Effect of Gentling on Development of Adrenal Regeneration Hypertension in Immature Female Rats.* (28542)

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Stress and the concomitant secretion of adrenocorticotrophic hormone as well as administration of cortical steroids have been shown to increase blood pressure on the one hand, and to depress growth on the other(1, 2,3). A series of curious observations have indicated that handling and gentling of animals improves response to stressors(4,5,6,7, 8,9) and causes superior growth as compared to rats not so treated(6,7,9,10,11,12,13,14). These findings suggested that gentling might affect the development of adrenal-regeneration hypertension, inasmuch as the pathogenesis of this form of experimental hypertension has been related to some functional characteristic of the regenerating adrenal cortex, and curiously enough, occurs only in young animals during their most active growth period.

Methods. Forty-four female Charles River rats were treated in the following manner: 2 groups of 12 rats each were unilaterally nephrectomized and adrenalectomized and contralaterally adrenal-enucleated(15) (Groups 1 and 2). Two groups of 10 intact rats (Groups 3 and 4) served as unoperated controls.

All groups received *ad libitum* a synthetic diet, calculated to yield 4.2 Cal/g and containing 0.8% NaCl. The adrenal-enucleated groups were given 1% saline to drink, the intact rats tap water. The rats were kept in single cages in an air-conditioned room maintained at 26°C. One operated (Group 1) and one intact (Group 3) group of rats was gentled in the following manner: the individual rat was placed on the lap of the investigator (LLB) and stroked, with the thumb of the right hand, from the nose along the back to the root of the tail, with a frequency of about 20 strokes per minute. The duration of gentling during the first week of the experiment was 5 minutes per day. From the second week to the end of the experiment, which lasted 6 weeks, the duration was 6 minutes per day, except for several weekends and the days on which blood pressure measurements were made.

Food and water intake were measured throughout the experiment. Blood pressure was measured prior to operation at 25 days of age, and weekly thereafter by the method of Friedman and Freed(16). The animals were under light ether anesthesia for this measurement as well as for determination of body weight and body length. All animals were sacrificed on the 43rd experimental day and their organs were excised, trimmed and weighed.

Results. Gentled, adrenal-enucleated rats (Group 1) showed a greater rise in mean blood pressure than did non-gentled, adrenal-enucleated animals (Group 2), but the levels were significantly different only on the 29th day of experiment (Fig. 1). A similar pattern of blood pressure rise was found in the gentled intact rats (Group 3), where a significant difference from the non-gentled rats (Group 4) was present on days 22, 36 and 43, but at no time was the blood pressure in these control animals in the hypertensive range.

Gentled rats, whether intact or adrenal-enucleated, did not show significantly greater body weight gain than their non-gentled counterparts. Both intact and adrenal-enucleated, gentled animals showed greater body length than non-gentled controls throughout the experiment, but the difference was never significant except for the intact rats on the 36th day of experiment (Group 3: 350.8 $\pm$ 3.62 mm, Group 4: 337.9 $\pm$ 4.20 mm, $p < 0.05$). Actually the body length of gentled rats bearing regenerating adrenals decreased toward the end of the experiment, so that the terminal mean value was identical to that of the non-gentled group.

Gentled, intact rats (Group 3) showed a

* This investigation was supported by a research grant from Nat. Heart Inst., Nat. Inst. Health, P.H.S.

TABLE I. Organ Weights of Gentled and Non-gentled Adrenal-enucleated and Intact Female Rats (mg/100 g body weight).

Group	Heart	Kidney(s)	Thymus	Adrenal(s)
Adrenal-enucleated gentled (Group 1)	387 ± 18*	808 ± 67	319 ± 27	13.7 ± 5.4
Adrenal-enucleated non-gentled (Group 2)	367 ± 16	744 ± 25	257 ± 21	12.8 ± 3.7
Intact gentled (Group 3)	297 ± 8	784 ± 30	208 ± 11	25.9 ± 1.2
Intact non-gentled (Group 4)	313 ± 6	769 ± 22	233 ± 18	23.5 ± 0.8

* Means ± S.E.M.

consistently higher food intake than non-gentled rats (Group 4) but only during the third week of the experiment was the difference significant (14.3 ± 0.88 g/day *vs* 11.3 ± 1.17 g/day, $p<0.05$). It is noteworthy that this increase in food intake coincided with the beginning of their blood pressure rise. In contrast, gentled rats bearing regenerating adrenals (Group 1) consistently ate less than their non-gentled counterparts, but this was statistically significant only during the sixth week of experiment. (Group 1: 16.9 ± 1.17 g/day, Group 2: 20.3 ± 1.17 g/day, $p<0.05$). At this time linear growth also showed a sudden increase in the intact as well as the adrenal-enucleated rats.

