Maturity of The Pituitary–Adrenal Axis in The Newborn Dog (37500)

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The functional development of the pituitary–adrenal system has been investigated to only a limited extent in the fetal and newborn dog. Following administration of ACTH to the fetal dog *in utero,* Jackson and Piasecki (1) reported that the levels of plasma cortisol and corticosterone increased in the fetus. Milković and Milković (2) found that pups 3 to 4 days of age responded to epinephrine with a moderate but significant fall in the content of adrenal ascorbic acid but not of cholesterol.

A report (3) from our laboratory describes experiments indicating that the feedback mechanism of homeostatic control of adrenal secretion appears to be functional at birth in the dog. In the present study, the maturity of the pituitary–adrenal axis has been tested by determination of the cortisol levels in adrenal glands of newborn pups following administration *in vivo* of ACTH or dexamethasone.

Methods. Nineteen litters of pups, taken within 24 hr after birth, were randomly divided into 2 control and 3 treated groups. For the studies of response to ACTH, the 17 animals of the first control group were given intramuscular injections of 1 ml of isotonic saline and the 39 animals of the first treated group were similarly given 2 units of ACTH in 1 ml of isotonic saline. For the experiments employing dexamethasone, all animals in the second control group and in the second and third treated groups were anesthetized with 25 mg/kg of pentobarbital administered intraperitoneally. The jugular vein of each pup was then cannulated and the 29 control pups were given 1 ml of isotonic saline. The 18 pups of the second treated group were given 0.01 mg/kg of dexamethasone in 1 ml of saline and the 15 pups of the third treated group were similarly given 0.02 mg/kg of dexamethasone.

All animals were kept in a warm environment without further handling until sacrificed by decapitation 3 hr after injection. Trunk blood was collected from each pup and the concentration of plasma cortisol was determined by the method of Silber and Porter (4) as modified by Peterson *et al.* (5). The adrenals were removed immediately after collection of the trunk blood and were trimmed of extraneous tissue. The gland pairs from each pup were then weighed, ground with a mixture of sea sand and dimethoxymethane (6), and allowed to stand 2 hr at room temperature or at —20° overnight. The mixtures were then filtered through fritted discs and the residues of the filtrates were chromatographed on paper, employing a modification of the method of Baggett, Kinsella, and Doisy (7). The cortisol content of the residues of eluates of appropriate cuts of the paper strips was determined by the method used for the plasma extracts.

Results and Discussion. The pup during the first 24 hr after birth responds to the administration of ACTH as indicated by an increase in the content of cortisol in the adrenals as well as a pari passu increase in the level of cortisol in the plasma (Fig. 1). It would seem, therefore, that the stress of delivery, the adjustment to extrauterine life and the experimental procedure did not elicit maximal production or release of cortisol in the newborn pup (2). Zarrow and co-workers (8) have emphasized the importance of the temporal relationship between the stress applied and the end point measurements as well as the brevity of the response in the new-

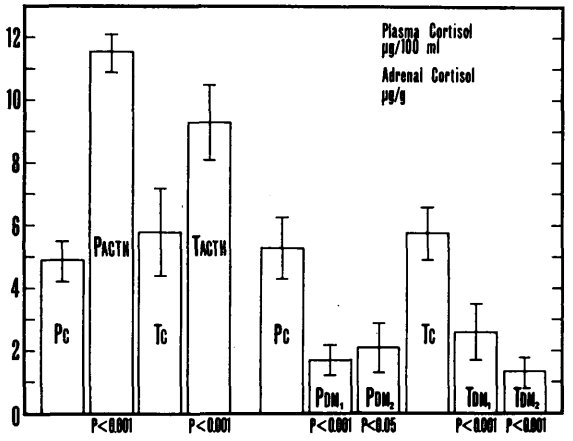

FIG. 1. Plasma Cortisol (P) and Adrenal Gland Cortisol (T) in the newborn dog following administration of ACTH (2U) and Dexamethasone (DM_1 = 0.01 mg/kg and DM_2 = 0.02 mg/kg). C = Control; I = Standard Error. [Some values of plasma cortisol levels have been taken from and recalculated from an earlier paper by Muelheims, Francis, and Kinsella (3).] The p values (t test nonpaired experiments) represent the difference between the control and treated groups.

born rat. In the pup, the effects of stimulation were still measurable several hours after administration of the trophic hormone. This may have resulted from the large amounts of ACTH used or from its slow release from the site of injection, which might have assured continued stimulation of the adrenals through the 3-hr period until decapitation was carried out and samples of plasma and adrenal glands were taken for analysis.

