Effects of Grouping on Levels of Circulating Antibodies in Mice. (28883)

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The purpose of this work was primarily to investigate the effects of grouping on antibody titer, and secondarily, to study the effects of social rank on antibody titer. Since under some circumstances adrenocortical hormones inhibit antibody responses, and since various stressors, such as grouping, may increase the output of these hormones, grouped mice should respond to a particular antigen with a lower titer of antibody than should isolated mice.

Adrenocortical hormones affect host resistance through several mechanisms. Antibody responses to various antigens were inhibited by cortisone in rabbits(1), guinea pigs(2), rats(3), and mice(4). Inflammatory responses were diminished or delayed in rabbits(5) and guinea pigs(6).

Indirect evidence that various stressors increase the output of adrenocortical hormones is considerable. For example, grouped mice had heavier adrenals(7) and lower numbers of circulating eosinophils(8) than did isolated mice. A more indirect case is that grouped mice were less resistant to infestation by *Trichinella spiralis* than were isolated mice (9). Also, mice stressed in shuttle boxes were protected against anaphylactic shock(10).

Direct evidence shows that grouping increases adrenocortical steroid output. Rats kept in colonies had higher levels of plasma corticosterone than those in groups of four (11). Also, groups of 20 had higher levels than did singly caged animals(12).

Available evidence, then, indicates that adrenocortical steroids may inhibit the antibody response, that various stressors cause increased production of adrenocorticoids, and that grouping often is a sufficient stressor to cause such an increase. It follows that grouping may inhibit the circulating-antibody response if the quantitative relations are adequate. Also, since social rank of mice shows an inverse relationship to adrenal weight(13) and a direct relationship to number of circulating eosinophils(8), it may be that a relationship exists between social rank and antibody titer.

Methods. Mice used in these experiments were C3H males. They were weaned at 20 days of age and placed in separate one-gallon jars; dry dog food pellets and water were supplied *ad lib.* Cardboard was placed between the jars so that the mice were visually as well as physically isolated. Mice used in experiments ranged in age from 9 to 15 weeks.

Mice to be grouped were individually marked, then placed in a metal can 3 feet in diameter for 4 hours per day for duration of the experiment. Experiment I involved 6 grouped mice and 6 isolated controls. Also, 5 mice were placed together in an 18 $\times$ 12 $\times$ 5 inch plastic cage for duration of the experiment. Experiment II consisted of 2 groups of 6 mice each and 5 isolated controls. All mice in these 2 experiments were injected with antigen. Experiment III involved 6 grouped mice and 5 isolated controls; mice in this experiment were not injected.

Grouped mice were observed for 20 minutes each day. The number of fights each mouse won or lost was recorded, as was the number of times he chased or was chased by another mouse. A mouse was ranked above another if he dominated in more than half of their interactions.

Mice to be tested for antibody response were injected i.p. with 0.5 ml of a 1:5 dilution of beef serum on the fifth day of grouping. Blood samples were taken from all injected mice before injection, and 4, 8, 11, 18 and 28 days after injection. Four or 5 drops of blood were obtained for each sample from behind the eye with a drawn-out piece of heparinized, 5 mm glass tubing. Each sample of blood was centrifuged, the plasma drawn off and placed in a precipitin tube along with an equal volume of saline, coded, and frozen for later use. A total of 204 plasma samples was collected.

TABLE I. Mean Logarithms of Antibody Titers.

Exp	Grouped	Mice	Days after injection†												
			0	4			8			11			18			28
			M	M	S.E.	P	M	S.E.	P	M	S.E.	P	M	S.E.	P	M
I	Yes	6	0	0	—	n.s.	.87	.27	n.s.	.98	.22	<.05	.50	.22	<.02	0
	No	6	0	.50	.25		1.30	.13		1.55	.05		1.20	.10	<.05	0
	Yes*	5	0	.40	.22	n.s.	.86	.22	n.s.	.66	.27	<.01	.60	.25		0
II	Yes	6	0	.55	.25	<.01	1.05	.07	<.001	1.10	.06	<.01	.33	.29	<.05	0
	No	5	0	1.15	.07	<.001	1.45	.07	<.02	1.45	.07	<.01	1.05	.06	<.01	0
	Yes	6	0	.17	.17		.88	.18		1.15	.07		.33	.21		0

* Grouped continuously rather than only 4 hr per day.
† A mean of zero indicates that all titers were below 10.
S.E. is standard error of mean.

