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Neonatal Cortisol Administration: Effect on Growth, the Adrenal Gland and Pituitary-Adrenal Response to Stress.* (30650)

SHAWN SCHAPIRO (With the technical assistance of Miss Carol J. Percin)
Developmental Neuroendocrinology Laboratory, V. A. Hospital, San Fernando, Calif., and Department of Psychiatry, Center for the Health Sciences, University of California, Los Angeles

The infant rat does not secrete ACTH in response to stress in the same manner as the adult until about the eighth postnatal day (1-3). The basis for this neonatal stress-non-responsive period probably lies within the central nervous system. The available evidence indicates, however, that the steroid feedback mechanism is operating during fetal (4) and early postnatal life(5-6) so that central mechanisms responding to changing internal signals (plasma cortical hormone levels) appear to be functioning before external signals (stress or noxious stimuli) are relayed to the pituitary gland. These observations are compatible with the view that endogenous hormones, acting in the classic feedback manner to produce a steroid blockade, may prevent stress from activating the neonatal pituitary. The "set" of the "corticostat" (that region of the central nervous system, presumably within the diencephalon, upon which adrenal cortical hormones act to moderate the secretion of ACTH) may render it more sensitive to cortical hormones during the early postnatal period.

This report compares the adrenal atrophy produced in infant and adult rats to graded doses of cortisol. In addition several observations were made concerning the effect of one injection of cortisol upon growth and the pituitary-adrenal response to stress.

Methods. Sprague-Dawley rats bred in the laboratory were used in all experiments. A. Effect of multiple injections of cortisol upon adrenal weight. Litters born the same day were paired to 8 pups each and from days 2-5 or 30-33 received daily I.P. injections of 0.10-2.5 mg/100 g cortisol acetate (cortef-Upjohn). On day 7 or day 35 all rats were killed, including a control group receiving saline, and the adrenals weighed individually. B. Effect of single doses of cortisol upon growth and the adult response to stress. Litters were paired to 8 pups at day 2 and half of each litter received a single subcutaneous injection of 1 mg cortisol acetate. They were weaned at 21 days of age and on day 44 some of each group were rapidly anesthetized with ether, decapitated, trunk blood collected and the adrenals removed and weighed. Others were anesthetized with ether and 20 minutes later decapitated and blood and adrenals also saved for corticosterone analysis(7). The thymi and brains were removed from some rats in each group, weighed and brain cholesterol(8) was determined. C. Effect of single dose of cortisol upon fertility. From day 55-75 daily vaginal smears were performed on 12 female rats which had received cortisol as infants. To assess fertility four cortisol treated females were placed with proven males for 20 days. Pregnancies occurred in all instances.

Results. A. Effect of cortisol upon adrenal weight: Fig. 1 illustrates the changes in adrenal weight that occurred in infant and 30-day-old rats on graded doses of cortisol. The changes are based upon comparisons with saline treated controls. At all but the highest dose level there was a greater effect upon the adrenals of the infants than upon the adults. B. Effect upon growth, fertility and the response to stress. Following a single injection of 1 mg cortisol into the 2-day-old

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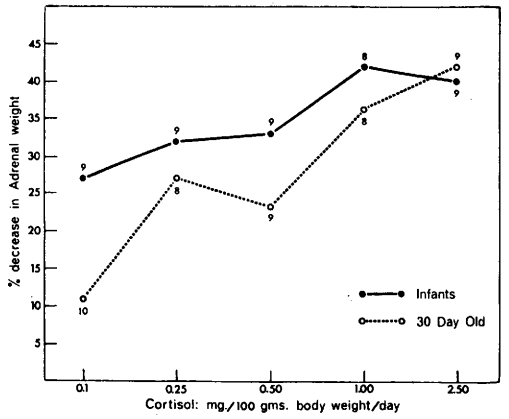

FIG. 1. Effect of graded doses of cortisol upon adrenal weight of infant and 30-day-old rats. Cortisol or saline given I.P. daily, for 3 days from day 2-5 or 30-33. All rats sacrificed on day 7 or 35 and adrenal weights compared to controls.

rat a rather unique physical change developed. The skin of these animals became blotchy with areas of sparse hair and hyperpigmentation; they also appeared more nervous. In addition to this rather characteristic "corticoid stigmata" there was a marked stunting of growth (Fig. 2-3). Frequently, although not always, the eyes and the perineal region of these "corticoid runts" were encrusted with an exudate and seemed to be infected. The pituitary-adrenal response to stress was normal in these runts as shown in Table I. Brain myelinization, as determined by cholesterol, was decreased, while the thymus weight was diminished only slightly in proportion to body weight; the adrenal glands were substantially heavier on a body weight basis (Table I). Vaginal smears performed daily indicated normal ovarian cycles and four runted females became pregnant. Three of these delivered grossly normal offspring which were nursed successfully. The remaining female (weight 80 g) was observed walking in the cage dragging a dead extruded fetus. Laparotomy revealed 6 enlarged fetuses, well formed, whose weight averaged 7.5 g.

