

Strain and Sex Differences in Response of Inbred Mice to Adrenal Cortical Hormones.* (19730)

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Function of the adrenal cortex in both animals and man has been measured by the degree of eosinopenia which follows its stimulation(1,2). Speirs and Meyer(3,4), developed a bioassay for cortical hormones based upon this response in mice. Their technic is highly specific for corticoids, sensitive to doses as low as 0.5 μ g and can be used for quantitative estimation. The assay is sensitive, rapid, and easily performed.

This report demonstrates that mouse strains differ in their responses to cortisone; thus for accurate assays it is imperative to use certain inbred strains, highly sensitive to cortisone, rather than mice selected at random.

Method. Male mice of 10 inbred strains or their hybrids, and females of 2 strains were tested by the method of Speirs and Meyer mentioned above. Inbred mice were adrenalectomized in a one-step operation and pellets of 11 desoxycorticosterone acetate implanted subcutaneously. A few days postoperatively, mice received a subcutaneous injection of epinephrine, followed in 4 hours by a dose of adrenal cortical hormone. Eosinophil counts were taken immediately before and 3 hours after injection of the corticoid. The percentage of decrease in number of eosinophils during this period was correlated with quantity of hormone injected.

Precautions were taken to stabilize environmental factors. Mice chosen were similar in age (2 months) and weight (20-25 g), and were bled usually the third and tenth day after adrenalectomy. For at least 20 hours previous to and during the run mice were kept in a dark, constant-temperature cabinet (80°F).

Preliminary experiments showed that the amount of adrenalin needed to eliminate non-specific responses varied with strains. Three to 5 μ g for C57BR/cd, C57BR/a, C57BL/6,

and BR/cd X BL/6 hybrids (called BBF₁) was found effective if the animals were newly adrenalectomized but not always so 3 to 4 weeks later. When the dose was increased to 20 μ g all such non-specific responses were eliminated without producing highly toxic effects so this dose was adopted for these strains. With 129/Rr's 10-20 μ g was used, DBA/1's required 30, and B ALB/c's 40 μ g to eliminate non-specific responses. Larger doses such as 20 μ g for 129's and 40 μ g for B ALB/c's were probably responsible for occasional difficulty in bleeding or death.

Hormones, injected subcutaneously, were: .03 cc of cortisone acetate, dissolved in benzyl alcohol diluted with at least 9 volumes of sesame oil; .5 cc of cortisone (free alcohol), dissolved in distilled water.

Results. Strain differences. Strains injected with cortisone acetate and cortisone (free alcohol), are included in this comparison, because as Fig. 1 (plotted from data of Speirs and Meyer(4)) shows no significant difference occurred in response to these 2 types of hormone preparation in one strain tested—

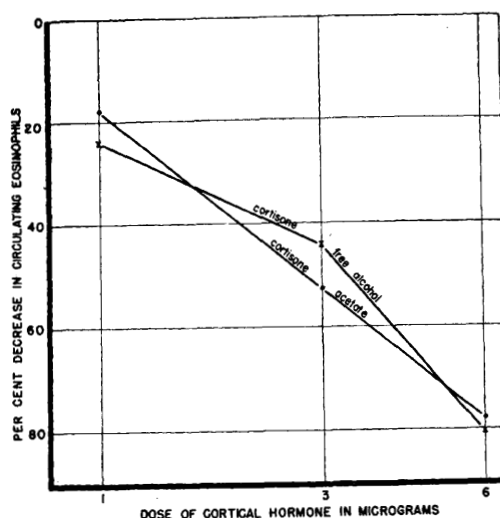


FIG. 1. Three-hour response of adrenalectomized male C57 brown ed mice to cortisone acetate; cortisone (free alcohol).

