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Postnatal Changes in the Pituitary-Adrenal Axis of the Rat*† (32994)

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Contrary to the findings of Jailer (1) and Schapiro *et al.* (2), recent data from both this laboratory (3-6) and Levine's laboratory (7-8) indicate that the adrenal gland of the neonatal rat responds to certain stressors. The degree of response decreases at around day 7 of age and thereafter gradually increases to the adult pattern. A similar "biphasic" pattern for the growth of the adrenal gland was reported in the rat more than 50 years ago when the adrenal weight data were expressed as a function of body weight (9).

In addition, certain correlations have been found to exist between "early handling" of the neonate and adrenocortical function in the young adult rat (6, 10). Although a causal relationship between the two events has yet to be established, it is assumed that such may exist.

The present study is designed to examine the development of adrenocortical activity in the neonatal rat and to determine whether the

decrease in adrenal activity at 7 days of age in the rat is due to changes in the adrenal gland, the pituitary, or both.

Materials and Methods. All rats used in these experiments were of the Purdue-Wistar strain. The litter size was reduced to 6 on the morning the litter was found and the pups were considered to be 1-day old at this time. The lighting schedule consisted of 13 hours light (7 a.m. to 8 p.m.) and 11 hours dark. The rats were born and housed in special rooms and no one except the experimenter was allowed to enter on the day of the experiment. Food and water were supplied *ad libitum*. All rats were killed at 10:30 a.m. Wherever possible, a split-litter technique was used so that control and experimental values were obtained from the same litter.

Results. Expt. 1: Compensatory Hypertrophy. In this experiment animals were used at 2, 5, 7, 10, 15, and 30 days of age. Two rats were unilaterally adrenalectomized (right adrenal gland removed) and 2 others were sham operated. Both groups were killed 72 hours later and the left adrenal removed and weighed to the nearest 0.2 mg. Two control rats were killed at the time of the operation

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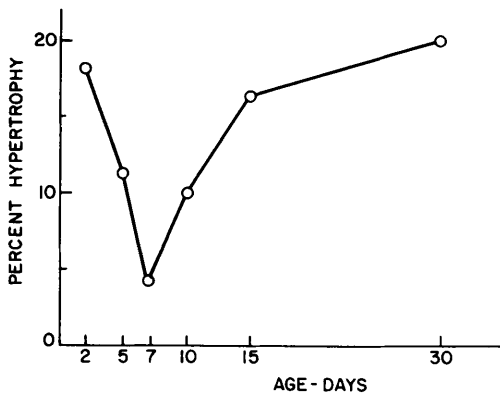


FIG. 1. Compensatory adrenal hypertrophy in the rat following unilateral adrenalectomy. Age in days at the time of the operation is plotted against % hypertrophy of the remaining gland.

and the left adrenal gland was weighed. Percentage hypertrophy was calculated from the following equation (11):

% hypertrophy =

$$\frac{\left[\begin{array}{c} \text{mg \% left adrenal} \\ \text{(adrenx rat)} \end{array} \right] - \left[\begin{array}{c} \text{mg \% left adrenal} \\ \text{(sham op. rat)} \end{array} \right]}{\text{mg \% left adrenal of the initial control rat}} \times 100.$$

The results of this experiment are presented in Fig. 1. Ten to 12 rats were used in each group, and the curve for percentage hypertrophy is a replication of the one obtained by Jackson (9) and by us for the normal growth of the adrenal gland when expressed as a function of body weight. Both the 2- and 5-day old groups showed a significant hypertrophy of 18.2 ± 3.8 and $11.4 \pm 4.6\%$ respectively. The response decreased to $4.5 \pm 2.7\%$ at 7 days of age, which was not significant at the 5% level, and thereafter increased progressively at 10, 15, and 30 days of age to values of 10.1 ± 2.1 , 16.6 ± 3.2 , and $20.0 \pm 4.1\%$ respectively.

