

## Effect of Postnatal Malnutrition on Plasma Corticosteroid Levels in Male Albino Rats<sup>1</sup> (38747)

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Recent research from this laboratory has been aimed at elucidating the functional changes which accompany growth retardation in postnatally malnourished animals. For example, it has been shown that rats which are suckled by dams given a low protein diet have altered behavior, cholinergic brain chemistry, and brain mitochondrial respiration during as well as after the suckling period (1-3).

Two recent studies have shown that nutritional restriction imposed during both pre- and postnatal periods leads to increased basal levels of plasma corticosteroid during the fourth week of postnatal life in rats (4, 5). The following experiment was performed to show whether nutritional insult imposed solely during the postnatal period would also alter plasma corticosteroid levels and whether such nutritional privation would alter corticosteroid level prior to the fourth week of life.

**Materials and Methods.** Sperm positive Holtzman albino rats weighing 209-263 g were purchased at 3-4 days of pregnancy. The rats were housed in a temperature regulated room ( $23.1 \pm 0.8^\circ$ ) with a 12-hr light: 12-hr dark cycle (lights on from 0700-1900 hr). Pregnant rats were given a 25% casein diet until parturition. Male pups were cross-fostered 24 hr after birth, and litter size was kept at eight throughout the study. During the lactation period, the experimental dams were given a diet containing 12% casein which was sufficiently low in protein so as to reduce milk supply for the pups designated DL while the dams of the control pups continued to receive a 25% casein diet. At 21 days of age the pups

were weaned, placed in individual cages, and given either a 9% or 25% casein diet. The control pups which were weaned onto the 9% casein diet were designated DW, while the DL pups which were weaned onto the 9% casein diet were designated DL-DW. All groups had ad libitum access to food and water. A detailed description of the nutritional design and housing conditions has been presented elsewhere (6).

Blood and adrenal glands were obtained between 0800 and 1000 hr. One rat was taken from each litter, with experimental and control animals being sampled in alternating sequence. After a rat was removed from its litter, it was replaced by a female pup of approximately the same weight. The rats were decapitated within 1 min after they were removed from their cages, and their blood was collected in heparinized capillary tubes. The tubes were centrifuged at room temperature for 3 min. The plasma-containing portion of each tube was sealed with plastic caps then refrigerated for approximately 2.5 hr. The tubes were then frozen at  $-15$  to  $-20^\circ$  until use. After a rat's blood had been collected, the adrenal glands were removed, trimmed of fat and weighed to the nearest 0.1 mg.

Plasma corticosteroid was measured on an Aminco-Bowman spectrophotofluorometer by a microfluorometric method (7). Corticosterone (1, 2, 5 or 10  $\mu\text{g}/100$  ml) was used as the standard. The assay was linear in concentrations of 1-50  $\mu\text{g}/100$  ml. A mean recovery of 94.5% was obtained in quintuplicate determinations when 2.5 ng of corticosterone was added to 25  $\mu\text{l}$  of plasma containing 4.0 ng of the steroid. Precision was tested by analyzing two sets of plasma (five aliquots each). The mean, standard deviation, and standard error for each set of samples was 6.2, 0.65, 0.29  $\mu\text{g}/100$  ml and 4.0, 0.83, 0.37  $\mu\text{g}/100$  ml, respectively.

<sup>1</sup> Supported in part by funds provided through the State University of New York, National Institutes of Health Training Grant No. 5T01GM-01221-10, and Public Health Research Grant No. HD-02581 from the National Institute of Child Health and Human Development.

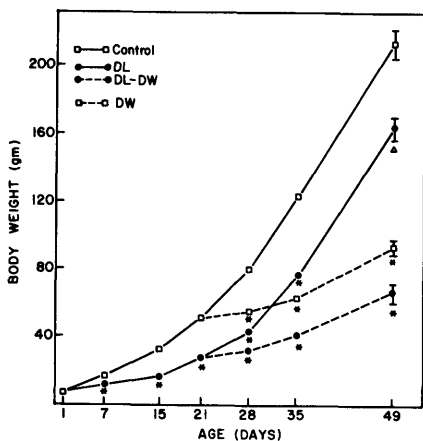


FIG. 1. Body weights of control and malnourished male rats. Abbreviations and symbols: (DL) rats malnourished during lactation; (DW) rats malnourished after weaning; (DL-DW) rats malnourished during lactation and after weaning; vertical bars,  $\pm 1$  SEM; asterisk,  $P < 0.001$  compared to controls.

Fluorescence emission peaks (515 nm) were identical for both plasma and standards. Although corticosterone is the major glucocorticoid produced in rats, cortisol may also be present under some conditions (8). Since the assay does not differentiate corticosterone and cortisol, the term "corticosteroid" is used throughout.

The *t* test (two-tailed) for unpaired data was used for all statistical comparisons.