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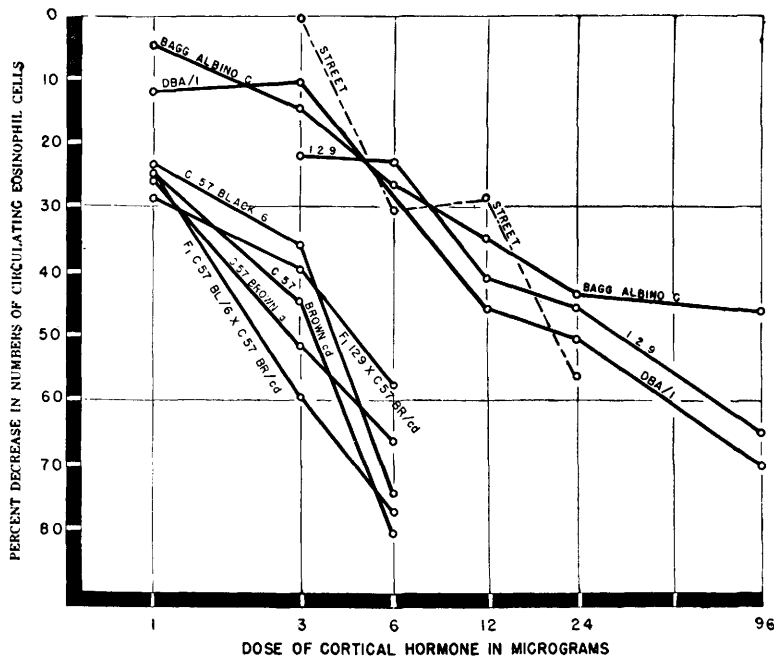


FIG. 2. Three-hour response of adrenalectomized inbred strains to adrenal cortical hormone.

C57BR/cd. The similar effects of these preparations have also been observed in BBF₁ (unpublished).

The average responses of 9 strains tested are plotted in Fig. 2. Table I gives the number of animals per point, standard deviation, standard error, and slope of each curve. (Method given by Dorfman(5)). From the figure it is apparent that responses fall into 2 distinct groups. 1. Sensitive strains: respond significantly to 1 µg and maximally to approximately 6 µg of cortisone. (C57BL/6, C57BR/cd, C57Br/a, BBF₁, F₁ 129 X BR/cd). 2. Insensitive strains: require 3-6 µg for a significant response and at least 96 µg to approach maximum eosinopenia. (129/Rr, DBA/1, B ALB/c, Street).

An additional type is represented by the strain Small, in which cells are too few to use in calculations. Of 24 counts made, 64% had less than 60 eosinophils per mm³ blood.

Variability of response according to dose and strain is brought out in Table I. The standard deviation of most groups of animals in sensitive strains is comparatively small (from 3.5 to 16) for the high dose of 6 µg. For the same dose, in insensitive strains, it is

from 16-23. Strains such as the Street, F₁ BR/cd X 129, and C57BL/6 seem more variable than others.

Sex differences. Table II shows the percent decrease in circulating eosinophils of females (BBF₁, C57BR/cd) to cortisone (free alcohol) and cortisone acetate is less consistent than that of males. In addition, their standard deviation is generally greater except for the 3 µg injection of cortisone acetate, but this response is much smaller than for male animals.

Eosinopenia and behavior under stress. The C57BR/cd is an active mouse which jumps, squeals, and tries to bite when handled. It gives maximum eosinopenia after 6 µg of cortisone—a highly sensitive response. On the other hand, the 129/Rr although active, is a mild-mannered mouse which is easily handled and rarely if ever attempts to bite. Thus it is a good laboratory animal. Furthermore its light color makes tail veins easy to see and it bleeds well. Unfortunately for assay work, it is less sensitive to cortisone, requiring a dose 16 times as large as the Brown to give maximum eosinopenia.

Fig. 3 shows the hourly response of normal

TABLE I. Standard Deviation, Standard Error, and Slope of Response of Some Inbred Mouse Strains Injected with Cortical Hormone.

