the disease to the time of bleeding to procure the serum.

Inoculations of monkeys with African yellow fever virus were given subcutaneously six hours after the serum had been given intraperitoneally. The amount of the virus injected was 0.3 cc. per kilogram of body weight in experiments I, II, and III, and 0.2 cc. per kilogram in Experiment IV.

The temperatures of the monkeys were taken twice each day. To eliminate the personal equation as far as possible, only temperatures of 40°C. or above were considered as signifying fever except when otherwise stated in the tables. In the column headed "Prevented fever" the plus sign (+) signifies that the monkey had no fever and remained well during an observation period of 30 days after inoculation. The minus sign (−) in this column indicates that fever developed, and was probably due to yellow fever, unless explained in a footnote. In the column "Prevented death" a plus sign (+) shows that the animal did not die. Survival was not necessarily due to the action of the serum, for very rarely recoveries follow infection with the strains of African virus in use, as for example in the case of one of the controls receiving no serum. A minus sign (−) in this column signifies that the monkey died of yellow fever. The diagnosis in all fatal cases of yellow fever was determined by finding characteristic lesions on postmortem examination and on histological examination of the tissues.

In the tests of sera for their power to protect guinea pigs against leptospirae a strain of *L. icteroides*, Brazil 49, was used in Experiments A and B, and one of *L. icterohaemorrhagiae*, Rat I, in Experiment C. Both these strains were given us by H. R. Muller, of The Rockefeller Institute for Medical Research. Strain Rat I had been isolated recently from a wild rat of New York.

In the Pfeiffer tests 1 cc. of the serum to be tested was mixed with 1 cc. of an active culture of leptospirae, and 1 cc. of the mixture was injected immediately into the peritoneal cavity of a guinea pig. Fluid was withdrawn from the peritoneal cavity of the guinea pig 30, 60, 90, and 120 minutes after inoculation and examined under the dark-field microscope. If the Pfeiffer phenomenon was absent, the result was recorded with a minus sign (−) under "Pfeiffer phenomenon." In the cases in which the record shows a plus sign the Pfeiffer phenomenon was present and unmistakable.

The guinea pigs which had been inoculated in the Pfeiffer tests were kept under observation to determine whether they were protected against experimental leptospiral jaundice by the sera injected. In the column "Prevented fever" a plus sign means that the guinea pig remained free from fever and a minus sign that it developed fever. In the column "Prevented death" a plus sign indicates survival, not always due to the serum, and a minus sign means death from experimental leptospiral jaundice. Footnotes explain the irregular results. The diagnosis of leptospiral jaundice in the guinea pig was made by the observation of the jaundice, extreme hemorrhages, and other characteristic lesions, and usually by the observation of leptospirae in the tissues or body fluids.

The tests of the sera of the fourteen persons who had recovered recently from yellow fever in the epidemic in Rio de Janeiro gave the following results:

10 persons—the serum protected against African yellow fever virus, but not against leptospirae (tests with *L. icteroides* in 8 instances and *L. icterohaemorrhagiae* in 2).

2 persons—the serum protected against leptospirae but not against African yellow fever virus (both tests with *L. icterohaemorrhagiae*).

2 persons—the serum protected against neither African yellow fever virus nor leptospirae (tests with *L. icteroides* in one instance and *L. icterohaemorrhagiae* in the other).

The results with the second and third groups of the sera in the above classification were clear cut and regular. Those with the first group showed a few irregularities in the tests in guinea pigs, as is explained in the notes accompanying Table III.

In two cases, in which the guinea pigs died too soon for diagnosis, the definitely negative Pfeiffer test may be accepted as indirect evidence of the lack of protective power in the serum, since in the other cases negative Pfeiffer tests were followed by failure to protect and positive Pfeiffer tests by protection of the guinea pigs. Two guinea pigs inoculated with serum and leptospirae developed fever and recovered. That occasional recoveries are to be expected after inoculation with the strain of *L. icteroides* used, in the absence of a protective serum, is shown by the behavior of a control guinea pig which received the same strain with normal horse serum and recovered (Table IV).

The two species of leptospirae, *L. icteroides* and *L. icterohaemorrhagiae*, appear to be so closely related that they may be used interchangeably in immunological tests for leptospiral jaundice, as in this investigation of ours, and we have already referred to the work of investigators who obtained strong evidence of their serological identity. It will be noticed in Table IV that anti-icteroides serum, even when diluted, gave positive Pfeiffer reactions with both species of leptospira. This serum had been prepared by The Rockefeller Institute by immunizing a horse against a number of strains of *L. icteroides*.

