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POSTNATAL ADRENAL AXIS OF YOUNG RATS SUBJECTED TO PRENATAL LOW PROTEIN DIET

by

David Thomas Magrane

A Dissertation Submitted to the Faculty of the

COMMITTEE ON ANIMAL PHYSIOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

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GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by **David Thomas Magrane**

entitled **Postnatal Adrenal Axis of Young Rats Subjected to Prenatal Low Protein Diet**

be accepted as fulfilling the dissertation requirement of the degree of **Doctor of Philosophy**

Robert B. Forbes 4/10/72

Dissertation Director Date

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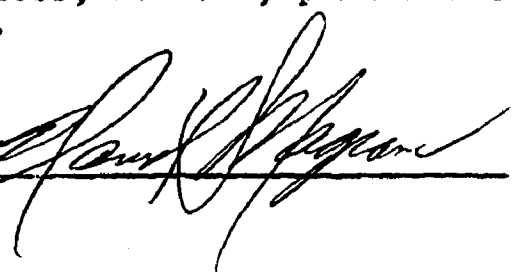
SIGNED: 

PREFACE

The treatise presented here was conceived as an idea of cooperative research between two graduate students, Charles Lewis and myself, to design a research proposal that would fulfill the requirement of original research for The University of Arizona Graduate College, and accomplish our feeling for pertinent research, applicable to human problems. An animal nutrition study was proposed with the intention of possible correlations to human dietary inadequacies and consequent behavioral development. It is hoped that these studies will help precipitate a better understanding of human malnutrition.

I wish to acknowledge with gratitude Dr. Robert B. Chiasson for his guidance and critical evaluation of the text. Drs. Donald E. Ray and Gerald H. Stott have read the dissertation and offered constructive criticisms. The guidance provided by Drs. Robert O. Kuehl and Donald E. Ray with the statistical analysis is greatly appreciated.

Finally, I am indebted to Charles Lewis for his cooperation during the course of this research.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

Pregnant rats fed a diet of 4% protein during the second half of gestation (LPD II) produced offspring with significantly lower body weights and smaller adrenals when compared to the offspring of rats fed a diet of 20% protein during gestation. However, adrenal activity in LPD II rats, as measured by plasma corticosterone (B) titers and relative in vitro release of adrenal corticoids, was greater than controls. A 4% protein diet fed to pregnant rats during the first half of gestation was without measurable effect on the offspring. Body weight, plasma B, and relative in vitro release of corticoid differences between LPD II and controls were no longer significant by postnatal (PN) day 7, but the adrenal weights remained significantly less than controls until PN 21. All neonatal adrenal glands were at their minimal level of activity at PN 7, as evidenced by relative adrenal weights, level of plasma B, and response to exogenous ACTH. Since PN 7 rat pituitaries contain an adequate concentration of ACTH (7-10 mU/ mg), the paucity of hypothalamic corticotrophic releasing activity assayed during the first two weeks of postnatal life is suggested as the cause of this adrenal nadir.

Pituitary weights, pituitary ACTH concentration, and hypothalamic corticotrophic releasing activity in neonatal rats were not affected by the LPD II treatment. All rats responded to exogenous ACTH *in vitro* and *in vivo* with significant adrenal corticoid release over basal levels at PN 0, PN 7, PN 14, and PN 21. With one exception, significant treatment differences were not found after exogenous ACTH administration. This exception was a reduced *in vitro* adrenal corticoid release after an *in vivo* ACTH injection in LPD II rats when compared to their controls. It is concluded that a low protein diet fed to rats during one-half of gestation is not detrimental to the subsequent development of the neonatal adrenal axis.

INTRODUCTION

Inadequate nutrition or excessive amounts of glucocorticoids during periods of hyperplastic development can result in permanent changes in various tissues, especially the brain. These changes may be reflected in morphological, physiological, biochemical, and behavioral alterations. Using brain deoxyribonucleic acid (DNA) content as an index of cell number, postnatal malnutrition of rats (Winick and Noble 1965), mice (Howard and Granoff 1968), and children (Winick and Rosso 1969) caused a reduction in the number of brain cells. Brain weight, ribonucleic acid (RNA), and cholesterol are generally reduced by malnutrition. The quantitative reduction of postnatal food intake was also effective in reducing selected brain enzymes in rats (Swaiman, Daleiden, and Wolfe 1970).

A single injection of cortisol in rats at two days of age resulted in a complex of developmental abnormalities on day 44 including a decrease in brain cholesterol, body weight, locomotor activity and a delayed development of cerebral cortical dendritic spines (Schapiro 1968). At 60 to 70 days, these same rats exhibited a deficient response to antigenic challenges. A deficit in hypothalamic growth hormone releasing factor was reported in five to six-week old rats that had received an injection of

cortisol at two days of age (Sawano, et al. 1969). Corticosterone pellets, implanted subcutaneously in two-day old mice, caused a reduction in total brain weight, cholesterol, RNA, and DNA when analysis was performed on postnatal day 7 (Howard 1965). Corticosterone implants in young rats also caused a lasting reduction in body size and impairment in the ability to maintain normal glucose levels upon physiological demand (Taylor and Howard 1971). Despite the impairment in the regulation of blood glucose, adult plasma corticosterone levels were not significantly reduced from control rats.

The effect of protein restriction during prenatal life is less well known, but the available results indicate similar size and chemical impairment. Zamenhof, van Marthens and Margolis (1968), and Zeman (1970), have shown that pregnant rats maintained on a low protein diet produced offspring with small body and brain weights and their brains had fewer cells than their controls. Although the total amounts of water, fat, protein, and ash were less in these rats, the percent composition of these components were not significantly different from that of their controls (Allen and Zeman 1971). Cerebral weight and total cerebral DNA were reduced in second generation progeny (F_2) of female rats (F_0) fed a diet of 8% protein during gestation (Zamenhof, van Marthens, and Grauel 1971). Dietary protein restriction to one generation of rats

also caused retardation of growth and behavioral development in the next generation (Cowley and Griesel 1966). Rats given a diet of 8% protein beginning at mid-gestation produced offspring with 25% less brain norepinephrine at day 24 than that of their control litter mates (Shoemaker and Wurtman 1971). Prenatal restriction by itself was not effective in reducing brain norepinephrine, but it did magnify the effect of postnatal malnutrition.

The hypothalamus is known to be influenced in its development by several hormones. Thus, thyroxine enhances the development of the tadpole median eminence (Etkin 1963) and androgens masculinize the hypothalamic regulation of gonadotrophins in mice and rats (Barraclough and Gorski 1961). The numerous correlations between "emotionality" and adrenocortical activity suggest an influence of adrenal steroids on neural organization. The hypothalamus acts as a modulator of adrenocortical function via its control over the pituitary release of adrenocorticotrophic hormone in the adult and probably in the fetus (Jost 1966; Jost, Dupouy, and Geloso-Meyer 1971). For maternal corticoids to affect its regulation, the fetal hypothalamus must be capable of concentrating corticosterone from maternal origin. This ability was demonstrated by experiments of Zarrow, Philpott, and Denenberg (1970) in which the relative uptake of ^{14}C-4-corticosterone injected into a pregnant rat was greater in the fetal

hypothalamus, after 30 minutes, than in the rest of the brain. The ability of the hypothalamus to concentrate corticosterone may indicate an influence of this steroid over fetal hypothalamic development.

The response of the neonatal adrenal axis to a chronic prenatal maternal stress has not been reported. Alteration of adrenal regulation might explain behavioral changes that were observed in offspring of pregnant rats with adrenal hypersecretion in response to stress situations (Thompson 1957). The present study will examine the adrenal axis of postnatal rats born of mothers fed a low protein diet during one-half of gestation. A low protein diet was chosen since it is known to depress neuron development, induce behavioral changes, and elevate maternal glucocorticoid levels. The quantification of hypothalamic corticotrophic stimulating factor, pituitary adrenocorticotrophic hormone concentration, and basal and stimulated release of corticoids from the adrenal glands through the third week of postnatal life will be reported.