Strain	Cortisone used	Dose											
		1 μ g			3 μ g			6 μ g			12 μ g		
		Avg resp.	S.D.	S.E.	Avg resp.	S.D.	S.E.	Avg resp.	S.D.	S.E.	Avg resp.	S.D.	S.E.
C57 BR/a	Acetate	25	18.5	7.7	51	10.9	5	66	16.5	7.5			
C57 BR/cd	Free alcohol	24	22.8	9.5	44	27	11	80	3.4	1.4			
C57 BL/6	Acetate	23	35.9	15	35	31.6	12.8	74	15.4	5.9			
BBF ₁	"	22	14.4	6.5	59	23	8.8	77	8.8	3.4			
129 X BR/cd	Free alcohol	28*	25.7	14.8	39	18	6.4	57	21.4	8.2			
B ALB/c	Acetate	4	8.5	4	14	17.5	7.3	26†	16.2	5.1	34	11.8	5
DBA/1	"	11†	13.7	4.6	10	10	3.6				45†	18.5	8.4
129/Rr	"				21	16.1	6.7	22	22.9	9.5	40	20	6.2
Street	"				0	0	0	30	23.1	9.6	28	31.6	13
											56	13.9	6.3
											46†	27.7	8.7
											70	5.9	2.7
											66	19.3	6.9
													64.9
													41.5
													68.4
													76
													81.8
													86.9
													12.7
													20.3
													23.2
													64.9

Note: Usually 6 animals per point.

* 3 animals.

† 9-12 animals.

TABLE II. Sex Differences in Response to Cortisone.

Strain (adrenalectomized)	Dose in μ g cortisone	Avg % decrease in eosinophils		No. of animals		Stand. dev.		Stand. error	
		δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ
F ₁ hybrid	1 (free alc.)	19	20	7	6	19.1	17.4	7.3	7.3
	3 " "	62	40	7	6	11.6	16	4.8	6.7
C57 BL/6	6 " "	73	59	6	6	7.9	15.1	3.4	6.3
C57 BR/cd	1 (acetate)	14	7	8	9	15.6	12.4	5.6	4.3
	3 " "	48	12	6	6	21.4	7.1	7.6	3
(BBF ₁)	6 " "	64	37	12	11	15.1	24.2	4.3	7.3
C57 BR/cd	1 (free alc.)	7	28	6	4	11.8	24.4	5	12.2
	3 " "	64	78	3	3	14.8	12	8.7	7
	6 " "	80	87	6	4	3.5	4.8	1.4	2.4

animals to stress. Under mild stress of handling (warming and taking blood from a nick in the tail vein) the number of circulating eosinophils in the 129 is not disturbed nearly so much as in the Brown. The 129 shows a decrease for only one hour, rather than 4, and reaches a maximum of —31% in contrast with —85% in Browns. When stress in 129's is increased by injecting 10 μ g of epinephrine, their response is almost identical with that of the mildly stimulated Browns. If further increased to 20 μ g, the same rapid decrease occurs in 4 hours, but the numbers remain low for a longer period, with a decrease of 64% still present at 8 hours (not plotted).

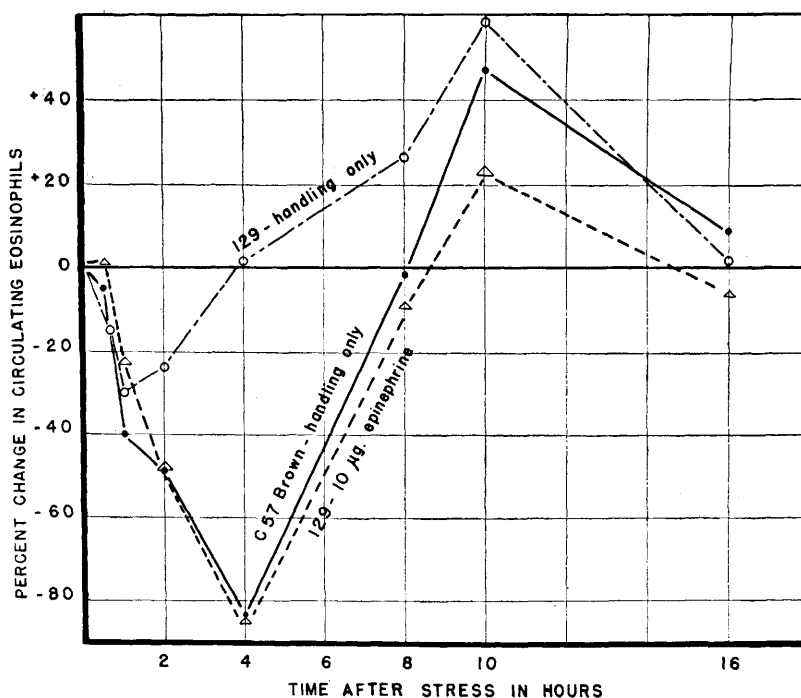


FIG. 3. Hourly response of normal 129 and C57 brown ed male mice to stress.