The absence of protective power against African yellow fever virus in four of the sera from recent cases of yellow fever in Rio de Janeiro (D.A., M.J.S., R.B.S., and C.W.G.) is in our opinion strong evidence against the presence of yellow fever in these cases, in view of the evidence already presented showing that African yellow fever and American yellow fever are immunologically the same. That some of the

sera sent from Rio de Janeiro should be from cases other than of yellow fever casts no reflection on the ability of the diagnosticians, for in many cases it is impossible to distinguish by symptoms between yellow fever and leptospiral jaundice, and mild yellow fever may simulate a number of infections.

As evidence of the lack of protective power in these sera we have the results following the inoculation of one monkey in each case, and the confirmatory observations, already mentioned, of Hudson, Philip, and Davis (21) in the case of D.A. The evidence from the inoculation of one monkey in each case seemed conclusive, however, as there was no suggestion of even partial protection by amounts of serum varying from 3.2 cc. to 5.9 cc. according to the weights of the animals.

Observations which bear on the reliability of such negative protection tests have been tabulated by Hudson (22) and relate to sera of 23 West African natives who had recovered recently from yellow fever during observed epidemics. Of 28 monkeys, each of which had received 4 cc. of serum and 0.25 cc. to 1.0 cc. of blood virus (Asibi strain), only one succumbed to yellow fever. The possibility of error of diagnosis in the case of the person supplying the one negative serum cannot be completely ruled out. Hudson gives the estimated number of spontaneous recoveries of *rhesus* monkeys after inoculation with the Asibi strain of virus as about two per cent.

Two of the sera from Rio de Janeiro (M.J.S. and C.W.G.) had strong protective power against *L. icterohaemorrhagiae*, and in one of these cases (C.W.G.) the occurrence of a definite relapse after discharge from the hospital is against the diagnosis of yellow fever and suggestive of leptospiral jaundice, which so commonly has an after-fever. H. R. Carter (23), after a wide experience with yellow fever, said of that disease, "I have never seen a relapse, but other men have. They must be rare." Although the possibility cannot be entirely excluded that the protective substances in either serum were due to an earlier attack of leptospiral jaundice, it is highly probable that in both cases the illnesses taken for yellow fever were in reality leptospiral jaundice (Weil's disease).

M.J.S. had a mild attack and was discharged from the hospital as cured on the seventh day after the onset, according to data published by Barreto (24).

C.W.G. came to New York after his recovery and permitted us to draw an additional specimen of blood. He also supplemented the information which had been sent us by Dr. F. L. Soper. The illness of C.W.G. was characterized by high fever, headache, great weakness, vomiting, backache beginning with the

second day, moderate albuminuria from the fourth day, and jaundice from the seventh. During the first four days the symptoms were such that the physicians were of the opinion that the disease was not yellow fever, but on the seventh day a definite diagnosis of yellow fever was made. The patient was allowed to get up on the 13th day and to leave the hospital on the 15th. On reaching home he again developed high fever and headache. On the 17th day he was able to leave his bed. Convalescence was slow, and he was still distinctly jaundiced on the 48th day, when he visited our laboratory in New York. In Rio de Janeiro he worked at the water front and docks in places where there were many rats. An investigation of this case in Brazil has been published by R. A. Warner (25).

As the sera from most of the 14 persons in Brazil who had recently had symptoms like those of yellow fever possessed strong protective power against African yellow fever virus, the conclusion seems justified that the yellow fever of America is the same disease as that of Africa. The same conclusion has been reached by Theiler and Sellards (26), Hudson, Bauer, and Philip (27) and Hudson, Philip, and Davis (21) as the result of their protection tests with American sera.

B. Sera Taken Soon after Suspected Yellow Fever

Four of the sera sent us came from a town in southern Brazil, and were from cases in which yellow fever was only suspected. In each case the illness was mild and a diagnosis could not be made from the symptoms. These specimens were tested with the results recorded under the heading "Doubtful cases" in Table III Two of the sera protected completely against yellow fever and two did not protect. at all. One of the sera which protected against the virus and the two others were tested for power to protect against *L. icteroides*, and none protected. We were evidently dealing with two cases of yellow fever, and two cases of infection other than yellow fever or leptospiral jaundice.