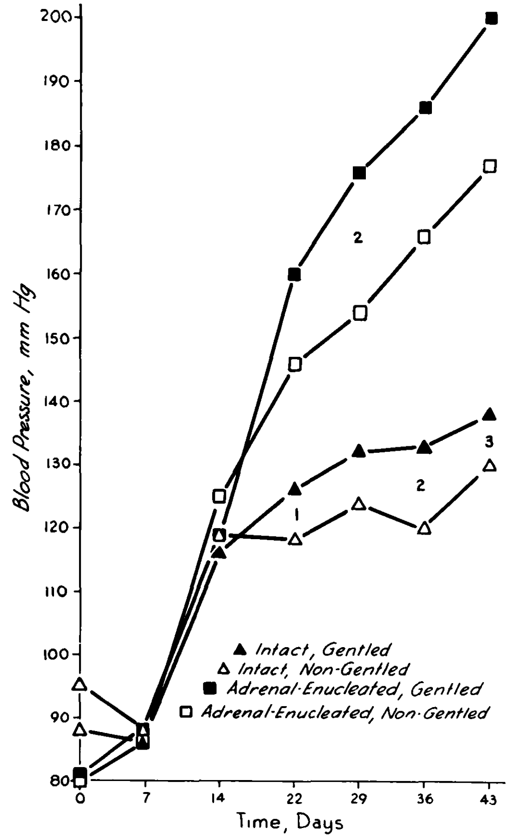

FIG. 1. Blood pressure response to gentling in adrenal-enucleated and intact female rats (1) $p<0.05$, (2) $p<0.02$, (3) $p<0.01$.

No significant differences in water intake between the gentled and non-gentled intact rats were observed. The saline intake of the adrenal-enucleated, gentled rats was significantly lower than that of the non-gentled controls only during the fifth week of the experiment (Group 1: 42.0 ± 3.27 g/day, Group 2: 54.5 ± 4.90 g/day, $p<0.05$).

Although differences in mean organ weights of adrenal-enucleated (Groups 1 and 2) and intact rats (Groups 3 and 4) were not significant, the findings warrant mention, since they are suggestive of certain functional trends (Table I). For example, in the adrenal-enucleated rats mean heart weight of gentled animals was greater than that of non-gentled rats, contrasting sharply with the values for intact rats, where the reverse was true. A similar relationship obtains for thymus weight. Weights of the adrenals were almost identical in the gentled and non-gentled, adrenal-enucleated (Groups 1 and 2) and intact (Groups 3 and 4) rats, but it is worth noting that the weights of the regenerated glands were much less than the weights of both adrenals in the intact animals.

Discussion. The present results show that if gentling has an effect on either adrenal regeneration hypertension or growth it is one of enhancement rather than inhibition. The presence of larger thymus glands in adrenal-enucleated, gentled rats than in non-gentled, adrenal-enucleated controls, lends further indirect support to the thesis that during adrenal-regeneration hypertension an abnormal pattern of steroid secretion occurs in the regenerating glands, for, if the hypertension were solely the result of higher corticosterone secretion, the thymus would be expected to be smaller in the gentled animals. The different response to gentling of rats bearing regenerating adrenals from that of intact animals probably relates to some disruption of the CNS-pituitary axis as a result of the enucleation procedure and the subsequently altered pattern of steroid secretion.

The statement of Ruegamer *et al.*(11) and Weininger(7) that gentled rats did not eat more than non-gentled controls could be confirmed for the adrenal-enucleated rats only. In our experience gentled intact animals ate somewhat more than non-gentled controls throughout the experiment, a fact which may have contributed to their slightly better growth.

Summary. 1. Gentling acts as a stressor and increases blood pressure in adrenal-enucleated as well as intact rats. 2. At the same time gentling seems to promote growth, a paradoxical situation since growth is generally considered to be depressed by the activities of ACTH and glucocorticoids. 3. This paradox may be explained by one of the currently held concepts of the mechanism of adrenal-regeneration hypertension, namely the secretion of a hypertensive steroid other than corticosterone which is, however, ACTH-dependent.

The authors are indebted to Miss Mary Hyde, Miss Marjorie Boyd, Miss Lillian Vitanza and Mr. David Zandor for technical assistance during this investigation. The illustrations were prepared by Mr. Melford Diedrick, Dept. of Medical Illustration, and by Mr. Emmanuel Kaufman, Dept. of Pathology.

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Received May 23, 1963. P.S.E.B.M., 1963, v113.

Assimilation of Sulfur Compounds by *Pasteurella multocida.** (28543)

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Sulfur assimilation is an essential step for biosynthesis of cystine and methionine in microorganisms, and many species are able to utilize sulfate as a sulfur source(1). Some strains (virulent and avirulent) of *Pasteurella pestis* have an absolute requirement for cystine and methionine while other strains (A1122 and the "spontaneous mutant") do not assimilate sulfate, although less oxidized sulfur compounds such as thiosulfate, sulfite or sulfide can satisfy all the sulfur requirements of the "spontaneous" mutant or are used for synthesis of cysteine (cystine) by strain A1122(2). The pattern of utilization of inorganic sulfur compounds by another species of *Pasteurella (P. multocida)* is summarized below.

* Supported by grants from Rockefeller Foundation and Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

† On leave from Inst. Nacional de Microbiol.