Expt. 2: ACTH-Induced Hypertrophy. The failure of the 7-day group in Expt. 1 to show a significant hypertrophy could be caused by the inability of the adrenal to respond or to failure of the pituitary to secrete ACTH. The ACTH question was attacked in this experiment.

A single injection of ACTH² suspended in 5% beeswax-oil was given to rats at 2, 7, and 21 days. Again a split-litter technique was

used so that 2 rats from each litter received the ACTH, 2 the vehicle and 2 received no treatment. All rats were killed 48 hours after the injection and both adrenal glands were removed and weighed at autopsy. Percentage hypertrophy was calculated as in Expt. 1 except that absolute adrenal weights were used due to the body weight inhibition seen in the ACTH treated rats (12). Ten rats were used for each dose and age group.

The percentage adrenal hypertrophy \pm SE was 42.5 ± 3.6 , 49.3 ± 6.0 , and 25.9 ± 4.1 after 5 IU of ACTH per 100 gm of body weight in the 2, 7, and 21-day rats, respectively. At 10 IU the percentage hypertrophy increased to $85.0 \pm 8.9\%$ for the 2-day old rats, $100.6 \pm 7.6\%$ for the 7-day old, and $38.0 \pm 4.9\%$ for the 21-day old rats. At the highest dose used (20 IU of ACTH/100 gm of body wt.) the 2-day old rats gave an increase in adrenal response of $88.4 \pm 7.3\%$, the 7-day old a $133.9 \pm 8.6\%$ increase, and the 21-day old a $71.6 \pm 6.4\%$ increase. The failure to get an increased response in the 2-day old rat with 20 IU of ACTH as compared with 10 IU cannot be explained at this time. However, the results were replicated in a second experiment. The slopes of the three curves indicate that the response in the 7-day old rat is more sensitive than in the other two age groups (Fig. 2). Student *t* tests were

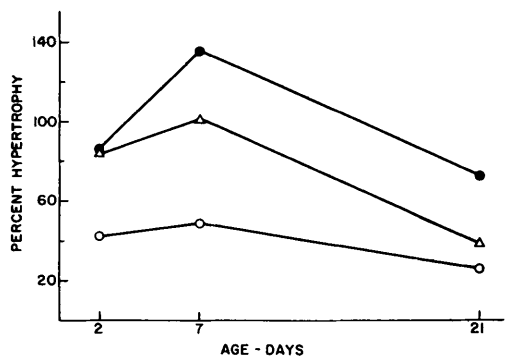


FIG. 2. Percentage hypertrophy following treatment with a single injection of ACTH in beeswax-oil. (○) 5 IU/100 gm of body wt.; (△) 10 IU/100 gm of body wt.; and (●) 20 IU/100 gm of body wt.

² The ACTH was obtained through the courtesy of Dr. James W. Bastian, Armour Pharmaceutical Co., Kankakee, Illinois.

conducted both between and within age groups. All values with the exception of the 10 vs 20 IU in the 2-day old group, 5 vs 10 IU in the 21-day old group, and the effect of 5 and 10 IU of ACTH in the 2 vs 7-day old groups were significant at the 1% level or better. This study established the fact that the 7-day old adrenal is capable of responding to ACTH with a greater hypertrophy than either the 2 or 21-day old.

Discussion. The biphasic pattern of response seen following unilateral adrenalectomy (Fig. 1) is identical with that seen in the normal growth of the adrenal gland when the weight of the gland is expressed as a function of the body weight. In both instances the 7-day old rat showed a depressed value as compared with older and younger rats. However, since the injection of ACTH caused a significant increase in the weight of the adrenal of the 7-day old rat (Fig. 2), it is obvious that the depressed response following unilateral adrenalectomy is not due to an inability of the adrenal gland to respond by a weight increase. Thus, the adrenal gland of both the 2-day old and 7-day old rat can respond to ACTH although the response in the 7-day old is greater at the higher dosages of ACTH.

