

dependently of the hormonal environment of the animal and are inhibited specifically by glucocorticoids. On the other hand, edema formation and its inhibition by glucocorticoids are definitely influenced by the level of circulating thyroid hormone.

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Infantile Manipulation and Aggregate Living in the Rat: Effect of Electric Shock on Blood Histamine Level.* (32178)

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There is ample evidence that histamine injection results in pituitary-adrenal activation(1,2,3). As an outgrowth of these studies, it has been proposed that endogenous histamine participates in the response to stressful stimulation(4,5). Despite the voluminous literature on histamine now available, it appears that this notion has not been adequately tested(6).

The present study was undertaken to investigate the effects of differential neonatal experience and post-weaning group living on the concentration of blood histamine in the rat following noxious stimulation administered in early adulthood. It employed a reliable chemical method to measure blood histamine levels in animals raised under four different living conditions, then later subjected to electric shock.

Methods. Subjects comprised 20 litters of Sprague-Dawley rats[§] culled to 10 pups each at birth. Half of the neonates were manipu-

lated during the period 1 to 7 days of age, inclusive. In this procedure, pups were carefully removed from the nest and placed in separate, cardboard containers for 3 minutes each day. All animals were weaned at 21 days of age and assigned by sex to one of 4 experimental categories. These were: (a) Infantile manipulation followed by post-weaning isolation; (b) No infantile manipulation followed by post-weaning isolation; (c) Infantile manipulation followed by post-weaning group living (4 to 6 rats per cage); (d) No infantile manipulation followed by post-weaning group living (4 to 6 rats per cage).

In the tenth week of life, all animals were subjected to electric shock. Shock parameters were 1.0 ma delivered through a grid to the feet for 30 seconds. At time zero, and at 5-, 10-, and 20-minute intervals following initiation of shock, representative animals were sacrificed by cervical spinal separation. With prior practice, it was possible to obtain a blood sample, containing arterial and venous components, within 10 seconds following removal of the animal from its cage. This permitted the investigators to secure

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[§] Pregnant, primiparous, cesarian-derived females were secured from the Charles River Breeding Laboratories, North Wilmington, Mass.

TABLE I. Mean Blood-Histamine Levels (γ %) at Stated Intervals Following Electric Shock.

Post-shock time (min)	Datum	Male				Female			
		Treatment group*				Treatment group			
		MI	NI	MG	NG	MI	NI	MG	NG
0	Mean	.376	.439	.272	.409	.823	.892	.250	.416
	S.D.	\pm .216	\pm .292	\pm .197	\pm .233	\pm .124	\pm .301	\pm .083	\pm .245
	N	5	7	6	5	4	5	6	6
5	Mean	1.070	1.312	2.022	2.228	2.756	1.436	3.206	2.375
	S.D.	\pm 1.659	\pm 1.353	\pm 1.530	\pm 1.514	\pm 1.026	\pm .774	\pm 2.227	\pm 1.446
	N	6	8	5	4	4	6	6	6
10	Mean	2.188	1.664	2.330	2.720	1.554	1.558	2.646	2.603
	S.D.	\pm 1.457	\pm .877	\pm 1.332	\pm .697	\pm .291	\pm .280	\pm .772	\pm .571
	N	5	4	6	6	4	4	6	6
20	Mean	1.046	1.527	.977	.797	.897	.667	.272	.234
	S.D.	\pm 1.049	\pm 1.845	\pm .731	\pm .944	\pm .493	\pm .094	\pm .079	\pm .135
	N	7	7	6	6	4	6	6	6

* MI: Infantile manipulation followed by post-weaning isolation. NI: No infantile manipulation followed by post-weaning isolation. MG: Infantile manipulation followed by post-weaning group living. NG: No infantile manipulation followed by post-weaning group living.

accurate resting values uninfluenced by anesthetic or cardiac puncture procedures. It was necessary, of course, to weigh this advantage against the possibility that non-hematic tissue fluid would enter the sample at decapitation. Since all animals were subjected to a uniform procedure for histamine assay, it was felt that the principal effect of contamination with extraneous body fluid would be to increase within-group variability. Thus, any substantial difference in group values would remain a function of differential experimental treatment. Each blood sample was collected in a beaker containing 0.5 ml of a 10% sodium oxalate solution. The sample was then transferred to a coded test tube and frozen immediately. A 2.0 ml aliquot was subsequently analyzed for histamine content utilizing a modification[¶] of Shore's method (7).