C. Sera Taken Long after Yellow Fever

The testing of the sera from recent cases of yellow fever at Rio de Janeiro indicated that the Brazilian disease was the same as that of Africa. The assumption was also that it was identical with the disease studied by Reed in Cuba and suppressed by Gorgas in Panama. The fact, however, that the ocean passage between Senegal and Brazil may be as short as six days permits of the possibility of the transport of the African yellow fever to Brazil, although there is no evidence of this at the present time. Fortunately the relationship of the present yellow fever of Africa and Brazil to the historic yellow fever of America

need not be left to speculation. Direct evidence has been obtained by the examination of the sera of persons who passed through attacks of American yellow fever many years ago.

Serological evidence of the unity of the American yellow fever of the past and the African yellow fever of the present has been secured by Bauer and Hudson (28). Two sera obtained 23 years after attacks of yellow fever in the Panama Canal Zone and one taken 26 years after an attack in Tampico, Mexico, protected monkeys against African yellow fever. A fourth serum taken 26 years after yellow fever in New Orleans failed to protect. The tests were made in duplicate.

The results of the tests of five sera from persons who had had yellow fever in the Americas many years ago are given under the heading "Former epidemics" in Table III. The particulars regarding the sources of these sera are as follows:

G.E. had yellow fever in La Union, Salvador, in July, 1919, soon after arriving from Honduras. The attack was very severe and lasted 14 days. It was characterized by much vomiting, heavy albuminuria, and jaundice. According to the patient there were other cases of yellow fever in La Union and in Honduras at the time. Blood was obtained for us by Dr. J. E. Elmendorf, Jr., and information regarding the case was supplied by Dr. Peralta Lagos. The interval between the attack and the bleeding was 9 years and 3 months.

C.H.H. had yellow fever in Aracajú in the State of Sergipe, Brazil, in May, 1923, according to his statement. We are indebted to Dr. B. E. Washburn and Dr. Hargreaves of Kingston, Jamaica, for obtaining blood. The blood was drawn 5 years after the attack.

D.O.L. had a very severe attack of yellow fever in the city of Panama in June 1905. He was attended by several doctors who had had a large experience with yellow fever, including Colonel W. C. Gorgas, and Dr. H. R. Carter. We are indebted to Dr. W. E. Deeks and Dr. R. C. Connor for putting us in touch with D.O.L., and to Captain J. W. Smith of the Medical Corps of the U. S. Army for drawing a specimen of his blood. The interval between the attack of yellow fever and the drawing of blood was 23 years and 5 months.

I.J.K. contracted yellow fever in Morropón, Peru, in April, 1920, during an epidemic, and had a mild attack. He spent a few months in West Africa in 1926. In his case the interval between the disease and the bleeding was 8 years and 7 months. A different specimen of serum of I.J.K. was tested by Hudson, Bauer, and Philip (27) and found to protect against African virus, Asibi strain.

P.S.R. had yellow fever in Havana, Cuba, in May, 1899, 30 years before the specimen of serum was taken.

The results obtained in these tests were as follows:

Serum taken 8½ years after an attack of yellow fever in Peru protected a monkey completely against African yellow fever. Serum taken 9 years after yellow fever in Salvador permitted the appearance of fever in the monkey but prevented death. A specimen obtained 23 years after an undoubted attack of yellow fever in Panama failed to protect when injected in the usual amount, but prevented death, while permitting fever, when used in double quantity. The same amount of normal human serum failed to prevent the death of a control animal in this experiment and in each of three other experiments (Table IV). A serum taken five years after yellow fever in Northern Brazil failed to protect. The evidence with regard to the diagnosis in this case was meager. Serum taken 30 years after yellow fever in Cuba did not protect.

The results obtained in these tests and those of the other investigators cited show the historic yellow fever of America to have been the same as the present African yellow fever.

Protective Power of African Yellow Fever Sera against American Virus

If we were right in our conclusion, derived from observation of the protective power of American sera against African virus, that the yellow fever of America is the same as that of Africa, then the sera of persons who have recovered from yellow fever in Africa should protect likewise against American yellow fever virus.

To demonstrate this by experiment proved difficult owing to the low virulence of the F.W. strain of American yellow fever virus for monkeys, as the strain exists in this laboratory. Only twice in our experience has death resulted from the inoculation of a monkey with the F.W. strain. In using this strain in protection tests it was necessary, therefore, to base our conclusions on the presence or absence of fever in the experimental animals after inoculation. Under these circumstances the results of the tests would of necessity be inconclusive in relation to the individual specimen of serum, but they should permit conclusions with regard to a group of sera when compared with an adequate series of control tests.