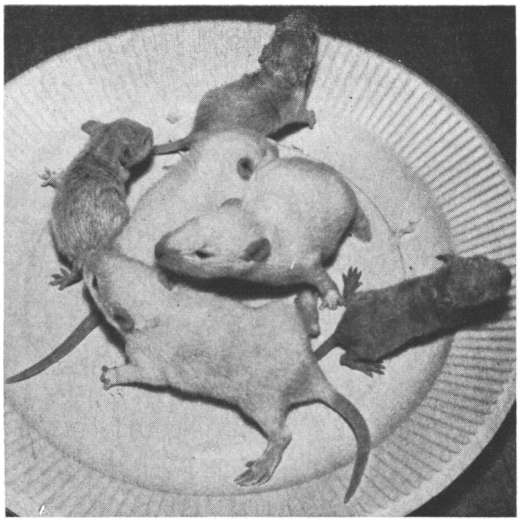

FIG. 2. Litter mate rats, 17 days of age. The 3 in the center are controls, the other 3 received one injection of cortisol acetate when 2 days old.

Discussion. The mechanism whereby steroid hormones act to inhibit the secretion of ACTH in response to stress is thought to involve an adjustment in the hypothalamic stimulus to the anterior pituitary(9).

The precise central localization of the steroid sensitive site(s) within the CNS ("corticostat") which adjusts ACTH secretion is yet unclear, although evidence suggests it may be within the hypothalamus and/or the reticular formation(10). Regardless of its location, the data in Fig. 1, indicating greater adrenal atrophy in infant rats than adults in response to graded doses of cortisol, would support an interpretation that there has been a more effective suppression of ACTH secretion in the neonatal animals. While this may argue for a greater sensitivity of the "corticostat" to steroids during this time alternative possibilities have not as yet been excluded. For example, the metabolic half-life of cortisol in the circulation may be considerably longer in the infant than the adult(11) and there-

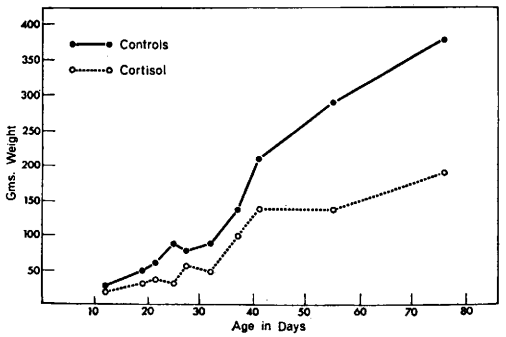

FIG. 3. Body weight of rats receiving 1 mg cortisol acetate on day 2. Each point represents 5-10 animals. In all cases one-half of each litter received cortisol and the other served as controls.

TABLE I. Effect of Neonatal Cortisol Acetate Upon the Rat at 40 Days of Age.

Group		Body wt (g)	Adr wt (mg)	Adr / BWt × 10	A* SCS	B* SCS	A* ACS	B* ACS	Brain chol (mg %)	Thymus wt (mg)	Thymus/ BWt
Cortisol	N	(36)	(20)	(19)	(7)	(31)	(8)	(14)	(6)	(9)	(9)
	Avg	120	21.0	2.65	23.9	50.8	21.7	85.4	1260	363	3.93
	SE	± 16	± 1.8	± .12	± 3.7	± 5.3	± 2.6	± 2.1	± 20	± 8	± .15
Control	N	(33)	(19)	(19)	(8)	(34)	(10)	(13)	(6)	(10)	(10)
	Avg	171	24.7	2.17	20.1	55.4	25.4	83.5	1380	554	4.11
	SE	± 11	± 1.1	± .09	± 2.5	± 2.4	± 2.4	± 1.1	± 30	± 32	± .32
Signif.		.001	.001	.001	NS	NS	NS	NS	.05	.001	NS

* Blood (SCS) and adrenal gland corticosterone (ACS) determined immediately (A) and 15 minutes after (B) ether anesthesia for 3 min.

fore its feedback activity may be prolonged. Furthermore, and perhaps related to the above, the blood brain barrier may be more permeable to cortical hormones during the neonatal phase of development and accordingly more hormone may reach the brain to exert an influence over the functional activity of the "corticostat."

