

sperm formation. This extract did not increase testis weights in the unoperated immature animal. Restoration and maintenance of spermatogenesis was successfully accomplished in the hypophysectomized mature rat. A marked seminal vesicle weight increase was observed in nearly all of the treated animals.

## 11732

### Immunological Distinction Between St. Louis and Japanese B Encephalitic Viruses.

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Experiments by Perdrau published posthumously indicated that St. Louis encephalitic virus, Strain No. 3, and Japanese B encephalitic virus, Strain No. 2, furnished by us, were immunologically identical.<sup>1</sup> These results are contrary to our previous findings<sup>2</sup> and hence have been checked in this laboratory.

Perdrau's technic was followed in preparing immune sera. The standard technic for setting up neutralization-tests used in both sets of experiments was continued. Serum-virus mixtures were tested after 4 hours according to our method, and again after 20 hours according to Perdrau's method.

We found at once that immune sera prepared as above<sup>1</sup> were equal or superior to ours in neutralizing capacity and that 20 hours' standing reduced the activity of the serum-virus mixtures one hundred- to one thousandfold, yet brought out any neutralizing effect more dramatically. Most important, however, was the ready confirmation of Perdrau's results with St. Louis No. 3 and Japanese No. 2 strains (Table I). Further tests in animals showed that the St. Louis strain had retained its distinguishing characteristics, whereas the Japanese virus behaved in every way like the St. Louis virus. Questions arose, therefore, as to whether (a) our previous findings<sup>2</sup> were erroneous, (b) the Japanese virus had become altered through repeated passage, or (c) the Japanese virus had at some time been erroneously labeled.

That an error had occurred in labeling was indicated by the following tests. Seven strains of virus were secured from outside

<sup>1</sup> Perdrau, J. R., *J. Path. and Bact.*, 1940, **50**, 545.

<sup>2</sup> Webster, Leslie T., *J. Exp. Med.*, 1938, **67**, 609.

TABLE I.  
Complete Cross-Neutralization of St. Louis No. 3 and Japanese No. 2 Viruses.

Virus	Serum	Incubation hr	Fate of mice injected intracerebrally with serum-virus mixtures in dilutions					Neutralization in MLD
			10-2	10-3	10-4	10-5	10-6	
St. Louis No. 3	Normal*	4	4/4†	4/4	4/4	4/4	4/4§	
	Equine encephalomyelitis		4/4	4/4	4/4	4/4	4/4	6
	Rabies		4/4	4/4	4/4	3/3	4/4	0
	St. Louis No. 3		4/4	4/4	4/4	1/4	0/4	10-100‡
	Japanese No. 2		4/4	4/4	3/4	1/4	0/4	10-100
	Normal	20	4/4	4/4	4/4	1/4	0/4	
	" brain		4/4	4/4	4/4	1/4	0/4	0
	Equine encephalomyelitis		4/4	4/4	4/4	2/4	0/4	0
	Rabies		4/4	4/4	4/4	3/4	0/4	0
	St. Louis No. 3		1/4	0/4	0/4	0/4	0/4	100+
Japanese No. 2	Japanese No. 2		1/4	0/4	0/4	0/4	0/4	100+
	Normal	20	4/4	4/4	3/4	0/4	1/4	
	" brain		4/4	4/4	2/4	1/4	0/4	0
	Japanese No. 2		0/4	0/4	0/4	0/4	0/4	100+
	St. Louis No. 3		0/4	0/4	0/4	0/4	0/4	100+
	" " Broun '33		0/4	0/4	0/4	0/4	0/4	100+
	" " "		0/4	0/4	0/4	0/4	0/4	100+
	convalescent 434		4/4	1/4	0/4	0/4	0/4	10-100
	St. Louis convalescent 435		4/4	1/4	0/4	0/4	0/4	10-100
	St. Louis convalescent 437		4/4	4/4	0/4	0/4	0/4	1-10

\*Normal = Normal rabbit serum.

Equine encephalomyelitis = Rabbit immunized with mouse brain equine encephalomyelitic virus.

Rabies = Rabbit immunized with mouse brain rabies virus.

St. Louis No. 3 = Rabbit immunized with mouse brain St. Louis No. 3 virus.

Japanese No. 2 = Rabbit immunized with mouse brain Japanese No. 2 virus.

Normal brain = Rabbit immunized with normal mouse brain.

†4/4 = Four of 4 tested mice died from the experimental infection.

‡1 MLD = Highest dilution fatal to 50% or more of controls.

§The dilution in italics signifies the titration-end point, 1 MLD.

TABLE II.  
Partial Cross-Neutralization of St. Louis No. 3 and Broun 1933 Viruses.

Virus	Serum	Incubation hr	Fate of mice injected intracerebrally with serum-virus mixtures in dilutions					Neutralization in MLD
			10-2	10-3	10-4	10-5	10-6	
St. Louis No. 3	Normal	20	4/4	4/4	4/4	1/4	0/4	
	" brain		4/4	4/4	4/4	1/4	0/4	0
	St. Louis No. 3		4/4	1/4	0/4	0/4	0/4	10-100
	" " Broun '33		3/4	0/4	0/4	0/4	0/4	10-100
St. Louis Broun '33	Normal		4/4	4/4	4/4	0/4	0/4	
	" brain		4/4	4/4	4/4	0/4	0/4	0
	St. Louis Broun '33		1/4	0/4	0/4	0/4	0/4	100+
	" " No. 3		4/4	4/4	0/4	0/4	0/4	1-10

See Table I for explanation of footnotes.

