Adrenal Response to Hypophysectomy in the Newborn Dog* (34114)

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In the course of studies of the fetal and newborn dog, in which adrenal secretory rates were determined by means of collection of effluent blood from the gland, it became apparent that it would be useful to employ a preparation in which the initial secretory rate was low. An earlier investigation of the hypothalamic–pituitary–adrenal axis in the newborn dog had established that feedback control is operative (1). Prompt responsiveness of the newborn rat to stimuli which included heat and electric shock (2), histamine (3), and a handling procedure (4) has been shown. The secretory rate, determined by collection of left adrenal venous blood after preparing the animal for adrenal drainage, appeared to reflect the stress of the experimental preparation. Hypophysectomy preceding preparation for adrenal drainage seemed to offer a means of obtaining low initial adrenal secretory rates. In addition, it also seemed of interest to make some estimate of the lapse of time between hypophysectomy and the finding of the lowest secretory rates.

Methods. Pups delivered spontaneously were taken from their mothers from 5 min to 12 hr after birth.

Hypophysectomy was carried out under anesthesia with ether by means of a modification of the transbuccal approach described by McLean (5), with removal of the hypophysis by aspiration. Completeness of hypophysectomy was determined in each animal by inspection. All animals were kept in a warmed box until taken for adrenal drainage.

Adrenal venous drainage was carried out in pups anesthetized with 50 mg/kg of pentobarbital administered intraperitoneally. After opening the abdominal cavity, the following vessels were ligated: inferior and superior mesenteric and celiac arteries, and both renal arteries and veins at the hilum of the kidney. These animals were, in effect, eviscerated and nephrectomized. The tip of a polyethylene catheter (PE50) was introduced into the right renal vein and passed through the inferior vena cava into the left adrenal vein. The tip was secured by a ligature around the left adrenal vein. The left lumbodorsal vessels distal to the adrenal were ligated. Blood from the catheter was allowed to drain into graduated centrifuge tubes. The rate of collection and the hematocrit of each aliquot of blood were determined. The levels of cortisol in plasma were determined by the method of Silber and Porter (6) as modified by Peterson *et al.* (7). Cortisol secretory rate was calculated from the formula: plasma concentration $\times$ blood flow $\times$ $\frac{(100\text{-hematocrit})}{100}$.

All animals, at the time of craniotomy, received 0.05 mg of deoxycorticosterone intramuscularly and 5 ml of a solution containing 5 g of glucose and 0.9 g of sodium chloride per 100 ml of water intraperitoneally. The animals taken for adrenal venous drainage 16–20 hr after hypophysectomy received a second injection. subcutaneously, of the same solution 2–3 hr before adrenal drainage. The weights of all pups taken for adrenal drainage after hypophysectomy were within 1–3% of the weights found before

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hypophysectomy.

The animals were divided into 3 groups:

Group 1 (5 animals). After anesthetization with ether, the procedure was carried out to the extent that a hole was drilled through the sphenoid bone.

Group 2 (6 animals). The procedure for hypophysectomy was carried out but postmortem examination revealed that hypophysectomy had not been achieved. The difference between Groups 1 and 2 was in respect to the trauma to the pituitary gland and the hypothalamus and the subdural hemorrhage which the animals of Group 1 did not have.

Group 3 (20 animals). These animals were successfully hypophysectomized. The group was further divided into subgroup 3A (15 animals) which included animals taken for adrenal drainage 2–5 hr after the intracranial procedure, and subgroup 3B (5 animals) which included animals taken 16–20 hr after the intracranial procedure.

Results and Discussion. All animals included in this study which had been subjected to the intracranial procedure appeared to do well postoperatively. They were active and noisy after recovering consciousness. Deoxycorticosterone, water, and sodium chloride were administered in order to prevent the circulatory consequences of hypoadrenalism. The amounts employed preserved the weight determined initially.

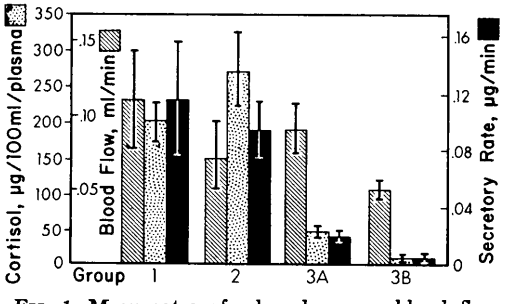

FIG. 1. Mean rates of adrenal venous blood flow, mean cortisol concentrations in adrenal venous blood, and mean cortisol secretory rates are shown for each group by the bars. The vertical lines are ± one standard deviation of the mean. Animals were divided into the following categories: Group 1, 5 animals in which, after anesthetization with ether, a hole was drilled through the sphenoid bone; Group 2, 6 animals in which the intracranial procedure was carried out but hypophysectomy was not achieved; Group 3A, 15 animals taken for adrenal drainage 2–5 hr after hypophysectomy; Group 3B, 5 animals taken for adrenal drainage 16–20 hr after hypophysectomy.