METHODS AND MATERIALS

Animal Care

Weanling female rats of the Sprague-Dawley strain were maintained at 22°C with a lighting regimen of 13 hours light and 11 hours dark. Prior to experimentation, all rats were given an antibiotic solution (Sulmet Drinking Solution, American Cyanamid Co.) in their drinking water to reduce bacterial infections. Dosage was 1 oz/gal of water. Tap water was given during the experimental procedure. Rats were fed lab chow (Purina Lab Chow, Ralston-Purina Co.) at all times, except when on an experimental diet.

Pregnant female rats were maintained in individual cages. A wire mesh screen was fitted to the bottom of each cage of a pregnant rat on the 21st day to prevent newborn pups from dropping through the cage floor. Paper towels were added at this time for nesting material.

Diets

The diets used in these experiments were prepared by The University of Arizona Department of Poultry Science

and adopted from those of Zeman (1970).* The control groups were fed a diet of casein, 27%; dextrose, 59%; corn oil, 8%; and salt mix, 6% (purified vitamins, 2% and minerals, 4%) ad libitum. The low protein diet was identical, except that casein was reduced to 6% and dextrose was increased to 80%.

Experimental Procedure

Three to five female rats were placed in large cages with two males. Vaginal smears were taken each morning and those females with sperm in their vagina were assumed pregnant. Sperm positive females were randomly assigned to treatments and placed on an experimental diet (4% protein) or a control diet (20% protein) during either the first half of gestation (day 1 through day 11), or the second half of gestation (day 12 through day 22). Lab chow was fed during the other half of gestation. The dietary regimen of a low protein diet during the first half of gestation is referred to as LPD I, and the dietary regimen of a low protein diet during the second half of gestation is designated LPD II.

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*The control and experimental diets proposed by Zeman contained 27% and 6% protein respectively. However, the diets that were prepared for these experiments were assayed in this lab and found to contain 20% protein for the control diet and 4% protein for the low protein diet.

Data was initially collected on body and gland weights, plasma corticosterone, and adrenal release of corticoids. The number of litters for each treatment and at each postnatal period varied from five to nine. Several litters were randomly discarded in order to balance subclass numbers. Each treatment group was thus limited to five litters. Litters consisted of various numbers of individuals, all of which were sacrificed on the assigned day. Within each litter, three males and three females were randomly selected for statistical analysis. Sample sizes for the analysis of hypothalamic corticotrophic releasing factor, pituitary adrenocorticotrophic hormone (ACTH) concentration, and adrenal response to ACTH were unequal. The maximal response to exogenous ACTH in vitro and in vivo was obtained from one litter (of at least eight pups) for each of the postnatal period-treatment combinations. Since the results show no important differences between rats on the control diet and LPD I, the samples for analysis of hypothalamic corticotrophic releasing factor, pituitary ACTH concentration, and adrenal response to ACTH were only collected from controls and rats on LPD II.

Tissue and Sample Collection

Young rats were decapitated at postnatal days 0, 7, 14, and 21. Samples for analysis thus obtained were designated by their postnatal period as follows: PN 0, PN 7,

PN 14, and PN 21. Twenty-one day old rats were isolated 24 hours prior to use to minimize stress effects due to transport. Individual pups were quickly sexed, weighed, and decapitated. Blood was collected in a heparinized test tube on ice, and after centrifugation at 2000 rpm for 15 minutes in a Clinical International Centrifuge at 8°C, a 0.5 ml sample of plasma was removed and frozen at -20°C. Blood was pooled from entire litters at PN 0 and from three rats of each sex at PN 7. Adrenals were quickly removed, trimmed of adhering fat, blotted, and weighed to the nearest 0.1 mg. Each adrenal pair was then halved and placed in 20 ml beakers containing 1.5 ml of 0°C Krebs-Ringer-bicarbonate-glucose buffer (Umbreit, Burris, and Stauffer 1959). To determine the basal release of corticoids, the beakers were placed in a water bath at 37.5°C in a Dubnoff metabolic incushaker. All incubations were run under an atmosphere of 95% oxygen and 5% carbon dioxide at a flow rate of 3.5 liters per minute. After a 30 minute preincubation, the media was aspirated via a collection bottle connected to a vacuum line, and 1.5 ml of fresh buffered ringers was added. The final incubation period ran for two hours. Following incubation, 1 ml of the incubation fluid was removed and frozen in 15 ml test tubes at -20°C until assayed.

Anterior pituitaries were removed (except for PN 0 rats), blotted, weighed to the nearest 0.1 mg. The glands

were pooled in a small test tube (13 x 100 mm) on ice, each containing a small amount of acid washed sand and 0.1 N HCl at a concentration of 1 pituitary per 0.025 ml of HCl. Hypothalami were removed and pooled in a procedure similar to that used for pituitaries and at the same concentration as that used for the pituitaries. The tubes were set in an ice water bath and allowed to extract in a refrigerator at 2°C for 24-48 hours. The tubes were then centrifuged in a cold room and the extract was stored at -20°C until assayed.

Maximal response to exogenous ACTH was studied *in vitro* and *in vivo*. In the *in vitro* experiments, a 30-minute preincubation was followed by two one hour incubations. The first 60-minute incubation served to determine a basal corticoid release. ACTH was added for the second incubation period at a concentration of 50 mU/ 10 mg of adrenal tissue. One ml from each incubation was removed and stored frozen at -20°C. ACTH was administered *in vivo* to rats whose endogenous secretion of ACTH was blocked by dexamethasone sodium phosphate (Decadron, Merck, Sharp, and Dohme). The dexamethasone was injected six hours prior to the ACTH at a concentration of 100 µg/ 100 gm body weight. ACTH (5 U/ 100 gm body weight) was given 30 minutes prior to decapitation. Following decapitation, blood was collected for plasma corticosterone assay, and the adrenals were removed, cleaned, blotted, weighed, halved, and placed

in beakers to test for corticoid release. After a 30-minute preincubation step, the media was aspirated and fresh media was added for the final one hour incubation period. Plasma and incubates were frozen and stored prior to use.

Hormone Analysis

Corticosterone in the plasma (plasma B) and corticoids released into the incubation media were assayed fluorometrically by using the semi-micro-method of Guillemin, Clayton, Lipscomb, and Smith (1959) modified as follows:

1. The isooctane, water, and NaOH washes were omitted since their removal from the procedure did not affect the results of the assay.
2. Chloroform extraction of steroids was performed by gentle inversions of the test tubes for 60 seconds.
3. The development of fluorescence was made with 1.5 ml per sample of a fluorescent reagent consisting of concentrated sulfuric acid and absolute ethanol in a ratio (v/v) 65 to 35 respectively.
4. A series of standards were run with the samples and consisted of a distilled water blank, 25, 50, 75, and 100 µg of corticosterone standard. The blank was set on 0 on the fluorometer and the 100 µg standard was set on 100.
5. Centrifugation after each extraction was made with a Sorvall GLC-1 centrifuge for 5 minutes at 2500 rpm.

A Farrand Model A fluorometer with the optical systems suggested by Guillemin, et al. (1959) was used for corticoid determinations.

The *in vitro* assay for corticotrophin releasing ability from a crude hypothalamic extract was adopted from Saffron (1961) as modified by Chan, deWied, and Saffron (1969). Hypothalamic corticotrophic releasing activity is estimated by the ability of hypothalamic extract to stimulate the release of ACTH from incubated pituitary halves. An aliquot of the pituitary incubate is then added to incubated adrenal quarters and the release of corticoids into the media is measured. A direct relationship is assumed between the content of corticotrophic releasing factor and the stimulation of ACTH release from the pituitary. Further modifications included a two step pituitary preincubation period. The first step was a 30-minute period followed by a second step of 60 minutes. Rinses and fresh media were added after each step. Also, the release of corticoids from adrenal quarters was assayed fluorometrically. Hypothalamic extract was added at a concentration of one hypothalamic equivalent per 0.025 ml of 0.1 N HCl. Four rats were used for each assay.

ACTH concentration in the extract of anterior pituitaries was assayed using an adaptation of the *in vitro* method of Saffron and Schally (1955). Fluorometric values of corticoids released from adrenal quarters after addition of the pituitary extract were compared to values released by the standard. ACTH (Acthar, Armour Pharmaceutical Co.)

was used as the standard in doses of 10 mU for the high dose and 3.3 mU for the low dose.