TABLE III.  
Lack of Cross-Neutralization Between St. Louis and Japanese B Encephalitic Strains.

Virus	Serum	Incubation hr	Fate of mice injected intra-cerebrally with serum-virus mixtures in dilutions							Neutralization in MLD
			10-2	10-3	10-4	10-5	10-6	10-7		
St. Louis No. 3	Normal	20	4/4	4/4	0/4	0/4	0/4	—		
	St. Louis No. 3		0/4	0/4	0/4	0/4	0/4	—	10+	
	Japanese Nakayama		4/4	3/4	1/4	0/4	0/4	—	0	
Japanese Nakayama	Normal	4	4/4	4/4	4/4	4/4	4/4	1/4		
	St. Louis No. 3		4/4	4/4	4/4	4/4	4/4	1/3	0	
	St. Louis convalescent		4/4	4/4	4/4	4/4	3/4	0/3	0	
	Normal	20	4/4	4/4	3/4	2/4	1/4	0/4		
	St. Louis No. 3		4/4	4/4	4/4	2/4	0/4	0/4	0	
	St. Louis convalescent		4/4	4/4	3/4	0/4	0/4	0/4	1-10	
Japanese Nakayama	Normal	20	4/4	4/4	0/4	0/4	0/4	0/4		
	Nakayama		0/4	0/4	0/4	0/4	0/4	0/4	10+	
	St. Louis No. 3		2/4	0/4	0/4	0/4	0/4	0/4	1-10	
Jap. Sasaki	Normal	4	4/4	4/4	4/4	3/4	0/4	0/4		
	St. Louis No. 3		4/4	4/4	4/4	1/4	0/4	0/4	1-10	
	Normal	20	4/4	4/4	2/4	1/4	0/4	0/4		
	St. Louis No. 3		4/4	2/4	0/4	1/4	0/4	0/4	1-10	
Jap. Sakurai	Normal	20	4/4	4/4	0/4	0/4	0/4	0/4		
	Japanese Nakayama		0/4	0/4	0/4	0/4	0/4	0/4	10+	
	St. Louis No. 3		4/4	4/4	0/4	0/4	0/4	0/4	0	
Jap. Sawatani	Normal	20	4/4	4/4	2/4	0/4	—	—		
	Japanese Nakayama		0/4	0/4	0/4	0/4	—	—	100+	
	St. Louis No. 3		4/4	3/4	0/4	0/4	—	—	1-10	
Jap. Calinina			10-1	10-2	10-3	10-4	10-5	10-6	10-7	
	Normal	20	—	4/4	4/4	1/4	0/4	—	—	
	Japanese Nakayama		0/4	0/4	0/4	0/4	—	—	—	
	St. Louis No. 3		4/4	3/4	1/4	0/4	—	—	—	

See Table I for explanation of footnotes.

— = Dilution not tested.

sources. Dr. G. O. Broun kindly supplied St. Louis strains from the 1933 and 1937 epidemics, Dr. E. H. Lennette, the Japanese strains Nakayama and Sasaki, Dr. R. Kobayashi, the Japanese strains Sakurai (1939) and Sawatani (1939), and Dr. T. Mitamura the Japanese strain Calinina (1935). These viruses proved free of lymphocytic choriomeningitic virus.

In animals the strains behaved according to previous descriptions<sup>2</sup> except that the response of *Macacus rhesus* monkeys to the Japanese strains was somewhat less explosive.

Immune rabbit sera were prepared with the St. Louis Broun 1933 strain and the Japanese Nakayama strain.

Neutralization tests shown in Table II indicate that St. Louis No. 3 virus was neutralized equally well by homologous and St. Louis Broun 1933 immune sera (10 to 100 M.L.D.), but that the Broun strain was less effectively neutralized in the St. Louis No. 3 (1 to 10 M.L.D.) than in the homologous immune sera (10 to 100 M.L.D.). Thus there appeared slight immunological differences between the 2 St. Louis strains.

Table III shows that (1) St. Louis No. 3 virus was not neutralized by the Japanese Nakayama immune serum and (2) the 5 Japanese strains were neutralized either not at all or only in the M.L.D. dilution by St. Louis No. 3 immune serum and human St. Louis convalescent sera.

Mice immunized with St. Louis No. 3 virus were not immune to as little as 1 M.L.D. of the Nakayama virus.

Thus, on present evidence, we regard the relationship between St. Louis and Japanese B virus to be as stated in 1938.<sup>2</sup> They are closely related with respect to the epidemiology of the diseases which they produce. Together with louping-ill and equine encephalomyelitic viruses, they possess similar filterability-characteristics with Elford membranes. In animal species, St. Louis, Japanese, and louping-ill viruses show general similarity and Japanese B and louping-ill viruses appear identical. And finally, St. Louis, Japanese, and louping-ill viruses, in spite of an occasional cross-neutralization not exceeding 1 to 10 M.L.D., remain immunologically readily distinguishable.

### 11733

#### Androgenic Assay of the Human Fetal Adrenal.\*

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It is indicated from the study of castrate men and women that there is an extragonadal source of sex hormones of some abundance. This was suspected to be the adrenal long before the actual demonstration by Reichstein<sup>1</sup> that small amounts of androgenic material

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\* The autopsy material for this study was collected at the Baltimore City Hospitals and the Johns Hopkins Hospital, Baltimore.

<sup>1</sup> Reichstein, T., *Helv. Chim. Acta*, 1936, **19**, 223.