These differences in behavior between the 2-day old and 7-day old adrenal may be explained on the basis of a system deficient in ACTH. Under such circumstances the response could become more sensitive the longer the rat is deficient in ACTH. Since we have already reported that the 2-day old rat will respond to certain types of stressors and not to others (5) it may be inferred that some link in the hypothalamo-pituitary-adrenal axis is not complete. This is supported by the findings of Glydon (13) who reported that the formation of the hypophyseal portal system does not begin until day 5 of age.

Numerous physiological functions which depend upon the pituitary such as thyroid function (14) or those which depend upon adrenal function such as inhibition of epidermal histidase (15) also reflect this pattern.

We conclude, then, that several facets of the hypothalamo-pituitary-adrenal, axis fail to function in the 7-day old rat: the pituitary

gland because of an inability of CRF to get to the pituitary; and the adrenal gland because of the relative lack of ACTH in the circulation. Why then is the adrenal gland active in the 2-day old rat? Since the adrenal gland is active in the fetus (16-18) and does not show minimal activity until day 7 of age, it might be assumed that the system seen in late fetal life extends into the first 5 days after delivery. The important event at day 5 of age is the beginning of the hypothalamic-pituitary portal system. The fetal and early postnatal maintenance of the pituitary levels of ACTH could be the result of a diffusion of CRF to the pituitary which becomes more difficult and fails with the growth and development of the rat. Thus the 7-day old rat is showing signs of ACTH deficiency due to a failure of CRF to get to the pituitary gland.

Summary. The adrenal gland of the 2-day old responds to unilateral adrenalectomy by a significant hypertrophy of the remaining gland. Treatment with ACTH in the same age animal also causes a significant hypertrophy. The response in the 7-day old to unilateral adrenalectomy is significantly depressed as compared with that obtained in the 2-day old rat. Following treatment with ACTH, adrenal hypertrophy is greater in the 7-day old rat than in the 2-day old. It is concluded that the pituitary-adrenal axis is relatively refractory in the rat at about day 7 of age and is functional in both the younger and older animal.

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Blood Flow and Peripheral Resistance in Normotensive and Hypertensive Dogs* (32995)

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The level of the peripheral resistance is frequently used in the estimation of the severity of hypertension or the response to drugs affecting the peripheral circulation. Yet when measurements of resistance are followed from day to day in normal or hypertensive dogs, there are unexplained variations. In particular the hypertensive animal will frequently show levels of resistance well within the normal range, suggesting that the condition may be undergoing variations in severity. Observing the behavior of the circulation over periods of time, it was noted that level of resistance appeared to vary inversely with the cardiac output. The present study investigated this relationship in dogs before and after the development of hypertension and as it is influenced by drugs.

Material and Methods. Trained conscious dogs with chronically implanted catheters in the aorta and right heart have been used. Blood pressure and cardiac output measurements were made at intervals of 3–5 days in the fasting state without restraint or premedication using methods previously described in detail (1). Five dogs were studied repeatedly in the normotensive state and then again 3 weeks after the application of a Goldblatt

clamp to one renal artery with a contralateral nephrectomy. Two other dogs were studied repeatedly in the normotensive state and at each session, measurements were repeated after ganglion blockage with pentolinium (25 mg i.v. and 2.5 mg s.c.). This dose was chosen as it produced paralysis of the pupillary light reflex. After these measurements had been made, an infusion of norepinephrine (0.3 $\mu\text{g}/\text{kg}$ per min) was administered through a third catheter in the ascending aorta for 5 min with a cardiac output and blood pressure measurement being made in the final minute. The pupillary light reflex remained paralyzed at the completion of the infusion.

Results. From one day to another, the cardiac output, both in the normotensive or hypertensive state, showed considerable variation (Fig. 1). The mean blood pressure also showed some fluctuation and varied from 90 to 126 mm Hg in the normotensive and from 136 to 187 mm Hg in the hypertensive state. There was, however, no tendency for it to change with cardiac output (Fig. 2). The peripheral resistance, however, clearly fell in an exponential fashion as cardiac output increased. With the development of hypertension, the curve shifted to the right to give a higher level of resistance for any given level of cardiac output (Fig. 1).

Since it was thought that these findings

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