Statistical Treatment

Relative potency for both the pituitary ACTH concentration and the hypothalamic releasing activity were calculated by the factorial method of Bliss (1952). The hypothalamic potency is expressed as a percentage of the ACTH released by the non-stimulated hemi-pituitaries whose value was taken as 100%.

Data were statistically evaluated by analyses of variance techniques utilizing the following general model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + (TP)_{ij} + (TS)_{ik} + (PS)_{jk} + (TPS)_{ijk} + e_{ijkl}$$

Where, μ = overall mean with equal subclass number

T_i = i^{th} treatment (Control, LPD I, LPD II)

P_j = j^{th} postpartum stage (0, 7, 14 or 21 days)

S_k = k^{th} sex (male, female)

$(TP)_{ij}$, etc. = interactions among main effects

e_{ijkl} = random errors distributed normally and independently, with means of 0 and common variance

The three-factor interaction was omitted for analyses involving basal and stimulated corticoid release to *in vitro* ACTH, basal and stimulated corticoid release to *in vivo* ACTH, and basal and stimulated plasma 'B' levels

to *in vivo* ACTH. Analyses of relative potency of pituitary ACTH and hypothalamic CRF data included only main effects for postpartum stage and treatment.

Duncan's New Multiple Range Test (Duncan 1955) was used to test for significance among means where the F test in the analysis of variance was significant. Kramer's (1956) modification of Duncan's method was used for unequally replicated treatments. All tests were made at the $P < 0.05$ level.

RESULTS

Dietary Influence on Mother Rats

Although few analyses were made on the mother rats, several subjective notes were taken during the course of the experiments. When placed on diets low in protein, these animals exhibited signs of stress including hair discoloration behind the neck, little or no body weight gain, and a sluggish behavior. A general reduction in abdominal fat deposits at term was noticed in pregnant rats on diet restriction during the second half of gestation. Mother rats placed on the experimental diet in the second half of pregnancy had about 100% greater litter loss than controls. Litter loss is expressed as a percent of the total known gravid rats within each treatment and included those litters lost through fetal reabsorption, death of the dam at birth, refusal or inability of the dam to suckle the young, and cannabalism. The number of rats per litter averaged 9.3 for controls, 8.8 for LPD I pups, and 8.3 for LPD II litters.

All maternal rats examined had hypertrophied adrenal glands at term. A sampling revealed that their mean relative adrenal weights were 48 mg/ 100 gm body weight when fed the low protein diet during the second half of

gestation and 35 mg/ 100 gm body weight for those on a control diet. Mean diurnal variation of adult plasma B was not altered by the diet since measurements at 8:00 A.M. and 8:00 P.M. revealed the normal degree of variation. Respective values for all rats measured were 8 and 35 µg/ 100 ml of plasma with the values for the low protein restricted rats not significantly higher than that of their controls.

Body and Gland Weights

The effect of a low protein diet during pregnancy on the body weight of pups (Table 1) was not evident when the four postnatal means were averaged within each treatment and compared. However, Duncan's New Multiple Range Test (NMRT) revealed that young rats on LPD II had significantly lower body weights at PN 0 than those of controls. Averaging the means at each postnatal day disclosed a difference in body weight between the sexes which was found at PN 0, PN 7, and PN 21, with the males being larger than the females. Although not indicated in Table 1, the mean body weight for all males was 20 gm, and for all females 19 gm. The difference between the sexes proved to be statistically significant.

An effect of protein restriction on the absolute adrenal weights was revealed in rats from LPD II (Table 2). There was a significant reduction in adrenal weight from

TABLE 1

MEAN BODY WEIGHT

Postnatal Day	Animals Used[a]	Treatments: Controls	LPD I	LPD	Average of Postnatal Day Means
0	Total	6.2[b]	5.8	3.9[c]	5.3[d]
	Males	6.3	6.1	4.1	5.5[h]
	Females	6.0	5.5	3.8	5.1[i]
7	Total	12.8	13.4	13.1	13.1[e]
	Males	13.1	12.9	13.4	13.5[h]
	Females	12.6	12.9	12.7	12.7[i]
14	Total	26.8	24.9	24.3	25.3[f]
	Males	27.0	25.9	24.4	25.8[i]
	Females	26.7	23.8	24.2	24.9[i]
21	Total	35.3	33.6	34.0	34.3[g]
	Males	36.1	34.9	35.1	35.3[h]
	Females	34.4	32.3	32.9	33.2[i]
Average of Treatment Means		20.3 20.6 19.9	19.4 20.2 18.6	18.8 19.3 18.4	

[a]Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b]All values expressed in gm.

[c]Significantly different from control value of the same postnatal day.

[d,e,f,g]Column comparisons between means with a different superscript are significantly different, $P < 0.05$.

[h,i]Inter-sex comparisons between means at each postnatal day.

TABLE 2

MEAN ABSOLUTE ADRENAL WEIGHT

Postnatal Day	Animals Used[a]	Treatments									Average of Postnatal Day Means
		Controls			LPD I			LPD II			
0	Total	2.2[b]			2.0			0.9[c]			1.7[d]
	Males		2.2			2.1			1.0		1.7
	Females			2.2			2.0			0.8	1.7
7	Total	2.6			2.8			2.3[c]			2.6[e]
	Males		2.6			2.9			2.2		2.6
	Females			2.7			2.7			2.3	2.6
14	Total	6.2			6.5			5.1[c]			5.9[f]
	Males		6.2			6.5			5.1		5.9
	Females			6.3			6.5			5.1	5.9
21	Total	11.9			11.1			10.8			11.3[g]
	Males		11.7			11.2			10.8		11.2
	Females			12.1			11.0			10.8	11.3
Average of Treatment Means		5.7	5.7	5.8	5.6	5.7	5.6	4.8[c]	4.8	4.8	

[a]Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b]All values expressed in mg.

[c]Significantly different from control value at the same postnatal day.

[d,e,f,g]Column comparisons between means with a different superscript are significantly different.

5.6 mg for all LPD I rats to 4.8 mg for all those in LPD II. The mean adrenal weight of all controls was 5.7 mg which was not significantly different from LPD I. At each postnatal day studied, there was a lag in adrenal growth for rats on LPD II which was significant at PN 0, PN 7, and PN 14. There was no significant difference between the sexes in the adrenal weights at any of the postnatal periods.

The mean relative adrenal weights, expressed in mg/ 100 gm body weight (mg%) are tabulated in Table 3. An average of the control treatment means is 28.3 mg%, which was not significantly different from LPD I. The average value of 28.9 mg% for all pups on LPD I is significantly different from the average treatment mean of 23.4 mg% for all LPD II rats. This difference in relative adrenal weight occurs at all postnatal days except the 21st. A comparison of the average of the means at each postnatal day reveals that the lowest mean relative weight of 19.7 mg% at PN 7 represented a one-third decrease from the PN 0 value. A reestablishment of the relative adrenal weight occurred at PN 21 with values approximating those at PN 0. Although there was no significant difference in adrenal weights between males and females at any postnatal period, the mean relative weights for all males (26.1 mg%), and all females (27.6 mg%), proved to be significantly different.

TABLE 3

MEAN RELATIVE ADRENAL WEIGHTS

Postnatal Day	Animals Used[a]	Treatments									Average of Postnatal Day Means
		Controls			LPD I			LPD II			
0	Total	35.4[b,d]			35.0[d]			23.2[c,e]			31.2[d]
	Males		34.3			33.5			23.8		30.5
	Females			36.6			36.4			22.6	31.9
7	Total	20.7[e]			20.8[e]			17.6[c,f]			19.7[e]
	Males		20.0			20.9			16.5		19.1
	Females			21.5			20.7			18.6	20.2
14	Total	23.3[f]			26.5[c,f]			21.0[c,g]			23.6[f]
	Males		22.9			25.3			20.8		23.0
	Females			23.7			27.6			21.1	24.1
21	Total	33.7[d]			33.4[d]			31.7[d]			33.0[d]
	Males		32.4			32.4			30.7		31.9
	Females			35.1			34.4			32.7	34.1
Average of Treatment Means		28.3	27.4	29.2	28.9	28.1	29.8	23.4[c]	23.0	23.8	

[a] Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b] All values expressed in mg/ 100 gm body weight.

[c] Significantly different from control value of the same postnatal day.

