Role of Heredity in Experimental Disseminated Encephalomyelitis in Mice. (20906)

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Differences in the susceptibility of various stocks of mice to experimental encephalomyelitis have been noted(1-3). These observations prompted a systematic examination of mouse stocks of more certain genotypic composition, with the aims of 1) probing the possible genetic limits of the susceptibility-resistance spectrum of mice to this malady and 2) examining the mode of inheritance of such differences in susceptibility as was feasible. It is the purpose of the present paper to report the results of this examination.

Methods and materials. 1) *Test for susceptibility to experimental encephalomyelitis.* Mice were tested at the age of 6-8 weeks by a series of 6 weekly subcutaneous injections of proteolipide A and B(4,5) prepared from mouse brains. This chemical fraction which has been found to contain the encephalitogenic activity(3,4) was mixed with a Freund-type adjuvant, following precisely the methods already described in detail(3). All animals were observed daily for manifest signs of the malady, and if they showed no sign 12-15 days after the sixth injection their brains were removed and examined for histopathological lesions. Positive signs were confirmed by pathological findings. Animals were scored as either positive reactors, or nonreactors, *i.e.*, as susceptible or resistant. 2) *Mouse genotypes examined.* Four inbred lines were available. These lines were obtained by selective brother-sister mating of Rockefeller Institute mice by Dr. Webster of this Institute, as already described(6). Since Dr. Webster's death in 1943 they have been continued in uninterrupted brother-sister mating by one of us (H.A.S.). Selected on the basis of progeny tests, these 4 lines represent the possible combinations of resistance and susceptibility to a bacterial disease, salmonellosis, and a virus disease, St. Louis encephalitis, and are as follows:

1. BRVR (bacteria resistant, virus resistant)
2. BRVS (*idem* susceptible)
3. BSVS (" susceptible, " ")
4. BSVR (*idem* resistant)

These mice have been maintained in the breeding colony on a modified Steenbock stock diet(6). Progeny were weaned at 4 weeks of age and either retained in the breeding room for subsequent matings or removed for

TABLE I. Disseminated Encephalomyelitis Developing in 4 Inbred Mouse Lines following 6 Serial Subcutaneous Injections of Mouse-Brain Proteolipide A + B.

Mouse line	Generations inbred	No. pos./ No. tested
BRVR	33	0/15
BRVS	35	2/15
BSVR	44	8/14
BSVS	44	15/15

TABLE II. Disseminated Encephalomyelitis Developing in F_1, F_2 and Backeross Progeny.

Generation	Exp.	Mating	No. pos./ No. tested	Total
Parent BSVS	1	—	57/ 57	57/ 57
BRVR	2	—	0/ 42	0/ 42
F_1	3	BRVR ♀ ♀ × BSVS ♂ ♂	0/ 7	0/ 40
	4	"	0/ 12	
	5	BSVS ♀ ♀ × BRVR ♂ ♂	0/ 10	
	6	"	0/ 11	
F_1, BSVS backcross	7	BSVS ♀ ♀ × F_1 ♂ ♂	15/ 41	15/ 41
F_1, BRVR "	8	BRVR ♀ ♀ × F_1 ♂ ♂	1/ 38	1/ 38
F_2	9	F_1 ♀ ♀ × F_1 ♂ ♂	15/231	15/231

TABLE III. Statistical Analysis of the Genetic Data.

Generation	Hypothetical resistance factors required	Res. : Susc. Expected	Res. : Susc. Found	Freq. of susc. Expected	Freq. of susc. Found	X^2	P
F_1 backcross to BSVS	1	1 : 1	26 : 15	.50	.37	1.006	>.3
	2, both	1 : 3	"	.75	"	10.76	<.01
	2, either one	3 : 1	"	.25	"	0.797	>.3
	3, any one	7 : 1	"	.125	"	5.19	<.03
F_2	1	3 : 1	216 : 15	.25	.0649	28.4	<.001
	2, both	9 : 7	"	.44	"	85.22	"
	2, any 2	11 : 5	"	.313	"	46.2	"
	2, either one	15 : 1	"	.0625	"	.00009	>.99

test. While on test the mice were fed a diet of commercial fox chow, bread and milk, and water. Matings of suitable genotype for purposes of genetic analysis were made, and are itemized below.

Experimental. Susceptibility tests on the 4 inbred mouse stocks are summarized in Table I. It is clear from this that BRVR mice were found to be 100% resistant and BSVS mice were 100% susceptible. The BRVS and BSVR mice were mixed in their response, for causes which have been left uninvestigated temporarily, and were excluded from further consideration. The BRVR and BSVS stocks were therefore selected as suitable material for a genetic analysis of the observed difference (100%) in susceptibility.