**Results and Discussion.** Growth retardation of rat pups malnourished during lactation (DL) was evident from the seventh postnatal day and did not catch up with controls despite 4 wk of postweaning rehabilitation. Protein restriction initiated after weaning was also followed by growth retardation (Fig. 1). Adrenal glands of the DL animals weighed less than those of controls from days 11–21 ( $P < .01$ ). However, if adrenal weight was expressed as mg/100 g body wt, the apparent retardation of the malnourished rats disappeared. The adrenal glands of both groups of rats which were malnourished after weaning were hypertrophied at 49 days of age (Table I). Growth stunting and adrenal hypertrophy caused by nutritional deprivation during early postnatal development is in accord with previously published reports (4, 6, 9).

Although food intake was not determined

TABLE I. ADRENAL WEIGHTS OF MALNOURISHED AND CONTROL RATS.

Age (days)	Treatment (n)	Adrenal weight (mg/100 g body weight $\pm$ SEM)
7	Control (10)	19.5 $\pm$ 2.20
	DL (7)	21.7 $\pm$ 3.10
11	Control (10)	13.8 $\pm$ 1.45
	DL (9)	12.5 $\pm$ 1.20
15	Control (10)	18.9 $\pm$ 0.80
	DL (9)	18.7 $\pm$ 3.10
21	Control (10)	22.1 $\pm$ 1.40
	DL (9)	25.8 $\pm$ 3.12
49	Control (10)	14.9 $\pm$ 1.30
	DL (8)	15.9 $\pm$ 1.80
	DW (10)	22.4 $\pm$ 2.20 <sup>a</sup>
	DL-DW (12)	22.3 $\pm$ 1.20 <sup>b</sup>

<sup>a</sup> Differs significantly from controls,  $P < 0.01$ .

<sup>b</sup> Differs significantly from controls,  $P < 0.001$ .

in the present study, earlier work from this laboratory (6) has shown that rats which are malnourished before and after weaning or before weaning alone, consume more food per unit metabolic body size than controls during the postmalnutrition (rehabilitation) period.

Basal levels of plasma corticosteroid were significantly elevated in the DL rats at 11 and 21 days of age. However, corticosteroid level of this group of rats returned to normal after 4 wk of nutritional rehabilitation. Both groups of rats which had been fed a low protein diet after weaning had significantly elevated corticosteroid values at 49 days of age (Fig. 2). The fact that corticosteroid level returned to normal after 4 wk of nutritional rehabilitation suggests that nutritionally-induced change in basal corticosteroid concentration in infancy is not permanent, but depends upon concurrent dietary restriction. This may be related to decreased activity of the hepatic steroid reductase system, which is known to decrease during periods of food restriction (10).

The finding of increased plasma corticosteroid levels in malnourished infant rats confirms and extends the earlier reports of Adlard and Smart (4) and Shoemaker and Dallman (5). In those reports, rats which were malnourished during both pre- and

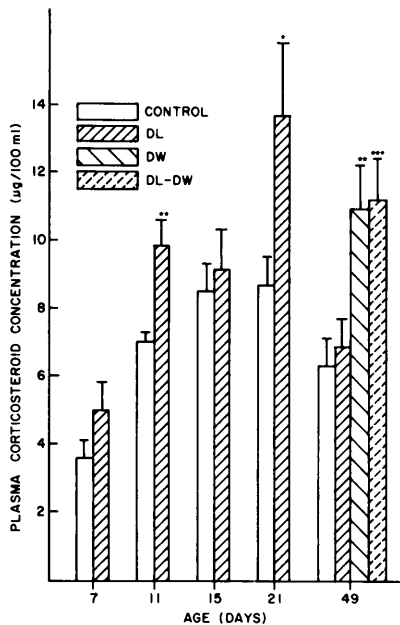


FIG. 2. Plasma corticosteroid concentration of control and malnourished male rats. Abbreviations and symbols: (DL) rats malnourished during lactation; (DW) rats malnourished after weaning; (DL-DW) rats malnourished during lactation and after weaning; vertical bars,  $\pm 1$  SEM; single asterisk,  $P < 0.05$  compared to controls; double asterisk,  $P < 0.02$  compared to controls; triple asterisk,  $P < 0.01$  compared to controls.

postnatal periods had significantly increased basal levels of corticosteroid at weaning or in the early postweaning period, while poststress corticoid levels were less than those of controls at either 28 days of age (5) or in adulthood (4). The present study demonstrates that increased plasma levels of corticosteroid may be present as early as 11 days of age and that gestational malnourishment is not a necessary antecedent condition for this result. Levine has shown that alterations in the pituitary-adrenal axis during early development cause long-lasting

changes in neural-endocrine response to stress (11). This suggests that increased postnatal corticosteroid levels such as those found in the present study may be important in determining subsequent abnormal adrenocortical functioning during stress in perinatally malnourished rats (4).

**Summary.** Basal morning plasma corticosteroid concentrations were significantly elevated in 11 and 21 day old male albino rats suckled by dams receiving a 12% casein diet when compared to control rats suckled by dams receiving a 25% casein diet. Rats which were either well-fed or malnourished during lactation then given a 9% casein after weaning had elevated corticosteroid levels and adrenal hypertrophy at 49 days of age. These changes may be related to altered adrenocortical responsivity found in adult rats malnourished in infancy.

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Received November 27, 1974. P.S.E.B.M. 1975, Vol. 149.