[d,e,f,g] Column comparisons between means with a different superscript are significantly different.

Mean absolute and relative pituitary weights are given in Tables 4 and 5 with the data expressed in mg and mg/ 100 gm body weight (mg%) respectively. These tables show no significant difference between experimentals and controls at PN 7, PN 14, and PN 21. Table 5 does show that the average of the means on PN 7 (3.8 mg%) was significantly different from the average of the means at PN 14 (3.5 mg%) and PN 21 (3.4 mg%). The respective mean relative pituitary weights for all males and females used in the experiments were 3.5 and 3.6 mg%, a difference which proved to be significant.

Plasma and Adrenal Basal Steroid Values

The basal values for plasma B (Table 6) show no significant differences when the means at each postnatal day were averaged within each treatment and compared. However, intra-treatment comparisons of plasma B by Duncan's NMRT at PN 0 revealed that the value of 24.6 µg/ 100 ml plasma (µg%) was significantly different from the control figure of 13.5 µg%. No significant difference was detected at the other postnatal days. A comparison of the average of the means from each postnatal period showed a significant decrease in plasma B levels from 18.4 µg% at PN 0 to 6.9 µg% at PN 7 and 9.4 µg% on PN 14. Twenty-one day old rats had a value of 18.0 µg%, which was comparable to PN 0 values.

TABLE 4

MEAN ABSOLUTE PITUITARY WEIGHTS

Postnatal Day	Animals Used[a]	Treatments									Average of Postnatal Day Means
		Controls			LPD I			LPD II			
7	Total	0.5[b]			0.5			0.5			0.5[c]
	Males		0.5			0.5			0.5		0.5
	Females			0.5			0.5			0.4	0.5
14	Total	0.9			1.0			0.8			0.9[d]
	Males		0.9			1.0			0.8		0.9
	Females			0.8			1.0			0.8	0.9
21	Total	1.1			1.1			1.2			1.1[e]
	Males		1.1			1.1			1.2		1.1
	Females			1.2			1.2			1.2	1.1
Average of Treatment Means		0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.8	0.8	

[a]Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b]All values expressed in mg.

[c,d,e]Column comparisons between means with a different superscript are significantly different, $P < 0.05$.

TABLE 5

MEAN RELATIVE PITUITARY WEIGHTS

Postnatal Day	Animals Used[a]	Treatments									Average of Postnatal Day Means
		Controls			LPD I			LPD II			
7	Total	4.0[b]			3.8			3.7			3.8[c]
	Males		3.9			3.7			3.9		3.8
	Females			4.0			3.9			3.5	3.8
14	Total	3.3			3.8			3.3			3.5[d]
	Males		3.4			3.6			3.3		3.4
	Females			3.2			4.0			3.3	3.5
21	Total	3.2			3.5			3.4			3.5[d]
	Males		3.0			3.2			3.3		3.2
	Females			3.5			3.8			3.5	3.6
Average of Treatment Means		3.5	3.4	3.6	3.7	3.5	3.9	3.5	3.5	3.4	

[a]Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b]All values expressed in mg/ 100 gm body weight.

[c,d]Column comparisons between means with a different superscript are significantly different, $P < 0.05$.

TABLE 6

MEAN BASAL PLASMA CORTICOSTERONE LEVELS

Post-natal Day	Animals Used	No.	Treatments: Control			LPD I			LPD II			Average of Postnatal Day Means
0	Total	5	13.5[a,c]			17.0[c]			24.6[b,c]			18.4[c]
	Males	--		---			---			---		---
	Females	--			---			---			---	---
7	Total	10	5.8[d]			7.8[d]			7.2[d]			6.9[d]
	Males	5		5.4			7.7			6.8		6.6
	Females	5			6.2			7.9			7.6	7.2
14	Total	30	9.2[d]			9.9[d]			9.1[d]			9.4[d]
	Males	15		9.1			10.3			8.8		9.4
	Females	15			9.2			9.5			9.4	9.4
21	Total	30	17.8[c]			15.8[c]			20.5[c]			18.0[c]
	Males	15		17.6			14.2			21.3		17.7
	Females	15			18.0			17.4			19.7	18.4
Average of Treatment Means			11.3	10.7	11.1	12.6	10.7	11.6	15.4	12.3	12.2	

[a]All values expressed in µg/ 100 ml plasma.

[b]Significantly different from control value of the same postnatal day, $P < 0.05$.

[c,d]Column comparisons between means with a different superscript are significantly different, $P < 0.05$.

Basal corticoid release (Table 7) at PN 0 was significantly greater in pups on LPD II than from controls or rats on LPD I. Significantly higher values of corticoid release were obtained at PN 0 when compared to the average of the means for other postnatal periods. This difference had disappeared by PN 7. No statistical difference in corticoid release between the sexes was obtained at any of the days studied or under any of the dietary regimes. However, the means of 7.3 µg% for all males and 7.9 µg% for all females were significantly different.

Hormone Content of Pituitary and Hypothalamus

The effect of diet restriction during the second half of gestation on the mean relative concentration of pituitary ACTH is illustrated in Figure 1. The anterior pituitary concentration in the controls increased from 10 mU/ mg of wet tissue on PN 7 to 29 mU/ mg on day 14, and finally to 69 mU/ mg on the 21st postnatal day. The respective experimental concentrations of 7, 32, and 66 mU/ mg were not found to be significantly different from the control values. Analysis of the means within both treatments indicated a difference between only PN 21 and the other two postnatal periods studied.

A pattern similar to that of the pituitary ACTH concentration was obtained when hypothalamic extracts were assayed for corticotrophin releasing activity. Figure 2

TABLE 7

BASAL CORTICOID RELEASE

Postnatal Day	Animals Used[a]	Treatments: Control	LPD I	LPD II	Average of Postnatal Day Means
0	Total	8.9[b,d]	8.3[d]	11.2[c,f]	9.4[d]
	Males	8.9	8.0	10.2	9.0
	Females	8.7	8.6	12.3	9.8
7	Total	7.6[d]	7.2[e]	6.5[d]	7.1[e]
	Males	7.6	6.6	6.0	6.8
	Females	7.6	7.8	7.0	7.5
14	Total	8.4[d]	6.6[c,e]	6.8[c,d]	7.3[e]
	Males	8.2	6.6	7.0	7.2
	Females	8.6	6.6	6.7	7.3
21	Total	6.1[e]	6.6[e]	7.6[e]	6.7[e]
	Males	5.4	6.4	7.4	6.4
	Females	6.7	6.9	7.5	7.0
Average of Treatment Means		7.7 / 7.5 / 7.9	7.2 / 6.9 / 7.4	8.0 / 7.6 / 8.4	

[a]Thirty animals per treatment (15 of each sex) were used at each postnatal day.

[b]All values expressed in µg/ 100 mg adrenals.

[c]Significantly different from control value of the same postnatal day, $P < 0.05$.

[d,e,f]Column comparisons between means with a different superscript are significantly different, $P < 0.05$.