Results. Mean histamine yield of each group at decapitation is tabulated in Table I. The marked temporal variation in mean histamine level occurring after electric shock was confirmed by a highly significant result

in a computer-programmed analysis of variance ($F = 30.1, df 3/158, p < .001$). A feature requiring special consideration in the statistical analysis was the tendency for variability to be proportional to the mean level of histamine recorded. While this would suggest the use of a logarithmic transformation, it was decided to retain the data in their original form to facilitate analysis of the response characteristics selected for examination. These were *total response* (TR) and *sensitivity of response* (SR).

Total response was defined as the shaded area in Fig. 1. This value, in γ /min units, was determined by the 4 mean, temporal levels of histamine as expressed in the following formula:

$$\text{Area} = \frac{1}{2}(-35x_0 + 10x_5 + 15x_{10} + 10x_{20})$$

Since a maximum post-stress time interval of 20 minutes was adhered to throughout the study, total response was expressed in γ units for clarity. This was accomplished by dividing the area by 20. Thus, in γ units the equation becomes:

$$\text{TR} = \frac{1}{8}(-7x_0 + 2x_5 + 3x_{10} + 2x_{20})$$

Analysis of TR revealed that group living rats had a significantly greater response to shock in comparison with isolated animals. The mean difference in TR between these living conditions was 0.74γ ; the 95% con-

[¶] This modification permitted the use of the assay on volumes approximately one-half those used by Shore. Sensitivity and recovery rates in the modified procedure were consistent with those reported for the original technique.

[¶] We wish to acknowledge the kind assistance of Dr. M. Cho, in standardizing the assay procedure.

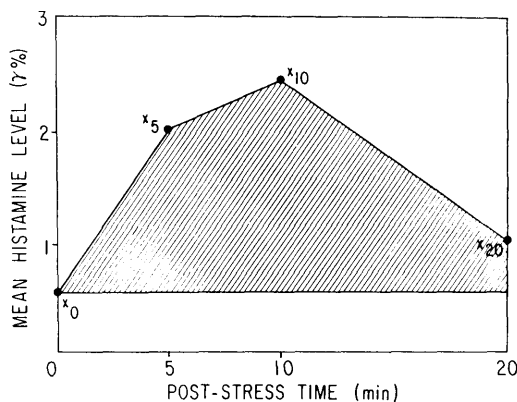


FIG. 1. Hypothetical plot of blood-histamine level in relation to post-stress time. (See formula in text.)

fidence interval in this case ranges from 0.40γ to 1.08γ . No other comparisons of TR were statistically significant, although infantile-manipulated rats responded with a somewhat more elevated level than those not subjected to this form of stimulation ($.05 < p < .10$).

Analysis of SR was undertaken to measure the rapidity of increase and subsequent fall in histamine level among the various groups. This characteristic was defined as: $SR = (x_5 - x_{20})$. On the basis of this calculation, it was found that group living led to significantly greater sensitivity of response than did isolated housing. The mean difference in SR level for grouped *vs* isolated rats was 1.27γ ; the 95% confidence interval for this value is between 0.22γ and 2.32γ . In addition, there was a substantial and consistent difference in sensitivity between the sexes, with females showing greater sensitivity than males. The mean excess sensitivity of females was 1.36γ ; the 95% confidence interval lies between 0.28γ and 2.43γ .