Antibody titers were determined by the ring precipitin test of Hanks(14), except that tests were incubated at room temperature for one hour, stored in the refrigerator overnight and then read.

At conclusion of each experiment both adrenal glands were removed from all mice, preserved in 10% formalin and later weighed wet on a Roller Smith 25 mg torsion balance. Results are expressed without regard to body weight since no correlation between body weight and adrenal weight was found for the 16 isolated mice used in these experiments.

Results. Antibody titers of both grouped and isolated mice (Exp. I) generally increased to the 11th day following injection, then declined (Table I). The mean logarithm of the antibody titer for each of the 2 groups was compared with that of the 6 isolated mice for each day samples were collected by the "t" test. Mean titers of the isolated mice were found to be significantly higher than those of either of the groups (P less than .05) for days 11 and 18. No significant differences were found for days 4 and 8. On day 28 the titers of all mice were below 10, which was the lowest dilution used.

To test this approach again, 2 additional groups of 6 mice each were grouped 4 hours per day, and 5 mice were kept isolated (Exp. II). Titers of both grouped and isolated mice generally increased to the 11th day following injection, then declined (Table I); "t" tests showed that the mean logarithm of the titers of the 5 isolated mice for each day samples were collected was significantly higher than those of the 2 groups (P less than .05) for days 4, 8, 11 and 18. Titers of all mice were below 10 by day 28.

Social rank, as determined by number of wins and losses in fights between individual mice of a group, was compared with antibody titer. In most cases it was not possible to rank all of the mice in a particular group; generally only the 2 or 3 top-ranking mice were determined. Inspection of the values of antibody titers indicated no correlation with position in the hierarchy except for the top-ranking mice. The mean logarithm of the titers of the 4 dominant mice from the above experiments was plotted with the mean values for the other 20 mice in these experiments for each day samples were taken (Fig. 1). Analysis of variance showed that the mean logarithms of the titers of the dominant mice were significantly higher than the mean values for the other mice (P less than .05)

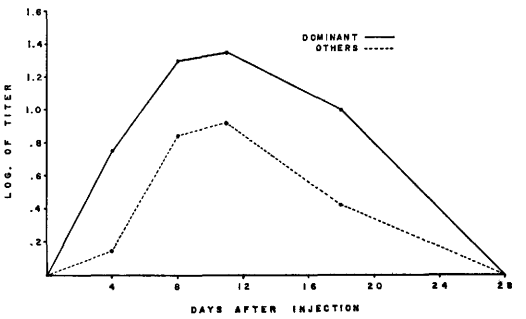

FIG. 1. Mean antibody titers of dominant mice and other mice from the 4 groups of Exp. I and II.

TABLE II. Mean Body Weights and Mean Adrenal Weights.

Exp	Grouped	Duration (days)	Injected	Mice	Mean body wt (g) Before	Mean body wt (g) After	Adrenal wt (g) Mean	Adrenal wt (g) S.E.
I	Yes	38	Yes	6	22.8	22.2	3.89	.14
	" *	38	"	5	27.4	30.6	4.29	.49
	No	38	"	6	24.3	30.2	2.95	.10
II	Yes	28	"	6	23.2	22.0	4.34	.36
	"	28	"	6	31.8	31.8	5.01	.37
	No	28	"	5	27.4	31.8	3.35	.17
III	Yes	38	No	6	22.7	25.3	4.20	.10
	No	38	"	5	24.8	33.4	3.12	.25

* Grouped continuously rather than only 4 hr per day.

for days 4, 8, 11 and 18.

Mean weights of the adrenals of the mice in groups were compared with those of the isolated mice within each experiment by the "t" test (Table II). In all cases the adrenals of the grouped mice were significantly heavier than those of the isolated mice (P less than .05).

