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Effect of Elevated Corticosterone Levels on Serum Gamma Globulin Concentrations in Newborn Rats[1] (37777)

J. A. PATT, JR.[2] AND R. J. EBERHART
(Introduced by A. Anthony)
Department of Veterinary Science, The Pennsylvania State University, University Park, Pennsylvania 16802

The intestine of the newborn rat has the capacity to absorb immunologically reactive antibodies present in the lacteal secretions of the dam. This capability is lost at about 20 days of age (1) but the changes in the epithelial cells associated with this "closure" and the mechanisms which trigger them are obscure. Because glucocorticoid hormones stimulate the maturation of adult levels of some enzymes and capacity for glucose absorption in these cells (2, 3), it has been postulated that these hormones may also influence the cessation of antibody transfer across the intestinal membrane. Plasma corticosterone increases after 18 days of age coincident with the beginning of intestinal closure (4). Similar correlations between elevated corticosteroid concentration and cessation of macromolecule uptake by the intestine have been shown for the young guinea pig and rabbit (5). Halliday (6) demonstrated that pharmacological doses of either cortisone acetate or deoxycorticosterone acetate in suckling rats prematurely terminated absorption of homologous antibodies against *Salmonella pullorum*. Large doses of cortisol administered to rat pups reduced the pinocytotic activity of intestinal cells (7), a capability believed necessary for the absorption of macromolecules (8). However, these studies used pharmacological doses of hormonal steroids other than corticosterone which is the predominant glucocorticoid in the rat (9). It was, therefore, decided to investigate the effects of corticosterone on the concentration of gamma globulin in the newborn rat. Rats were injected with ACTH rather than corticosterone as this treatment was expected to produce an elevation in serum corticosterone of some duration while avoiding administration of very high doses of the steroid. The increased serum corticosterone levels in the ACTH-injected rat pups would, therefore, be the result of endogenous secretion.

Materials and Methods. Sprague–Dawley rats were bred and maintained in our facility. All rats were allowed food and water *ad libitum*, and received 12-hr continuous light per day beginning at 7 AM.

The day of birth was designated Day 0. Beginning at 4 PM on Day 9 all pups in a given litter received either 0.2 ml sterile 0.9% NaCl or 0.1 USP U ACTH (Armour Acthar Gel) in 0.2 ml sterile 0.9% NaCl subcutaneously. Subsequently rats were injected at 8 AM and 4 PM each day and rats from each litter were decapitated at 5 PM on Days 9, 15, 18, 21, and 24. The blood was collected and the serum stored at −20° until it was assayed for corticosterone and gamma globulin. Blood from two or three rats killed on Day 9 and Day 15 was pooled to provide enough serum for a single determination. Twelve litters of rats were randomly assigned between both injection treatments, and all rats in each litter were divided among

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[2] Present address: Department of Biology, Kent State University, Ashtabula Regional Campus, Ashtabula, Ohio 44004.

the five age groups. The data were statistically analyzed as a 2 × 5 factorial design, and a Duncan's Multiple Range test was employed to distinguish significant differences among the means.

Corticosterone was assayed according to the fluorimetric method of Zenker and Bernstein (10) except that the samples were extracted with 25 ml redistilled dichloromethane instead of 45 ml chloroform and the organic phase was rinsed with 2.5 rather than 4.5 ml 0.1 *N* NaOH.

Serum gamma globulin concentrations were calculated after assaying total serum protein by the biuret method (11) and determining the percentage of gamma globulin in the serum proteins by cellulose acetate electrophoresis (12).

Results. The concentrations of corticosterone in the sera of saline-injected control rats

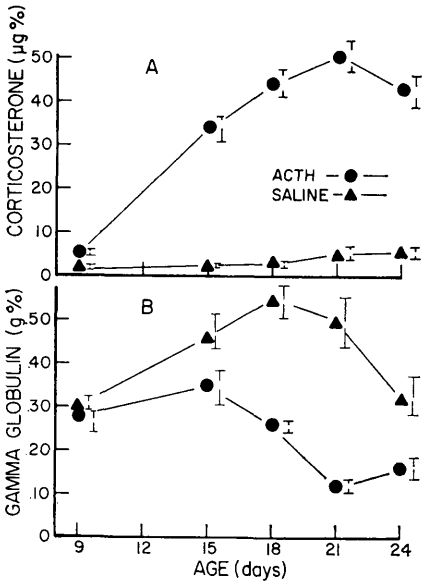

FIG. 1. Effects of exogenous ACTH in neonatal rats. Values are means ± SE. (A) Serum corticosterone levels in rats treated with 0.1 USP U ACTH in 0.2 ml saline, or with 0.2 ml saline. Rats were injected once at 9 days of age, twice daily thereafter, and were sacrificed 1 hr after the last scheduled injection of the day when the serum was collected. Six litters of rats were assigned to each treatment and each litter was divided among the various age groups. (B) Serum gamma globulin concentrations in the ACTH and saline-injected rats described above.