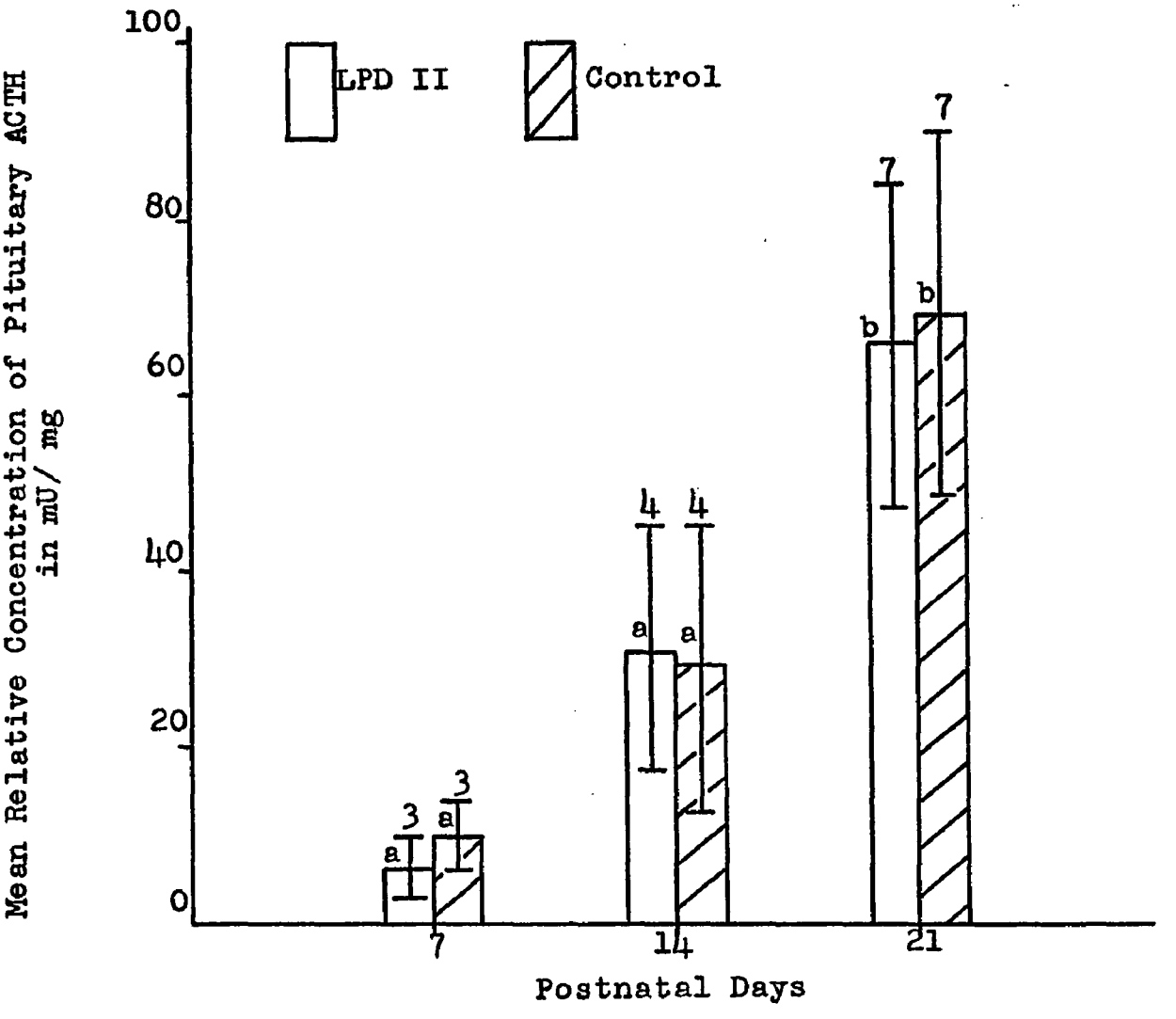

Fig. 1. Mean Relative Concentration of Pituitary ACTH

Effect of low protein diet during the second half of gestation on the ACTH concentration in the anterior pituitaries of postnatal rats. Vertical lines represent ± standard error of the means, and the numbers above each column refer to the number of litters analyzed per mean. Letters above each column mean indicate significance ($P < 0.05$). Comparisons between means with different letters are significantly different.

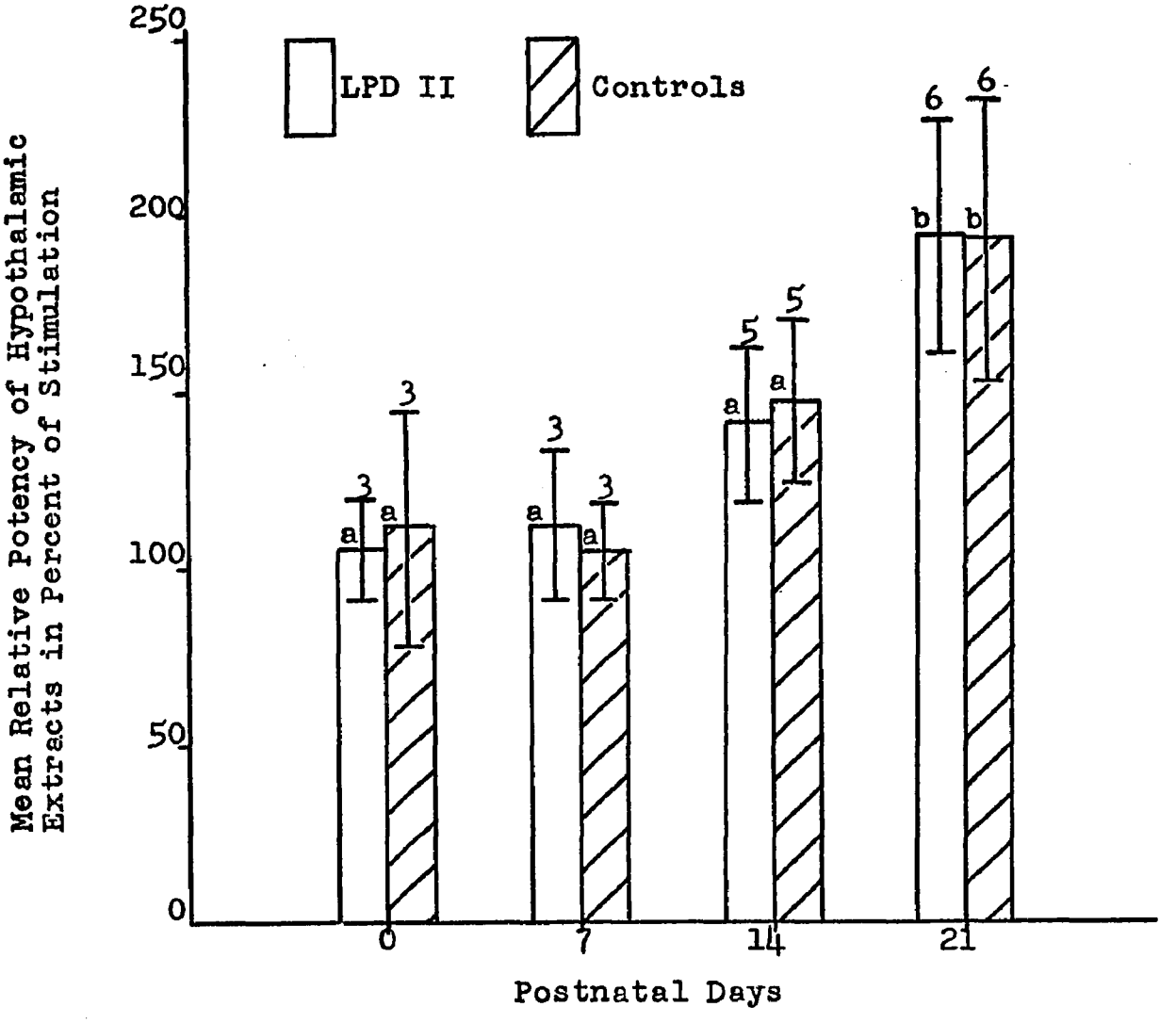

Fig. 2. Mean Relative Potency of Corticotrophic Releasing Activity in Hypothalamic Extracts

Effect of low protein diet during the second half of gestation on the relative potency of corticotrophic releasing activity from crude hypothalamic extracts. Vertical lines represent ± standard error of the means, and the numbers above each column refer to the numbers of litters analyzed per mean. Letters above each column mean indicate significance ($P < 0.05$). Comparisons between means with different letters are significantly different.

shows that hypothalamic extracts do not stimulate a significant release of ACTH from the pituitaries until PN 14, when hypothalamic potency of controls averaged 148%, compared to that of the non-stimulated pituitary halves (100%). A comparison of the corticotrophic stimulating activity of hypothalamic extracts at each postnatal day revealed no significant difference between controls and LPD II rats. Further analysis revealed that although extracts from 21-day old rats had significantly greater corticotrophic releasing activity than other days assayed, the extracts of PN 0, PN 7, and PN 14 were not different from each other.

Adrenal Response to ACTH

The effects of low protein diet during the second half of gestation on the response of pup adrenal glands to exogenous ACTH are presented in Tables 8 through 11.