Our results are in agreement with the results obtained following administration of ACTH to the neonatal rat. Milković and Milković (9), who used animals immediately following birth, and Jailer (10) and Rinfret and Hane (11), who employed animals from 4 to 7 days of age, all observed a fall in adrenal ascorbic acid. Schapiro *et al.* (12) also obtained a fall in the content of adrenal ascorbic acid and a concomitant increase in the adrenal content of corticosterone in rats 1–3 days old. Levine and co-workers (13) observed in 3-day-old rats an elevation in plasma and adrenal concentrations of corticosterone, and Allen and Kendall (14) reported a small increment in plasma corticosterone in animals 2 and 4 days of age. Our data are also in accord with that reported for the human newborn infant following the administration of ACTH (15–17). This is of practical significance since it indicates that the fetal and newborn pup may be utilized as an experimental model of studying various hormonal systems difficult to investigate directly in the normal human fetus and infant.

It is evident from our data that the administration of dexamethasone to the newborn dog resulted in a significant decrease in the levels of cortisol in the adrenal glands as well as in plasma (Fig. 1). De Wied (18) and Russell and co-workers (19) have shown that the inhibitory effect of dexamethasone on the synthesis of corticosteroids can result from a direct blocking action of the pituitary on the ACTH releasing mechanism. Since part of the response to stress involves stimulation of the adrenals by endogenous ACTH, the reduction in the amount of cortisol in the adrenal glands and plasma following injection of dexamethasone indicates the qualitative maturity of the pituitary in the newborn dog with respect to the production and release of ACTH. In fact, response of the neonatal canine adrenals to dexamethasone may be as great or greater than that of the adult rat (19). Our results with dexamethasone also establish that the pituitary–adrenal feedback system is operative in the newborn pup, probably rendering it capable of responding

to stress.

In contrast to our findings for the newborn dog, D'Angelo (20) did not obtain the expected suppressive effect of dexamethasone in the guinea pig during the first postnatal week. The adrenal glands did respond to ACTH as measured by hypertrophy of the glands and increase in plasma fluorescence-inducing corticosteroids. This difference between the newborn dog and guinea pig may be species dependent or may reflect differences in the experimental procedures employed. D'Angelo obtained his plasma samples by cardiac puncture under ether anesthesia, a possible stimulatory agent (21), and employed larger doses of dexamethasone. These differences and possibly others (20) do not permit resolution of the discrepancy.

Of interest are the actual levels of corticosteroids found in neonatal peripheral plasma and adrenal glands. We reported (3) that cortisol, identified by chromatographic means, was the principal free corticosteroid present in the plasma and adrenals of the newborn dog, confirming and extending the report of Jackson and Piasecki (22). The concentration in plasma averaged 5.2 μg/100 ml and in glands 5.6 μg/g of tissue (Fig. 1). If corrected for a 74% recovery obtained when cortisol was added to plasma, these values would be 6.5 μg/100 ml of plasma and 7.6 μg/g of tissue. Fonzo *et al.* (23) reported similar levels of 4.4 μg/100 ml of free cortisol measured in fetal mixed plasma from canine fetuses obtained by cesarian section at term.

Levels of corticosterone in adrenals of rats 3 days of age have been reported to range from 4.5–12 μg/g of tissue (13). Approximately the same levels, 10.4 μg/g of tissue were found in the adrenals of neonatal guinea pigs but the plasma concentration, 97.8 μg/100 ml, appears to be much higher in this species (20). 17-Hydroxycorticosteroid and cortisol levels, respectively, in the plasma of one day old piglets (24), 43.9 μg/100 ml, and sheep (25), 40 μg/100 ml, are intermediate between those of the rat and the guinea pig. In the newborn human, cortisol has been identified as the predominant circulating corticosteroid and values ranging from 5.5–26 μg/100 ml have been reported from plasma obtained at various times during the first 36 hr after birth (26). It would appear from the above that the levels of cortisol in the newborn dog are in the same range as those in the neonatal rat and the human infant.

Summary. The apparent changes in steroid hormone production and release by newborn canine adrenals following the administration of appropriate agents indicate that the pituitary–adrenal axis is functional at birth in this species, rendering it capable of responding to stress. The newborn dog may be employed as a useful experimental model in exploring the endocrinologic status of the newborn.

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