It should not be concluded that quiet disposition and poor sensitivity to injected cortisone are related. The DBA, an extremely active strain, is one of the least sensitive to injected cortisone.

Discussion. Two factors to be considered in comparing strains are sensitivity and variability.

When a strain sufficiently sensitive to detect physiological amounts of hormone is used for assay, the work and sources of error inherent in concentration processes may be reduced or eliminated. Sensitive strains frequently give satisfactory responses when non-extracted materials such as .5 cc urine from ACTH-treated patients is injected.

Less variability occurs as maximum eosinopenia is approached. Thus in sensitive strains 4 to 6 animals injected with 6 µg of cortisone give a very strong and reliable point, whereas smaller doses generally give values with more variability. With insensitive strains 24-96 µg are required to produce an eosinopenia large enough to manifest the reduced variability. (Note the exception that B ALB/c's are most variable at 96 µg).

Female mice have been found to respond like males by some workers. A few normal females (Tumblebrook Farm strain) gave an eosinopenia similar to that of males when injected with ACTH or foreign protein(6), and adrenalectomized females have been used for assay(7). Experience at the Jackson Laboratory indicates that females sometimes give results similar to males, but generally they are less reliable (Table II).

In choosing mice for the cortisone assay based on a decrease in circulating eosinophils, it must be obvious that non-inbred strains are less satisfactory because responses differ according to genetic constitution. In inbred strains, where individuals are genetically identical, this serious variable is eliminated. Their use also markedly reduces the number of controls necessary for assays.

After considering all factors, the B6F₁ male seems the best animal to use for assay work. Although it is rather wild and its veins are difficult to see due to the dark tail, it is very sensitive to cortisone, and its hybrid vigor shown in large litter production and resistance to toxic materials makes it economically the

preferable strain.

Summary. Adrenalectomized mice varied in response to cortisone according to genetic constitution. Some strains were: a) highly sensitive. They responded significantly to 1 μ g of cortisone, and maximally to 6 μ g. b) Relatively insensitive. They responded to 3-6 μ g but required 16 times the dose of a sensitive strain to approach maximum response. c) Too low in eosinophils to be practicable in calculations. Males were generally more reliable than females. Normal animals of 2 strains differed in response to mild stress. F₁ hybrid males from C57BR/cd X C57BL/6 are recommended for the assay described.

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Effects of Antibiotics on Daphnia.*† (19731)

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In his recent studies of hemoglobin formation in *Daphnia*, Fox has devised a method for estimation of the blood pigment in the living animal and shown that its concentration increases in response to lowering of the oxygen tension of the environment(1). It would appear that the hemoglobin system of such small aquatic animals may provide a useful tool for the study of certain problems of hemoglobin metabolism, among them aspects of nutrition. Interest in the adaptation of *Daphnia* to this kind of investigation has led to an attempt to cultivate the animals free of the natural microflora of the intestine, which, through synthetic

or degradational enzymatic activities, could preclude certain types of experiments. At the same time, it was recognized that success in the attempt might well create a nutritional problem, inasmuch as living bacteria in the gut will doubtless be necessary for normal growth of numerous species, including Cladocera(2), until their vitamin and other requirements have been more fully defined. Accordingly, it was decided to try to eliminate bacteria and fungi by the use of various combinations of antibiotics. The results that relate especially to the nutrition of *Daphnia* will be reported elsewhere. Meanwhile, in the course of the work it appeared that the tolerance of *Daphnia* for some chemotherapeutic agents parallels that of man. This fact, together with the several convenient qualities of *Daphnia* as a laboratory subject suggested that the findings might be of interest from a pharmacological point of view, and could perhaps be of use to those concerned with the toxicity of antibiotics.

An obvious deficiency of the microcrustacea

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