Figure 1 shows the mean (± SE) of the observed adrenal blood flow, of the determined concentration of cortisol in adrenal venous blood, and of the calculated adrenal secretory rate of cortisol for each group. The animals in Groups 1 and 2 displayed brisk secretory rates, undoubtedly reflecting the operative stress, which were not significantly[1] different. The animals taken for adrenal drainage 2–5 hr after hypophysectomy (Group 3A) displayed strikingly lower secretory rates than those of Groups 1 and 2 ($p<.001$). Since adrenal blood flow in this hypophysectomized group was not significantly different from that in Groups 1 and 2, the decrease in secretory rate was accounted for by the decrease in concentration of cortisol of the adrenal effluent blood. The findings support our earlier conclusion (1) that the hypophysis exerts a definite controlling influence on the adrenal gland of the newborn dog. Furthermore, hypophysectomy performed on the dog up to 12 hr after birth was effective in permitting the preparation of an animal with an initially low adrenal secretory rate.

It is surprising that a significant[1] difference in secretory rate was found between the animals of the 16–20-hr group and the 2–5-hr group ($p = .01$). The difference in secretory rates for these two groups was found to be significant[1] when the secretory rate of Group 3A was compared with a hypothetical rate calculated from the observed concentration of cortisol in Group 3B and the rate of blood flow of Group 3A ($p<.05$).

Factors which would determine the rate of decline in adrenal secretory activity after hypophysectomy would include completeness of extirpation and destruction of the gland, the duration of stimulation of the adrenal by residual active ACTH, and the length of the response of the stimulated gland after the

[1] *t* test, nonpaired experiments.

disappearance of ACTH. Although careful inspection for completeness of hypophysectomy was made at the conclusion of each experiment, the possibility of surviving pituitary tissue exists.

Estimations of the plasma half-life of ACTH in the rat and in man have been critically reviewed by Yates and Urquhart (8), and have been summarized by Wolf *et al.* (9), and by Scriba *et al.* (10). In these species the half-life has been found to range from about 4 to 10 min. The brevity of the period of persistent activity would lead one to anticipate that the ACTH circulating at the time of hypophysectomy should no longer be present 2–5 hr later. Any possible stimulatory effect of the anesthetic, ether (11, 12), should have been limited to the period ended by removal of the source of ACTH. Zarrow *et al.* (13) emphasized the brevity of the manifestation of responsiveness of the adrenal gland in the newborn rat. Urquhart reported a rapid decline in cortisol secretory rate when the rate of delivery of ACTH of the perfused isolated adrenal of the dog was reduced (14, 15). These factors of rapid inactivation of circulating ACTH and limited duration of responsiveness of the adrenal gland suggested that it was likely that the lowest levels of adrenal secretory rate would be reached 2–5 hr after hypophysectomy.

The adrenal secretory activity of the newborn may be controlled in a fashion analogous to that operating during maturity, or it may be influenced by factors derived from the recent maternal–placental relationship existing *in utero*. The striking fall in secretory rate after hypophysectomy establishes the importance of the hypophysis in the control of adrenal secretory rates during the 24 hr after birth. Highly significant differences were found in concentration of cortisol in adrenal effluent blood and in secretory rate between the animals 2–5 hr and those 16–20 hr after hypophysectomy.

That a maternal influence may be exerted on the fetal adrenal has been appreciated since the report of Ingle and Fisher (16) that adrenalectomy performed on the pregnant rat resulted in hypertrophy of the fetal adrenal cortex. Courier, Cologne, and Baclesse (17) also reported that the injection of corticosteroids into the pregnant rat resulted in smaller adrenal glands in the fetus. D'Angelo made similar observations in respect to the guinea pig (18). If a maternal–placental principle, influencing the fetus and waning during the period immediately after birth, was postulated to explain the less than complete response to hypophysectomy observed at 2–5 hr, it must have been a circulating factor other than one operating through the hypothalamic–pituitary components of the hypothalamic–pituitary–adrenal axis. The results reported here do not provide further insight but they do suggest that the decline in secretory rate is real and is slower than might have been anticipated.

Summary. Hypophysectomy in the newborn dog results in a prompt fall in the secretory rate of cortisol during collection of adrenal effluent blood. Due to the rapid rate of clearance of ACTH, lowest secretory rates were expected at 2–5 hr after hypophysectomy but, at 16–20 hr, an even lower rate was found. This unexplained observation was significant. The procedure of hypophysectomy recommends itself as a valuable means of exploring the endocrinologic status of the newborn.

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