Table 8 shows the maximal *in vitro* response to ACTH. After a one hour incubation to get a basal level of corticoid release, ACTH added to an additional one hour incubation significantly stimulated corticoid release at all postnatal days for each treatment group. Adrenal glands at PN 0 and PN 21 respond to ACTH by releasing corticoids to a greater degree than those at PN 7 and PN 14. With one exception (PN 21), there was no significant difference between control and experimental groups in the

TABLE 8

MEAN ADRENAL CORTICOID RELEASE IN RESPONSE TO ACTH ADDED IN VITRO

Postnatal Day	Treatment	No.	Basal Corticoid Release	Stimulated Corticoid Release	Percent Stimulation Over Basal[b]
0	Control	8	2.6 ± 0.3[a]	10.1 ± 0.8[d]	313 ± 48[d]
	LPD II	8	2.6 ± 0.3	10.1 ± 0.8[d]	320 ± 48[d]
7	Control	8	1.9 ± 0.3	6.4 ± 0.8[e]	222 ± 48[d]
	LPD II	11	2.3 ± 0.2	7.3 ± 0.7[e]	232 ± 41[d]
14	Control	8	2.1 ± 0.3	6.4 ± 0.8[e]	245 ± 48[d]
	LPD II	8	2.2 ± 0.3	6.3 ± 0.8[e]	203 ± 48[d]
21	Control	10	1.5 ± 0.2	9.1 ± 0.7[d]	573 ± 42[e]
	LPD II	11	1.8 ± 0.2	8.8 ± 0.7[d]	424 ± 41[c,f]

[a]All corticoid release values ± standard error are expressed in µg/ 100 mg adrenals.

[b]Percent stimulation calculated in the manner: stimulated corticoid release - basal corticoid release ÷ basal corticoid release x 100.

[c]Significantly different from the control value of the same postnatal day, $P < 0.05$.

[d,e,f]Column comparisons between means with a different superscript are significantly different.

release of corticoids by the stimulated gland as a percentage of basal corticoid release. The exception was a lower percentage of corticoid release by rats on LPD II when compared to their controls.

The *in vitro* release of corticoids after an *in vivo* injection of ACTH is given in Table 9. All rats responded with a significant *in vitro* release of corticoids after ACTH was injected when compared to saline injected littermates. An average of the two treatment means at each postnatal day showed a difference in response by Duncan's NMRT. The response at PN 7 is significantly lower than the other postnatal days and PN 14 is significantly less responsive than PN 0 (Table 10).

The percent of corticoid release following ACTH stimulation over that of the basal level was significantly lower than that of controls for LPD II pups only at PN 0 (Table 9). A reduced response to ACTH at PN 7 is again indicated when the percent of stimulation of all rats is combined within each postnatal period. These percent values are 301, 217, 377, and 388 for PN 0, PN 7, PN 14, and PN 21 respectively. Although not indicated in Table 9, absolute stimulated corticoid release averaged 9.3 $\pm$ 0.3 µg/ 100 mg/ hour for all controls and 8.3 $\pm$ 0.3 µg/ 100 mg/ hour for all LPD II rats. Their respective percent stimulated values averaged 346% $\pm$ 15 and 295% $\pm$ 15. In each comparison, these values were significantly different.

TABLE 9

MEAN ADRENAL CORTICOID RELEASE IN RESPONSE TO ACTH INJECTED IN VIVO

Postnatal Day	Treatment	Basal Corticoid Release[a]	Stimulated Corticoid Release	Percent Stimulation Over Basal[d]
0	Control	2.4 + 0.2[b] (4)[c]	11.5 + 0.6[f] (8)	376 + 28[f] (8)
	LPD II	3.1 + 0.3[e] (2)	10.0 + 0.6[e,g] (8)	226 + 28[e,g] (8)
7	Control	2.2 + 0.3 (2)	6.3 + 0.7[h] (6)	189 + 33[g] (6)
	LPD II	1.7 + 0.3 (2)	5.7 + 0.7[h] (6)	245 + 33[g] (6)
14	Control	1.9 + 0.2 (4)	9.9 + 0.6[g] (8)	409 + 29[f] (8)
	LPD II	1.7 + 0.2 (4)	8.0 + 0.6[e,i] (8)	368 + 29[f] (8)
21	Control	1.9 + 0.2 (4)	9.6 + 0.6[g] (8)	412 + 28[f] (8)
	LPD II	2.2 + 0.2 (4)	9.6 + 0.6[g] (8)	342 + 28[f] (8)

[a]Basal values were obtained by injecting saline into dexamethasone blocked litter mates.

[b]All corticoid release values ± standard error are expressed in µg/ 100 mg adrenals.

[c]Refers to number of rats per mean.

	LPD II	3.1 + 0.3 (2)	10.0 + 0.6 (8)	220 + 28 (8)
7	Control	2.2 + 0.3 (2)	6.3 + 0.7[h] (6)	189 + 33[g] (6)
	LPD II	1.7 + 0.3 (2)	5.7 + 0.7[h] (6)	245 + 33[g] (6)
14	Control	1.9 + 0.2 (4)	9.9 + 0.6[g] (8)	409 + 29[f] (8)
	LPD II	1.7 + 0.2 (4)	8.0 + 0.6[e,i] (8)	368 + 29[f] (8)
21	Control	1.9 + 0.2 (4)	9.6 + 0.6[g] (8)	412 + 28[f] (8)
	LPD II	2.2 + 0.2 (4)	9.6 + 0.6[g] (8)	342 + 28[f] (8)

[a]Basal values were obtained by injecting saline into dexamethasone blocked litter mates.

[b]All corticoid release values ± standard error are expressed in µg/ 100 mg adrenals.

[c]Refers to number of rats per mean.

[d]Percent stimulation calculated as in Table 8.

[e]Significantly different from the control value of the same postnatal day, $P < 0.05$.

[f,g,h,i]Column comparisons between means with a different superscript are significantly different.

TABLE 10

MEAN STIMULATED IN VITRO CORTICOID RELEASE BETWEEN TREATMENT MEANS AFTER IN VIVO ACTH INJECTION

Postnatal Day	PN 7	PN 14	PN 21	PN 0
Corticoid Release[a]	6.0[b]	8.9	9.6	10.7

[a]Any two means underscored are not significantly different.

[b]Values expressed in µg/ 100 mg/ hour.

There are no important differences in plasma B levels between controls and LPD II pups following in vivo ACTH injections (see Table 11). Differences at postnatal days occur within basal, stimulated, and percent stimulation over saline controls. The percent of stimulation of plasma B in response to an injection of ACTH in rats at PN 21 is 2.7 times greater than the rats at PN 14 and 5.7 times greater than PN 7 animals.

TABLE 11

MEAN PLASMA CORTICOSTERONE LEVELS IN RESPONSE TO ACTH INJECTED IN VIVO

Postnatal Day	Treatment	Basal Plasma Corticosterone[a]	Stimulated Plasma Corticosterone	Percent Stimulation Over Basal[d]
7	Control	3.4 ± 0.5[b] (2)[c]	6.9 ± 2.8[f] (3)	102 ± 44[f] (3)
	LPD II	3.1 ± 0.5 (2)	6.3 ± 2.8[f] (3)	105 ± 44[f] (3)
14	Control	4.4 ± 0.4 (4)	13.5 ± 1.7[g] (8)	207 ± 27[g] (8)
	LPD II	3.8 ± 0.4 (4)	12.0 ± 1.7[g] (8)	224 ± 27[g] (8)
21	Control	6.5 ± 0.4 (4)	41.2 ± 1.7[h] (8)	533 ± 27[h] (8)
	LPD II	6.4 ± 0.4 (4)	47.6 ± 1.7[e,h] (8)	643 ± 27[e,i] (8)

[a]Basal values were obtained by injecting saline into dexamethasone blocked litter mates.

[b]All plasma corticosteone values ± standard error are expressed in µg/ 100 ml plasma.

[c]Refers to number of rats per mean.

[d]Percent stimulation calculated as in Teble 8.

Day	Treatment	Corticosterone[a]	Corticosterone	Over Basal[d]
7	Control	3.4 ± 0.5^{b} $(2)^{c}$	6.9 ± 2.8^{f} (3)	102 ± 44^{f} (3)
	LPD II	3.1 ± 0.5 (2)	6.3 ± 2.8^{f} (3)	105 ± 44^{f} (3)
14	Control	4.4 ± 0.4 (4)	13.5 ± 1.7^{g} (8)	207 ± 27^{g} (8)
	LPD II	3.8 ± 0.4 (4)	12.0 ± 1.7^{g} (8)	224 ± 27^{g} (8)
21	Control	6.5 ± 0.4 (4)	41.2 ± 1.7^{h} (8)	533 ± 27^{h} (8)
	LPD II	6.4 ± 0.4 (4)	$47.6 \pm 1.7^{e,h}$ (8)	$643 \pm 27^{e,i}$ (8)

[a]Basal values were obtained by injecting saline into dexamethasone blocked litter mates.