Effects of injecting antigen and taking blood samples on adrenal weight were tested by comparing adrenal weights of grouped and isolated mice that had not been injected or bled with those that had. The mean adrenal weight of 5 isolated mice that were neither injected nor bled was not significantly different from the means of the isolated mice in the 2 experiments in which mice were injected and bled. Similarly, the mean for the 6 grouped mice that were neither injected nor bled was not significantly different from any of the means of the groups in the 2 experiments in which mice were injected and bled. Thus this small test does not indicate that the procedure itself altered the adrenal weight.

Discussion. Examination of Table I indicates that the duration of detectable antibody titers of grouped mice is almost identical to that of isolated mice. The primary difference is one of magnitude. Thus, it can be concluded that grouping is sufficient to cause a significantly lower level of circulating antibody to a beef serum antigen in grouped mice than in isolated mice.

Also we conclude that dominant mice respond to an antigen with a higher titer than do the other mice in their group. This result was expected since dominant mice have lighter adrenals than their subordinates and presumably are producing smaller amounts of adrenocorticoids(13).

In previous work Christian(7) found that the adrenal weights of mice grouped for 7 days were significantly higher than those of isolated mice. Evidence is given here that grouping mice for 28 and 38 days gives similar results. Grouping mice continuously has been shown to increase adrenal weights to the same degree as grouping them 4 hours per day for a 10 day period(13). Our evidence is that mice grouped continuously for 38 days also show an increase which is about the same as that of mice grouped 4 hours per day.

Summary. The effects of grouping and social rank on circulating-antibody titer were studied using C3H mice. Previously isolated mice were placed together in groups of 6 each for 4 hours per day and injected with beef serum on the fifth day of grouping. Antibody titers were determined from blood samples by the ring precipitin test. Grouped mice were found to have significantly lower titers of circulating antibody than did isolated mice. Dominant mice had significantly higher titers than the other mice in their groups. The adrenal glands of grouped mice, taken after 28 and 38 days of grouping, were significantly heavier than those of isolated mice.

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Rat Cerebral Cortical Oxygen Uptake Stimulating Factor from Sciatic Nerve.* (28884)

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A substance which stimulates oxygen consumption has been reported by Heller and Hesse(1) to be present in the sciatic nerve of the rat. This substance diffuses from the nerve during *in vitro* incubation in glucose-free medium at room temperature and is capable of increasing the rate of oxygen uptake of a fresh nerve subsequently tested in this medium. These authors(1) have named this material "activating" substance and the medium into which it has diffused "activating" medium.

The stimulation of oxygen uptake of rat sciatic nerve was observed with an "activating" medium prepared from the sciatic nerves of the rat. The experiments to be reported here were designed to test whether such a diffusible factor from nerve is capable of stimulating the oxygen uptake of rat cerebral cortical slices. In an attempt to determine the source of this "activating" substance the sciatic nerves were divested of their connective tissue sheaths (epineurium). The desheathed nerves and their sheaths were separately tested for effects on the oxygen uptake of cortex slices.

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Methods. I. *Manometry.* Manometers with a 1 mm bore, and 5 ml flasks were used with an American Instrument Co. circular Warburg apparatus. The temperature of the water bath was maintained at 37.34° ± 0.02°C and the flasks were shaken at 120 oscillations per minute. The rims of the center wells were greased with anhydrous lanolin and subsequently filled with 0.1 ml of 5% KOH. The surface area for absorption of CO_2 was increased by using a fluted filter paper. The flasks were gassed with pure oxygen for a period of 10 minutes, followed by a 10-minute temperature equilibration period, after which the manometers were closed. The first, or zero time, reading was taken 5 minutes later and subsequent readings were taken every 10 minutes for one hour.

II. *Solutions.* Two incubation media were used in these experiments. The millimolar quantities of their constituents are given in Table I. Fresh media for each experiment were prepared by pipetting measured quantities from stock solutions. The substrates for the Krebs III(2) medium were stored under refrigeration for no more than 4 days.

III. *Preparation of "activating" medium.* Three male Sherman rats were sacrificed by decapitation with a guillotine and their sciatic-peroneal nerves quickly dissected out.