Discussion. Exposure to electric shock is evidently followed by a significantly elevated level of blood histamine in the rat. In the present study, a factor contributing to the rise reflected in the over-all analysis was sex of the animal. Thus, females were more sensitive than males to this form of noxious stimulation, as indicated by a relatively more rapid increase and subsequent fall in circulating values. Relevant to this observation are the results of previous studies revealing sex dif-

ferences in rat histamine catabolism, where excretion rates of this amine in females were shown to be from 10 to 25 times higher than in males (8-11). This high ratio has been related to the mode of breakdown, rather than to increased histamine production in the female (10), a finding supported by the failure in the present study to demonstrate clear-cut sex differences in the total response measure.

The second factor influencing the rat's blood histamine response to electric shock was aggregation. Group living, in comparison with isolation, was associated with a more pronounced total response and greater sensitivity. This could reflect a high tissue concentration of histamine as well as great capacity to catabolize it in animals exposed to a group environment. Such a view is in agreement with Jencks (12), who reported that mice** reared in groups had a significantly larger population of subcutaneous mast cells than did isolated animals. Since this tissue component contains a rich store of histamine, Jencks' finding is in line with the present observation linking aggregate living with a copious histamine response to stress.

Finally, a numerically-greater effect on both sensitivity and total response was recorded for infantile-manipulated rats, in comparison with non-manipulated. However, it would appear that larger groups of animals than were employed in the present study would be required to permit an adequate test of the effects of infantile treatment on circulating histamine levels. Further study of this relationship would seem particularly promising in view of the small number of reports currently available concerning biochemical routes which may be involved in various early experience effects (14).

Summary. Exposure of young, adult rats to electric shock resulted in a significantly elevated level of blood histamine. The pattern of response was related to the animal's

** This finding has been confirmed in our laboratory using rats and a technique for determining the distribution of histamine-containing granules in the periosteal mast cells overlaying the skull (13). It thus appears that the group-living effect on histamine reservoirs is not confined to a single species or tissue type.

sex and pre-shock environment. Females were more sensitive to noxious stimulation than males, as reflected in a more rapid increase and subsequent fall in circulating levels. Post-weaning group living, compared to isolation, was associated with both greater sensitivity and a more pronounced total response. Infantile-manipulated rats had a numerically greater sensitivity and total response, in comparison with non-manipulated controls.

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Effect of Intravenous Angiotensin Infusion on Adrenal Medullary Function in Man. (32179)

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It has been shown in animal experiments that angiotensin can cause the release of catecholamines from the adrenal gland(1-5). In the cat, 0.1 μ g of angiotensin intravenously (I.V.) will produce this effect; an even smaller dose injected into the celiac artery can release up to 100 times its weight in epinephrine(1). However, the effect of an I.V. infusion of angiotensin on adrenal medullary function has not been investigated in man.

The purpose of this work was, therefore, to study in man the effect of an I.V. infusion of a moderate pressor dose of angiotensin (a) on the urinary excretion rates of catecholamines and 3-methoxy-4-hydroxy mandelic acid (V.M.A.) and (b) on the fasting blood glucose level.

Methods. (a) *Effect of angiotensin on catecholamine and V.M.A. excretion rates.* Studies were done on 2 male and 2 female volunteers, aged from 23 to 35 years. The subjects were normal or were patients who had recovered from minor illnesses and were in good health at time of the study. Subjects were kept at rest in bed in a hospital for 2 days, on the second of which the experiments were performed. Vanilla, vanilla-containing foods, bananas and coffee were excluded from the diet for the 2 days. With these restrictions, meals and fluids were permitted during the experiments. To compensate to some degree for depression in renal excretory capacity which angiotensin might induce, the inulin clearance was used throughout these experiments as a measure of glomerular filtration rate (G.F.R.). Excretion rates of catecholamines and V.M.A. were then expressed in μ g/hr/100 ml G.F.R.

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