[b]All plasma corticosteone values ± standard error are expressed in µg/ 100 ml plasma.

[c]Refers to number of rats per mean.

[d]Percent stimulation calculated as in Table 8.

[e]Significantly different from control value of the same postnatal day, $P < 0.05$.

[f,g,h,i]Column comparisons between means with a different superscript are significantly different.

DISCUSSION

Rats fed a diet deficient in protein during the second half of pregnancy produced offspring that were significantly smaller and lighter at birth than were control rats, or those deficient during the first half of pregnancy. This difference was eliminated during the first postnatal week. It is probable that the lower body weight at PN 0 is due to causes other than high corticosterone levels, although corticosteroids are known for their growth inhibiting effects (Schapiro 1968, Howard 1965, and others). Laine, et al. (1963) and Milkovic, Milkovic, and Efendic (1968), have demonstrated that hyperactivity of the maternal adrenal system by itself does not reduce fetal body weight. Burton and Jeyes (1968) have shown that the fetus has a high level of 11-hydroxysteroid dehydrogenase which converts as much as 90% of compound B to an inactive metabolite. The inactivation of this steroid thus may serve as a protective mechanism against high maternal corticosterone titers.

There is little effect on the adrenal axis of young rats by a prenatal diet deficient in protein. One observed effect was a reduced absolute adrenal weight in pups whose mothers were fed a low protein diet during the

second half of gestation. At PN 0, these adrenal glands were only 41% as heavy as the controls. However, by the end of the third week these experimentals had adrenals equal to 91% of the weight of controls.

The restriction in adrenal development may be explained by assuming that the low protein diet acted as a stressor thus inducing an increase in free maternal corticosteroids. These maternal steroids then entered the fetal circulation and inhibited fetal ACTH output, thus resulting in a decrease in adrenal weight. This speculation is supported by the following observations.

First, physical and behavioral signs of stress were seen in pregnant rats on low protein diet as well as adrenal hypertrophy and slight elevation in plasma B levels (see Results).

Second, several workers have established that maternal steroids do cross the placental barrier (Migeon, Bertrand, and Gemzell 1961; Zarrow, Philpott, and Denenberg 1970; Burton and Jeyes 1968).

Third, it is well known that the maternal steroid feedback inhibits fetal pituitary volume (Daikoku and Saigo 1964; Milkovic, Milkovic, and Efendic 1968) and pituitary ACTH content (Skelbelskaya 1968).

Fourth, fetal ACTH is known to stimulate the growth of the adrenals in the fetus (Jost 1966).

Finally, recent experiments implicate the fetal hypothalamus as a possible link between maternal steroids and the hypophyseal release of ACTH (Jost 1966; Jost, Dupoy, and Geloso-Meyer 1971). This suggests that corticotrophin release is regulated much the same way as it is in the adult rat.

It might be argued that the low adrenal weight seen in neonatal mothers treated from mid-gestation until birth is due to a proportional decrease in body weight. If the difference in body weights between controls and LPD II rats at PN 0 is expressed as a percent of the control value, the percent of body weight reduction of offspring on LPD II is 37%. This same calculation for adrenal weights discloses a 59% reduction. The smaller adrenal size is probably due to the inhibiting effect of maternal steroids over and above the effect of an inadequate nutrient supply. Adrenal growth when fetal ACTH is inhibited indicates some degree of autonomous growth.

Maternal glucocorticoids are important between gestational days 5 and 9 for the maintenance of pregnancy, fetal body weight, and fetal protein stores in pregnant rats fed a protein free diet (Berg, Sigg, and Greengard 1967; Morishige and Leathem 1972). The dietary regimen of 4% protein during days 1 through 11 of pregnancy as used in these experiments was not effective in interrupting

pregnancy nor were body weights reduced significantly in the offspring at birth. This might be expected since the competition for amino acids from the fetuses would be minimal during the first half of gestation. The lack of influence on the adrenal axis of rats from mothers treated during the first half of gestation supports the findings of Roos (1967) who has shown that adrenal organogenesis does not begin until the 12th day of gestation. No "carry-over" effects were seen in LPD I rats.

The values for absolute and relative adrenal weights at various ages (Tables 2 and 3) indicate that all adrenal glands are growing very little during the first week after birth. Their activity is similarly depressed (Tables 6 and 7). This reflects the well reported absence of adrenocorticotrophic stimulation during the first postnatal week (see review by Milkovic and Milkovic 1969). It was shown by Hiroshige and Sato (1971) that neonatal adrenals will respond to as little as 2 mU of exogenous ACTH. The pituitary at PN 7 contains an adequate supply of ACTH (7 to 10 mU/ mg) to stimulate the adrenal gland (Figure 1). It might be inferred from the assay of hypothalamic extracts (Figure 2) that the failure to release ACTH is due to an insufficient supply of a corticotrophic releasing substance. This concept was suggested by Bartova (1968). Using an <u>in vivo</u> assay, Hiroshige and Sato (1971) found no hypothalamic

stimulator of ACTH on the 7th postnatal day, but they did show its presence on the 2nd day after birth.

Plasma corticosterone in all treatments follows a pattern similar to that of relative adrenal weight. A ratio of plasma B to relative adrenal weight has been suggested as a measure of adrenal efficiency (Milkovic and Bates 1964). As illustrated in Table 12, the adrenal glands of rats subjected to LPD II produce steroids at a faster rate (per mg of tissue) than do controls of the same age. This hyperfunction is 2.5 times that of controls at PN 0. Both treatments show the normal adult ratio of 0.55 at PN 21.

TABLE 12

RATIO OF PLASMA B TO RELATIVE ADRENAL WEIGHT

	Postnatal Days			
Treatments	0	7	14	21
Controls	.38	.29	.36	.54
LPD II	.97	.47	.42	.58

Since corticoid release is primarily a function of ACTH the lack of adrenal stimulation is again evident at PN 7. Although the fetal release of ACTH was suggested as being repressed in LPD II rats, significantly higher plasma B and corticoid release was obtained from these

animals. It might be inferred from this evidence that inhibition was to the release of ACTH rather than to its storage. The stress of birth and the removal of this inhibition allowed the release of fetal pituitary ACTH following parturition. Using pregnant, adrenalectomized rats, the experiments of Hiroshige and Sato (1971) suggest that the high level of plasma B at day 0 is due to the release by the neonates adrenals during the birth process rather than from maternal origin. This would appear to be supported by the significant elevation of corticoids released in vitro by all rats at PN 0 (Table 7).

The mean relative pituitary weight of all females was found to be significantly greater than for males (Table 5). This difference in pituitary size is probably due to the greater gonadotrophic activity in prepuberal female rats when compared to males of the same age (Ojeda and Ramirez 1972).

No significant treatment difference was obtained in either pituitary ACTH concentration or hypothalamic corticotrophic stimulating potency. Both follow a similar pattern of hormone content. Significant hypothalamic corticotrophic was not detected until PN 14. The hypothalamic corticotrophic stimulating potency of 190% obtained at PN 21 was approximately two-thirds of the adult levels of 250 to 300%.

Rat adrenals are stimulated by ACTH to release steroids both *in vitro* and *in vivo* at all the postnatal days studied. This demonstrates that adrenals are responsive to ACTH even in periods in which they normally release small amounts of steroids. It is of interest that the response to ACTH at PN 0 and PN 21 is greater than the response at PN 7 and PN 14. This can be explained by the fact that ACTH maintains the fine structure of the adrenal gland and promotes RNA and protein synthesis (Farese 1968). The previous exposure of adrenal tissue to endogenous ACTH at PN 0 and PN 21 assures adequate enzyme levels and amounts of endogenous substrates to produce significantly more corticoids. There is an insufficient level of circulating ACTH at PN 7 and PN 14 to maintain adequate substrate and enzyme levels.

