

be due to a time factor since their experimental procedure consisted in the injection of 250 mg of estradiol on 4 consecutive days followed by a single injection of 5 to 10 mg of progesterone on the following day.

Consequently 12 virgin guinea pigs weighing approximately 200 g were castrated and hysterectomized under ether anesthesia. A period of 17 days was allowed to elapse between the operation and the initiation of the hormonal injections to assure the complete recovery of the animal and the recession of all sexual characteristics. At this time subcutaneous injections of estrogen were made for 7 days followed by a period of estrogen-progesterone treatment. Dosages and details

of administration are listed in Table I. Relaxation of the pelvic ligaments occurred in all injected animals between the 15th and 19th day of hormone administration.

The last 4 experimental animals were castrated and hysterectomized but received no hormone treatment. None of these animals showed any relaxation during the post-operative period of 37 days.

It appears from this experiment that castrated and hysterectomized virgin guinea pigs when given progesterone after an initial period of estrogen treatment show relaxation of the pelvic ligaments in the complete absence of both ovaries and uteri.

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Natural Resistance and Susceptibility to Russian Spring-Summer Encephalitis in Mice.*

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Webster¹ demonstrated that resistance and susceptibility in mice to *B. enteritidis* are inherited independently of resistance and susceptibility to St. Louis encephalitis virus in the same species. Consequently, by inbreeding and selection from a hybrid stock, he was able to derive 4 strains of mice exhibiting the 4 possible combinations of resistance and susceptibility to these two pathogens. For convenience these stable, uniform, inbred strains were designated as follows: Bacteria-resistant-virus-susceptible (BRVS) and, in the same manner, the three remaining strains, BRVR, BSVS, BSVR. These designations were valid as well in terms of the louping-ill virus in the instance of the BSVR and BRVS

lines,² but all strains were uniformly susceptible to mouse passage rabies virus,¹ to vesicular stomatitis virus,³ and to lymphocytic choriomeningitis virus.⁴ At the present time, then, designation of these strains of mice as "virus-resistant" or "virus-susceptible" is restricted to the viruses of St. Louis encephalitis and louping-ill.

Recently, Casals and Webster⁵ have demonstrated the existence of a close relationship between the viruses of louping-ill and Russian spring-summer encephalitis, a relationship based on similarities in (1) pathogenicity for various hosts, (2) complement-fixing antibody formation, (3) neutralizing antibody formation, and (4) cross-resistance following vaccination. The question arose as to whether this relationship could be further defined to include (5) natural resistance. This was

* These investigations were aided through the Commission on Neurotropic Virus Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.

¹ Webster, L. T., *J. Exp. Med.*, 1937, **65**, 261.

² Webster, L. T., *J. Exp. Med.*, 1933, **57**, 819.

³ Olitsky, Peter K., Sabin, Albert B., and Cox, Herald R., *J. Exp. Med.*, 1936, **64**, 723.

⁴ Casals, J., unpublished data.

⁵ Casals, J., and Webster, L. T., *Science*, 1943, **97**, 246.

TABLE I.
Inoculation of Russian Spring-Summer Encephalitis Virus in Different Strains of Mice.

Strain of mice	Fate of mice following subcutaneous injection of Russian spring-summer encephalitis virus in dilution:					
	10-4	10-5	10-6	10-7	10-8	10-10
BRVR	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S
BSVR	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S	S,S,S,S
BSVS	8,8,8,9	9,9,9,10	9,9,15,S	10,10,12,S	9,10,12,S	S,S,S,S
BRVS	8,8,8,8	7,8,8,9	7,8,8,9	8,8,9,9	8,9,11,12	S,S,S,S
W-Swiss		9,9,11,11	8,9,10,13	8,9,13,S	9,12,12,S	15,S,S,S

TABLE II.
LD₅₀ and Resistance Indices of Mice Following Injection of Russian Spring-Summer Encephalitis Virus by Subcutaneous or Intracerebral Routes.

Strain of mice	LD ₅₀ % (intracerebral)	Resistance index (intracerebral)	LD ₅₀ % (subcutaneous)	Resistance index (subcutaneous)
BRVR	<10-3	>2,000,000	<10-2	>10,000,000
BSVR			<10-4	>100,000
BSVS			10-8	10
BRVS	10-9.8	--3	10-8.3	5
W-Swiss	10-9.3	1	10-9	1

readily determined by testing the resistance and susceptibility of the 4 Webster strains mentioned above.

A number of tests were carried out in the following manner: Serial tenfold dilutions of mouse brain infected with Russian spring-summer encephalitis virus were made up in normal rabbit serum in the usual way, using physiological saline solution as diluent; then batches of mice from different strains were tested subcutaneously or intracerebrally by injection of 0.5 cc and 0.03 cc respectively of the several dilutions of virus. Unselected W-Swiss mice were included in each test as known susceptible controls. Four mice per dilution were infected and all animals employed in the tests were of the same age. Following injection the mice were kept under observation for 28 days and the survivors discarded.

Table I shows the results of one of the experiments. The figures in each column indicate the day of death of the mice; "S" represents those that survived the infection.

Other tests carried out under comparable conditions gave similar results which are sum-

marized in Table II. This table gives the LD₅₀ (50% mortality endpoint dilution) obtained by using the Reed and Muench method,⁶ as well as the Resistance Index (maximum number of fatal doses resisted) with relation to the control W-Swiss mice, whose Resistance Index is 1.

Since Resistance Indices of 10 or less are not significant, the values obtained in the tests indicated that BSVS and BRVS mice were susceptible to inoculation of Russian spring-summer encephalitis virus to the same extent as W-Swiss unselected mice, while BSVR and BRVR mice were resistant to inoculation of more than 2,000,000 intracerebral doses and to more than 10,000,000 subcutaneous doses of virus lethal to either W-Swiss or BSVS and BRVS strains.

Conclusion. Strains of mice selectively bred for susceptibility and resistance to louping-ill and St. Louis encephalitis viruses proved to be similarly susceptible and resistant to subcutaneous or intracerebral inoculation of Russian spring-summer encephalitis virus.

⁶ Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.