

ETIOLOGY OF YELLOW FEVER.

V. PROPERTIES OF BLOOD SERUM OF YELLOW FEVER PATIENTS IN RELATION TO *LEPTOSPIRA ICTEROIDES*.

By HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, May 2, 1919.)

Following the isolation of *Leptospira icteroides* from a case of yellow fever a series of experiments was instituted with a view to establishing the relation between the organism and the disease. As stated elsewhere,¹ the organism reproduces in experimental animals all the symptoms and lesions observed in man. From the standpoint of immunity the question arose whether or not the serum of yellow fever convalescents would have a specific influence upon the organism.

Blood was drawn from the median basilic vein of the patient and the serum mixed with *Leptospira icteroides* in the form of organ emulsion from infected guinea pigs or culture and injected into the peritoneal cavity of a normal guinea pig. After a period of 30 minutes to 1 hour the fluid was drawn from the peritoneal cavity by means of a sterile capillary pipette and examined under the dark-field microscope (Pfeiffer's phenomenon). The animals were allowed to live until the results of the inoculations were evident. As Table I shows, in some of the experiments, in which the amount of the infectious emulsion or culture used was too large, no certain protection of the animal from the final fatal infection was obtained with the serum of convalescents, notwithstanding the fact that such serum produced a definite Pfeiffer phenomenon in the peritoneal cavity. When a smaller quantity of the infecting material was used in combination with convalescent serum most of the animals were saved, while the controls with serum from patients not suffering from

¹ Noguchi, H., *J. Exp. Med.*, 1919, **xxix**, 585.

TABLE I.

Effect of Serum from Yellow Fever Patients upon Leptospira icteroides.

Case No.	Sex.	Age.	Severity of infection.	Day on which serum was taken.	Amount of serum.		Leptospira icteroides. Strain 1.		Pfeiffer phenomenon.	Result.
							Liver emulsion.	Culture.		
		yrs.			cc.	cc.	cc.			
9	M.	19	Mild. Recovered.	2nd	1	0.2		-	Died in 8 days.	
9	"	19	" "	10th	1	2		+	Survived.	
2	"	23	" "	12th	2	2		+	"	
14	"	23	Severe. "	10th	2	2		<+	Died in 7 days.	
Control.	"	21	Malaria.		2	2		-	" " 7 "	
"	"	20	"		2	2		-	" " 7 "	
"					Saline, 1 cc.	2		-	" " 7 "	
"					Saline, 2 cc.	2		-	" " 6 "	
7	M.	21	Severe. Recovered.	16th	1	0.1		+	Survived.	
12	F.	40	" "	11th	2	0.1		+	"	
9	M.		Diagnosis (?). Recovered.	30th	2	0.1		-	Died in 6 days.	
Control.					Ringer, 2 cc.	0.1		-	" " 10 "	
"					Ringer, 2 cc.	0.1		-	" " 9 "	
16	M.	25	Severe. Recovered.	19th	1		1	<+	All these sera were separated from the clot in 48 hrs. and then kept on ice for 2 to 4 wks. when they were tested. The negative results with the sera of Cases 26 and 29 may be due to deterioration through age.	
26	"	21	" "	18th	2		1	-		
29	F.	34	Mild. "	13th	1		1	-		
19	M.	25	Severe. "	24th	2		1	+		
30	"	21	Mild. "	14th	2		1	+		
31	"	22	Moderate. "	20th	1		1	+		
23	"	16	" "	22nd	2		1	+		
32	"	20	Mild. "	15th	2		1	+		
33	"	11	" "	7th	2		1	+		
8	"	21	Severe. "	14th	2		1	+		
20	"	20	Moderate. " (also malaria).	13th	2		1	<+		
34	F.	30	Mild. Recovered.	12th	2		1	+		

yellow fever, and with saline solution died with the typical symptoms.

The number of cases studied (eighteen) was limited, owing to the pressure of more urgent problems under investigation at the same time. It seems, however, to have been sufficient to establish the specific reaction which exists between *Leptospira icteroides* and the serum of yellow fever convalescents (fifteen positive, 83 per cent). In the case of one patient the serum did not have any effect upon the organism when tested on the 2nd day of the illness but was protective on the 10th day.

Sera from malarial patients in no case showed any action antagonistic to *Leptospira icteroides*. The malarial patients were all mountaineers and consequently non-immune to yellow fever. The negative results obtained with sera derived from doubtful cases of yellow fever, all so mild as to make it difficult to recognize the disease, might have been due to the absence of antibodies, or, if there were any, to the fact that they were too weak to produce a definite reaction under the experimental conditions, or, a not impossible assumption, to the existence of a variation among many strains.

As will be described later, ten normal sera from healthy, non-immune soldiers² were also examined before the vaccination of the latter with killed cultures of *Leptospira icteroides*, but none of them had any effect upon the organism.

SUMMARY.

The serum from a number of persons recovering from yellow fever in Guayaquil was studied with a view to establishing its possible immunological relationship with a strain of *Leptospira icteroides* derived from one of the yellow fever patients. For this purpose the serum of convalescents was mixed either with an organ emulsion of a passage strain, or with a culture of the organism, and inoculated intraperitoneally into guinea pigs.

² I am indebted for these specimens to Dr. Carlos A. Miño, Assistant Director of the Department of Health at Quito, to Dr. E. Salgado V., also of the Department of Health, and to Dr. Víctor M. Bayas, Surgeon of the "Bolivar" Regiment.

The Pfeiffer reaction was first studied, and then the animals were allowed to live until the controls, inoculated with the same emulsion or culture of *Leptospira icteroides* but without the serum, or with serum from patients suffering from other diseases than yellow fever, had died of the experimental infection with typical symptoms. A positive Pfeiffer phenomenon was observed in fifteen of the eighteen convalescent cases studied, or approximately 83 per cent. Sera from ten non-immune soldiers and from two malaria patients gave uniformly negative results. Protection from an ultimate fatal infection was afforded some of the guinea pigs which received the serum of yellow fever convalescents, while the control animals succumbed to the infection with typical symptoms. In one instance, in which the serum was tested on the 2nd and the 10th days of disease, a Pfeiffer reaction was demonstrated, as well as protective property against the infection, in the specimen from the 10th but not in that from the 2nd day.

From the foregoing observations of immunity reactions it appears highly probable that *Leptospira icteroides* is etiologically related to yellow fever.