Crosses were made in which BRVR mice were mated with BSVS mice of both sexes, as shown in Table II. It is to be noted that the F_1 hybrids were uniformly resistant, indicating that whatever the number of genetic factors involved those conferring resistance were dominant.

To determine the number of genetic factors and the mode of their inheritance, F_1 hybrids were mated *inter se* and also backcrossed to the parental lines. As Table II shows, the F_2 exhibited a 216:15 ratio of resistants to susceptibles, or a susceptible frequency of 0.0649. As indicated in Table III, this ratio is consistent ($P > 0.99$) with the hypothesis that two independent factors for resistance were segregating, the presence of either of which could confer resistance. The data here presented on the F_1 backcross to the susceptible parental line (BSVS) are insufficient to distinguish between the segregation of one or 2 factors, but do serve to exclude the participation of 3 or more factors.

Discussion. The resemblance in several features between experimental disseminated encephalomyelitis in various animal species, including mice, and certain of the demyelinating encephalitides of man, such as acute disseminated encephalomyelitis and multiple sclerosis, has been pointed out by Wolf, Kabat and Bezer(7) and by Ferraro and Cazzullo (8). Pratt *et al.*(9) have suggested that inheritance plays a role in the incidence of human disseminated sclerosis.

The data presented here indicate that in these mice the resistance to experimental acute disseminated encephalomyelitis is under genetic control. The details of this control are consistent with the hypothesis that two unlinked genetic factors are operative: the presence of either of these factors is sufficient to confer resistance and since both are dominant, resistance to the experimental disease is apparently effected by a single genetic factor.

Conclusion. An examination of 4 lines of inbred mice for susceptibility to experimental acute disseminated encephalomyelitis revealed a resistant line, BRVR, and a susceptible line, BSVS. The simplest explanation of the data here presented is that the 2 appear to differ by two resistance-conferring genetic factors either of which is adequate to bring about resistance.

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Effect of Continuous Injection of Glucagon upon Glycosuria of Partially Depancreatized Rats. (20907)

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Glucagon is a hyperglycemic principle occurring in extracts of pancreas. In the present study the continuous intravenous injection of glucagon caused a temporary increase in the urinary glucose of the mildly diabetic rat.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they were partially depancreatized at a weight of 275 to 280 g. After reaching a weight of 300 g they were force-fed a medium carbohydrate diet(1) by stomach tube each morning (8:30 to 9:00 a.m.) and afternoon (4:15 to 5:00 p.m.) Each rat was placed in a metabolism cage which restricted its activity so that the animal was unable to reverse its position. Each 24-hour dose of highly purified glucagon(2) was contained in one ml of physiological saline and was administered continuously by either intra-jugular or subcutaneous injection. A fresh solution was prepared every 24 hours.

It was found to retain its glycogenolytic potency during the period of administration. Saline only was injected during the control periods. Attempts to preserve asepsis were not uniformly successful. Infection of the vein at the site of the indwelling needle was found in about 40% of the animals at autopsy. The data of this report represent only those animals in which intravenous infusion was successfully maintained throughout the experiment. Urine was collected at the same hour (8:00 to 8:30 a.m.) each day for the quantitative determination of glucose(3).

Experiment. In Exp. 1, six normal rats were given glucagon by continuous subcutaneous injection in doses of 0.05 mg the 1st day, 0.1 the 2nd day, 0.2 the 3rd day, 0.5 the 4th day, and 1.0 mg the 5th day. These rats remained free from glycosuria during the control periods and during the injection of glucagon.

In Exp. 2, six mildly diabetic partially depancreatized rats were given glucagon by continuous subcutaneous injection every other day. Doses of 0.05, 0.1 and 0.2 mg per rat per day were tested with negative results. The experiment was extended on 5 similar animals using doses of 0.05, 0.1, 0.2 and 0.5 mg of glucagon per rat per day with negative results.

In Exp. 3, six mildly diabetic partially depancreatized rats were given glucagon by continuous intra-jugular injection at a dosage of 0.5 mg/rat/day for 8 days. In each rat there was a temporary increase in urinary

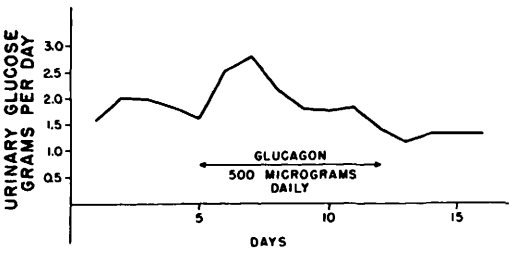

FIG. 1. Effect of continuous intravenous injection of glucagon upon glycosuria of 6 partially depancreatized rats. Average.

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