Differences in response to exogenous ACTH between treatments were generally not evident. The average *in vitro* release of corticoids in response to *in vivo* administered ACTH and the percent of stimulation above the basal level were both significantly greater in controls than in LPD II rats. Since there is no difference in plasma B levels between treatments (Table 11), the corticoid release in response to ACTH is unexplainable.

It is concluded that offspring from mothers fed a diet low in protein during one-half of gestation show no lasting quantitative alteration of the adrenal axis. It appears that the high degree of biological inactivation of corticosterone in the fetus (Burton and Jeyes 1968) offsets the increased level of maternal corticosterone. This inactivation serves to protect the fetus from detrimental effects of the steroid. There was no direct effect of nutrient deficiency on the adrenal axis of LPD II pups that may be separable from high corticoid titers.

LITERATURE CITED

Allen, L. H., and F. J. Zeman. 1971. Influence of increased postnatal food intake on body composition of progeny of protein-deficient rats. J. Nutr. 101: 1311-1318.

Barraclough, C. A., and R. A. Gorski. 1961. Evidence that the hypothalamus is responsible for androgen induced sterility in female rats. Endocrinology 68: 68-79.

Bartova, A. 1968. Functioning of the hypothalamo-pituitary-adrenal system during postnatal development in rats. Gen. Comp. Endocrin. 10: 235-239.

Berg, B. N., E. B. Sigg, and P. Greengard. 1967. Maintenance of pregnancy in protein-deficient rats by adrenocortical steroid or ACTH administration. Endocrinology 80: 829-835.

Bliss, C. I. 1952. The Statistics of Bioassay. Academic Press, New York.

Burton, H. F., and C. L. Jeyes. 1968. Corticosteroid metabolism in fetal and newborn mice. Canad. J. Biochem. 46: 15-20.

Chan, L. T., D. deWied, and M. Saffron. 1969. Comparisons of assays for corticotrophin-releasing activity. Endocrinology 84: 967-972.

Cowley, J. J., and R. D. Griesel. 1966. The effect on growth and behavior of rehabilitating first and second generation low protein rats. Anim. Behav. 14: 506-517.

Daikoku, S., and S. Saigo. 1964. Volumetric observation on the influence of maternal sham hypophysectomy on the fetal adrenal gland. Endocrin. Japon. 11: 55-61.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Etkin, W. 1963. Metamorphosis-activating system of the frog. Science 139: 810-814.

Farese, R. V. 1968. Regulation of adrenal growth and steroidogenesis by ACTH. In K. W. McKerns (ed.), Functions of the Adrenal Cortex. Appleton-Century-Crofts, New York. Pp. 539-581.

Guillemin, R., G. W. Clayton, H. S. Lipscomb, and J. D. Smith. 1959. Fluorometric measurement of rat plasma and adrenal corticosterone concentration. J. Lab. Clin. Med. 53: 830-832.

Hiroshige, T., and T. Sato. 1971. Changes in hypothalamic content of corticotropin-releasing activity following stress during neonatal maturation in the rat. Neuroendocrinology 7: 257-270.

Howard, E. 1965. Effects of corticosterone and food restriction on growth and on DNA, RNA, and cholesterol contents of the brain and liver in infant mice. J. Neurochem. 12: 181-191.

_____, and D. M. Granoff. 1968. Effect of neonatal food restriction in mice on brain growth, DNA, and cholesterol, and on adult delayed response learning. J. Nutr. 95: 111-121.

Jost, A. 1966. Problems in fetal endocrinology: the adrenal glands. Recent Progr. Hormone Res. 22: 541-574.

_____, J. P. Dupouy, and A. Geloso-Meyer. 1971. Hypothalamo-hypophyseal relationships in the fetus. In L. Martini, M. Mota, and F. Fraschini (eds.), The Hypothalamus. Academic Press, New York. Pp. 605-615.

Kramer, C. Y. 1956. Extensions of multiple range tests to group means with unequal numbers of replication. Biometrics 12: 307-310.

Laine, M. J., E. Naatanen, S. J. Simila, E. S. Raijola, and A. J. Raijola. 1963. Postnatal development of the young rats treated with ACTH during pregnancy. Ann. Med. Exp. Biol. Fenn. 41: 531-538.

Migeon, D. J., J. Bertrand, and C. H. Gemzell. 1961. The trans-placental passage of various steroid hormones in mid-pregnancy. Recent Progr. Hormone Res. 17: 207-248.

Milkovic, S., and R. W. Bates. 1964. Pituitary-adrenocortical system during growth of a transplantable pituitary tumor and tumor removal. Endocrinology 74: 617-626.

_____, and K. Milkovic. 1969. Responsiveness of the pituitary-adrenocortical system during embryonic and early postnatal periods of life. In B. Bajusz (ed.), Physio- and Pathology of Adaptation Mechanisms. Bergamon Press, Oxford. P. 28-47.

_____, _____, and S. Efendic. 1968. Significance of maternal corticosteroids in fetal adrenal development and organogenesis. Gen. Comp. Endocrin. 10: 240-246.

Morishige, W. K., and J. H. Leathem. 1972. Pregnancy maintenance with corticosterone in protein-depleted rats: A study of fetal protein composition. Endocrinology 90: 318-322.

Ojeda, S. R., and V. D. Ramirez. 1972. Plasma level of LH and FSH in maturing rats: Response to hemigonadectomy. Endocrinology 90: 466-472.

Roos, T. H. 1967. Steroid synthesis in embryonic and fetal rat adrenal tissues. Endocrinology 81: 716-728.

Saffron, M. 1961. Detection of corticotropin releasing activity. Meth. Med. Res. 9: 224-229.

_____, and A. V. Schally. 1955. In vitro bioassay of corticotropin: modification and statistical treatment. Endocrinology 56: 523-532.

Sawano, S., A. Arimure, A. V. Schally, T. W. Redding, and S. Schapiro. 1969. Neonatal corticoid administration: Effects upon adult pituitary growth hormone and hypothalamic growth hormone-releasing hormone activity. Acta Endokrin. Kbh. 61: 57-67.

Schapiro, S. 1968. Some physiological, biochemical, and behavioral consequences of neonatal hormone administration: Cortisol and Thyroxine. Gen. Comp. Endocrin. 10: 214-228.

Shoemaker, W. J., and R. J. Wurtman. 1971. Perinatal undernutrition: Accumulation of catecholamines in rat brain. Science 171: 1017-1019.

Skebelskaya, Y. B. 1968. The effect of corticosteroid concentrations in the blood of gravid rats on adrenocorticotropic function of the hypophisis of the fetus. Gen. Comp. Endocrin. 10: 263-268.

Swaiman, K. F., J. M. Daleiden, and R. N. Wolfe. 1970. The effect of food deprivation on enzyme activity in developing brain. J. of Neurochem. 17: 1387-1391.

Taylor, M. H., and E. Howard. 1971. Impaired glucose homeostasis in adult rats after corticosterone treatment in infancy. Endocrinology 88: 1190-1202.

Thompson, W. R. 1957. Influence of prenatal maternal anxiety on emotionality in young rats. Science 125: 698-699.

Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1959. Manometric Techniques and Tissue Metabolism. Burgess Publishing Company, Minneapolis, Minn.

Winick, M., and A. Noble. 1965. Quantitative changes in DNA, RNA, and protein during prenatal and postnatal growth in the rat. Devel. Biol. 12: 451-466.

_____, and P. Rosso. 1969. Effects of severe early malnutrition on cellular growth of human brain. Pediat. Res. 3: 181-184.

Zamenhof, S., E. van Marthens, and L. Grauel. 1971. DNA (cell number) in neonatal brain: Second generation (F_2) alteration by maternal (F_0) dietary protein restriction. Science 172: 850-851.

_____, _____, and F. L. Margolis. 1968. DNA (cell number) and protein in neonatal brain. Alteration by maternal dietary protein restriction. Science 160: 322-323.

Zarrow, M. X., J. E. Philpott, and W. H. Denenberg. 1970. Passage of ^{14}C-4-corticosterone from the rat mother to the foetus and neonate. Nature 226: 1058-1059.

Zeman, F. 1970. Effect of protein deficiency during gestation on postnatal cellular development in the young rat. J. Nutr. 100: 530-538.