1 hr after the last scheduled injection ranged from 1.4 µg/100 ml at 9 days of age to 5.5 µg/100 ml at 24 days (Fig. 1A), but the difference was not statistically significant ($P > 0.05$). Nine-day-old rats, which received a single subcutaneous injection of 0.1 USP U ACTH, had a serum corticosterone level of 4.5 µg/100 ml which was not statistically different from the Day 9 saline value. However, when rats received continued corticotropin injections twice daily up to 15, 18, 21, or 24 days postpartum, serum corticosterone levels were higher in these animals than in either saline-injected controls of the corresponding age ($P < 0.01$) or in 9-day-old ACTH-treated rats ($P < 0.01$). In the ACTH-injected rats the corticosterone concentrations increased from 4.5 µg/100 ml on Day 9 to 33.9 µg/100 ml on Day 15, 44.0 µg/100 ml on Day 18, and 50.6 µg/100 ml on Day 21; each of these values is significantly higher than the preceding one. The mean of 42.8 µg/100 ml on Day 24 was lower ($P < 0.01$) than that of Day 21.

Serum gamma globulin concentrations in control and ACTH-treated rats did not differ (0.30 and 0.28 g/100 ml, respectively, $P > 0.05$) at 9 days of age (Fig. 1B). However, the levels were lower in ACTH-treated rats than in the control group on Days 15, 18, 21, and 24 (Day 15, $P < 0.05$; Days 18, 21, 24, $P < 0.01$). Serum gamma globulin increased in control pups to 0.46 g/100 ml by Day 15 ($P < 0.01$ *vs* Day 9), peaked at 0.55 and 0.50 g/100 ml on Days 18 and 21, respectively, and declined to 0.31 g/100 ml by Day 24 ($P < 0.01$ *vs* Day 15, 18, 21). The serum gamma globulin level in the ACTH-injected rats increased to only 0.35 g/100 ml on Day 15 ($P < 0.05$ *vs* Day 9 control), and fell to 0.26 g/100 ml on Day 18. By 21 days the concentration had declined to 0.12 g/100 ml ($P < 0.01$ *vs* Days 9, 15; $P < 0.05$ *vs* Day 18), and the level remained low (0.16 g/100 ml) at 24 days.

Discussion. Repeated ACTH injections in suckling rats reduced the serum gamma globulin concentration. This effect was probably mediated through increased glucocorticoid activity. While the data do not indicate whether increased serum corticosterone

concentrations were transitory after each ACTH injection or were maintained through the interval between injections, it appears that over the course of the experiment ACTH-injected rats were subject to increased glucocorticoid stimulation. Since maternal antibodies obtained through the gut are the major source of immunoglobulins in the suckling rat (13), reduced gamma globulin levels in treated animals may have been due to corticosterone inhibition of gamma globulin absorption. These data, derived in rats in which increased corticosterone levels were achieved through stimulation of the animals' own adrenals, support and extend earlier studies which demonstrated reduced uptake of specific antibody (6) and reduced pinocytotic activity (7) after administration of high doses of steroids not normally secreted in significant amounts by the rat adrenal.

Although the adrenal cortex of 3-week-old rats does not yet produce adult type circadian rhythms of corticosterone secretion, the levels of plasma corticosterone are higher than those in 10-day-old rats (14, 15). It is possible that this increased adrenal activity during the third week of life, although below adult levels, may be sufficient to effect maturation of the newborn rat intestine.

Summary. Administration of 0.1 USP U ACTH to 9-day-old rats followed by similar treatments twice daily through Day 24 produced elevated serum corticosterone levels compared with saline-injected control values 1 hr after the last injection at 15, 18, 21, and 24 days of age. The concentration of serum gamma globulin was significantly lower in the ACTH-treated rats than in saline controls at 15, 18, 21, and 24 days postpartum, possibly due to premature termination of intestinal absorption of gamma globulin in the ACTH-treated group. The serum gamma globulin concentration increased in control rats between days 9 and 18, then declined between Days 21 and 24, while in the ACTH-treated rats, the level did not increase significantly between Days 9 and 15, and fell between 15 and 21 days of age. This experiment indicates that elevated concentrations of corticosterone, the primary glucocorticoid in rats, reduce the serum gamma globulin level during the neonatal period.

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