In Table V are shown the results of tests of the sera of six natives of the Gold Coast of West Africa. All of these persons had had yellow fever in observed yellow fever epidemics. We are indebted to Dr. Henry Beeuwkes, Director of the West African Yellow Fever Commission of the Rockefeller Foundation, for these sera.

TABLE V

Protection by Sera of Persons Recovered from African Yellow Fever against American Yellow Fever Virus

Serum	Time from attack to bleeding	Protected monkey from fever after inoculation with American yellow fever virus, F.W. strain*	Result of later test inoculation of monkey with African virus, Asibi strain	Results of other tests of the sera
African natives:				
K. Ot.	22 mos.	+	No reaction	Did not protect against *L. icteroides* (Table IV)
K. B.	23 "	+	No reaction	Protected against African yellow fever virus (Asibi)†
K. Owir.	20 "	+	Survived‡	
J. O.	19 "	+	No reaction	
K. N.	30 "	+	No reaction	
T. C.	33 "	+§		
Controls:				
American, recent recovery, M. A.	40 days	+	No reaction	Protected against African yellow fever virus (Table III)
American, recent recovery, C. W. G., double amount	47 "	−(11)‖	Survived‡	Protected against *L. icterohemorrhagiae*, but not African yellow fever virus (Table III)
Anti-icteroides, double amount		−(3.5)‖	No reaction	Protected against *L. icteroides* and *L. icterohemorrhagiae*, but not African yellow fever virus (Table IV)
Normal human, double amount		−(4.0)‖	No reaction	Similar specimens in Table IV

* The F. W. strain showed low virulence for monkeys and did not cause any deaths during this experiment.

† Tested in a later experiment with controls. Monkey showed fever but was protected against death.

‡ Had brief fever reaching 40°C. but recovered.

§ Had no fever but died on 25th day of observation from cause other than yellow fever.

‖ Fever indefinite or absent. The highest temperature of the monkey in the case of C. W. G. was 39.8°C.; in the case of the anti-icteroides serum, 39.7°C.; in the case of the normal human serum, 39.9°C.; in the case of the small dose of virus, 39.8°C.

TABLE V—*Concluded*

Serum	Time from attack to bleeding	Protected monkey from fever after inoculation with American yellow fever virus, F.W. strain*	Result of later test inoculation of monkey with African virus Asibi strain	Results of other tests of the sera
Controls—*Continued:*				
Normal *rhesus* monkey, double amount		−(4.0)	No reaction	Similar specimens in Table IV
No serum, full amount of virus		−(4.0)¶		
No serum, 0.01 amount of virus		−(3.5)‖	No reaction	

¶ Died from dysentery on 28th day after inoculation.

The methods of performing these tests and tabulating the results are in general the same as in the previous experiments (Tables III and IV). The regular amount of serum injected into the monkeys was 1.5 cc. per kilogram of body weight, and the double amount was 3.0 cc. The amount of virus-bearing blood injected was uniformly 0.4 cc. per kilogram, and it was injected subcutaneously six hours after the serum had been given intraperitoneally.

The results of the tests, though clearly unreliable in the individual case on account of the low virulence of the F.W. strain of virus used, show that the African sera as a group possessed protective power against American yellow fever virus. In no case did fever appear in a monkey which had received one of these six sera. The three control animals which received the full amount of virus without serum or after normal human or monkey serum all showed rises of temperature to 39.9°C. or over at the time when fever would be expected as the result of the inoculation. These observations are in agreement with the stronger evidence obtained by testing American sera against African virus.

Differences between the American and African Strains of Yellow Fever in Their Virulence for Monkeys

The two African strains we used in our experiments (French and Asibi) were similar to each other in virulence for monkeys, but they

differed markedly in this respect from the one American strain (F.W.). To facilitate comparison, we have summarized our experience with the inoculation of *rhesus* monkeys with the Asibi and F.W. strains.

In doing so we have considered only those cases in which the inoculum was blood taken on the first day of fever from monkeys experimentally infected with yellow fever in this laboratory. The amount of blood was not less than 0.1 cc., expressed as undiluted whole blood. If citrated blood was injected, the specimen was not over 24 hours old, and, if dried blood was used, it was prepared by drying in vacuum in the frozen state and was not over 100 days old. In fatal cases the animals were allowed to die or were killed when moribund.

Of 24 monkeys inoculated with African virus of the Asibi strain, all came down with yellow fever and only one survived. Of 20 monkeys receiving American virus of the F.W. strain, 15 developed fever and two of these died. These two, Monkeys V and W, had received dried blood 94 and 63 days old, respectively.