Administration of a single massive dose of cortisol had marked effect on body weight and appearance (Fig. 2-3). The dose used in the 2-day-old rat (wt. approximately 7 g) was roughly equivalent to 14 mg/100 g. The "corticoid runt" so produced, strikingly resembles the wasting condition produced in rats following neonatal thymectomy(12) or in mice following more chronic cortisol administration(13). While it is tempting to regard the "corticoid runt" as a functionally thymectomized animal it is apparent from Table I that the weight of the thymus was not specifically compromised. Whether certain of its immunological functions have been adversely affected is at present being investigated. Some effect upon central nervous system development is suggested by the decrease in brain cholesterol (Table I), apparent defective vision, and the presence of a distinct resting tremor. Changes in brain RNA-DNA in the cortisol treated mouse have been reported by Howard(13). The pituitary-adrenal cortical response to stress was practically identical in the "corticoid runts" and their controls (Table I). Thus, in contrast to cyclic gonadotropin secretion, which is permanently disrupted by neonatal treatment with testosterone propionate(14), neither neonatal cortisol nor testosterone propionate(15) appear to effect the pituitary-adrenal response to stress. Vaginal smears indicated normal cyclic gonadotropin function and this was supported by three of four successful pregnancies. One pregnancy was unsuccessful and was associated with enlarged fetuses. Whether this was coincidental or bears some relationship to cortisol treatment in early life is at present uncertain.

Summary. 1. The effect of graded multiple doses of cortisol (0.1-2.5 mg/100 g) on adrenal weight of 2-5-day-old and 30-33-day-old rats was compared. At all but the highest dose levels greater adrenal atrophy occurred in the infants. This suggests a greater sensitivity of the steroid feedback mechanism to cortical hormones during the early postnatal period. 2. The effect upon growth and development of a single massive injection of cortisol (1 mg) on postnatal day 2 was studied. Severe effects on growth and physical appearance were observed and a characteristic 'corticoid stigmata' and 'corticoid runt' are described. The secretion of ACTH in response to stress was normal in the 'runted' animals and ovarian cycles were maintained. Thymus weight was not disproportionately affected although the adrenal glands were somewhat enlarged. Brain cholesterol was decreased.

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Adrenal Hemorrhagic Effect of Thorotrast.* (30651)

WILEY H. MOSLEY AND LEIGHTON E. CLUFF
(With the technical assistance of Miss Susanne Bohnet)
Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Md.

Severe bilateral adrenal hemorrhage, the distinguishing characteristic of the Waterhouse-Friderichsen Syndrome, is clasically associated with meningococcal septicemia. It has also been reported with other acute fulminating infections(1), in association with severe non-infectious stress or shock(2,3) and as a complication of ACTH and of anticoagulant therapy(4,5). Experimentally, adrenal hemorrhage in animals has been observed following intravenous injection of lethal doses of a variety of bacterial toxins(6). In addition, adrenal hemorrhages have been noted in animals given endotoxins in the production of the generalized Shwartzman phenomenon (7,8,14). Among the factors contributing to the development of adrenal hemorrhage, the role of adrenal stimulation(9,10,11) and the association of adrenal hemorrhage with purpura, visceral hemorrhages and with multiple coagulation defects have been noted(6,7,12, 13).

It was previously reported that adrenal hemorrhage could be produced by the intravenous administration of endotoxin in rabbits pretreated with Thorotrast (thorium dioxide, colloidal) or ACTH(14). The studies described here were done to further examine the production of adrenal hemorrhage by Thorotrast alone. This effect was found to be directly related both to the dose of Thorotrast and the size of the animal. Adrenal hemorrhage was dependent upon the particulate material (thorium dioxide) in Thorotrast and was associated with histological evidence of vascular damage, adrenal stimulation, and multiple coagulation defects.

Materials and methods. Animals. Albino New Zealand rabbits supplied by a commercial distributor were given a standard laboratory diet and water *ad lib* throughout the experiments. Male animals were used in most experiments; a few female rabbits showed essentially the same response.

Thorotrast was a sterile, stabilized colloidal suspension of thorium dioxide (24 to 26% by volume) in 25% aqueous dextrin produced by Fellows-Testagar Co., Inc., Detroit.

Injection technique. All intravenous injections were made *via* the marginal ear vein.

Platelet counts. Platelet counts were determined in duplicate, using phase microscopy according to the method of Brecher and Cronkite(15).

Prothrombin times were performed according to the one-stage method of Quick(16). The results are reported in per cent, compared to a standard normal control.

Fibrinogen. Plasma fibrinogen levels were determined according to the thrombin method of Clauss(17).

Clotting time. Whole blood clotting times

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