In the cases of those animals that showed temperatures of at least 40°C., the incubation periods for the Asibi strain were, minimum 1.5 days, maximum 6, and average 2.7; for the F.W. strain, minimum 1.5 days, maximum 12, and average 4.3. The intervals between inoculation and death were, for the animals inoculated with the Asibi strain, minimum 2.5 days, maximum 10.5, and average 5. The two animals which died after receiving the F.W. strain did so 4.5 and 8 days after inoculation.

The Asibi strain of African virus proved to have a much higher virulence for monkeys than the F.W. strain of American virus. The former caused death in 23 of 24 monkeys inoculated, and the latter in only 2 of 20. It does not follow, however that there is a similar difference between these strains in their virulence for man, nor that these strains are representative in virulence of the African and American strains in general. Dr. N. Paul Hudson tells us that the figures of the laboratory of the West African Yellow Fever Commission of the Rockefeller Foundation showed, up to the end of 1928, that there was a marked variation in the virulence of African strains for *Macacus rhesus*. A strain (H.P.) obtained from a fatal case in a European killed only one-third of the monkeys inoculated with blood drawn from infected monkeys at the time of fever. The mortality was low also when this strain was transmitted from animal to animal by mosquitoes.

Comparison of the Lesions Produced by African and American Strains of Yellow Fever Virus

The gross and microscopic lesions produced by the Asibi and French strains of African yellow fever virus in our monkeys were in general those described by Hudson (29) for experimental yellow fever as produced in *M. rhesus* by the Asibi strain. The two animals (Monkeys V and W) which died as the result of inoculation with the F.W. strain of American virus showed gross and microscopic lesions such as are produced by the African strains. One animal (Monkey X) killed during a mild attack following inoculation with the F.W. strain showed only very slight changes.

Da Cunha and Muniz (30) speak of the varying extent of the liver lesions in monkeys after inoculation with a Brazilian strain of yellow fever virus. In some animals they found extensive necrosis of the hepatic cells and none in others. Although they found that their virus possessed less virulence than an African strain, it was sufficient at times to cause early death with extensive lesions like those following inoculation with the African strain.

CONCLUSIONS

1. The yellow fever now in South America, the present yellow fever of Africa and the historic yellow fever of Panama and other American countries are the same disease. This conclusion is based on cross immunity tests in monkeys with strains of yellow fever virus from Africa and Brazil and on tests of sera from 25 persons, who had recovered from yellow fever in various places and at various times, for the power to protect monkeys against African or Brazilian virus strains.

2. Cases of leptospiral jaundice (Weil's disease) were present among those diagnosed as yellow fever in the recent epidemic in Rio de Janeiro. This is shown by the isolation of cultures of leptospirae from the blood of two patients by H. R. Muller and E. B. Tilden of The Rockefeller Institute, and by the demonstration by us of protective power against leptospirae and absence of protective power against yellow fever virus in the sera from two persons after recovery. The isolation of leptospirae by Noguchi and other investigators from the

blood of occasional patients in past epidemics of yellow fever in a number of American countries indicates that leptospiral jaundice was present then as well and was diagnosed clinically as yellow fever.

3. The absence of protective power against leptospirae shown by the Brazilian sera which protected against yellow fever virus and the absence of protective power against yellow fever virus in the sera that protected against leptospirae point to the probability that American yellow fever is not the combined effect of leptospirae and yellow fever virus. The position of *L. icteroides*, isolated by Noguchi during yellow fever epidemics, now appears to be not that of a secondary invading microorganism in cases of virus yellow fever, but that of the incitant of a form of infectious jaundice, sometimes fatal, often coincident in its appearance with typical yellow fever and apparently indistinguishable from it clinically. This leptospiral disease has not hitherto been separated from true yellow fever. Noguchi's discoveries become, therefore, of the greatest significance in respect to the epidemiology and causation of yellow fever and of infectious jaundice, previously confused one with the other. In all outbreaks of supposed yellow fever hereafter the existence of the two kinds of jaundice, one due to yellow fever virus and the other to leptospirae will have to be taken into account. Only the former probably is spread by mosquitoes and requires anti-mosquito measures for its control.

4. The only difference observed by us between the American and African strains of yellow fever virus was a pronounced difference in virulence for monkeys. The virulence of the two African strains studied was very high while that of the one American strain was highly variable and usually low.

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