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Behavioral, morphological, and biochemical effects of protein deprivation

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**Behavioral, morphological, and biochemical
effects of protein deprivation**

by

Martha Beth Gillham

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY**

**Department: Food and Nutrition
Major: Nutrition**

Approved:

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In Charge of Major Work

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**Iowa State University
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TABLE OF CONTENTS

	Page
LIST OF SYMBOLS AND ABBREVIATIONS	xii
INTRODUCTION	1
REVIEW OF LITERATURE	4
Restriction of Protein or Calories?	4
Reproductive Performance	7
Maternal adjustments	7
Food intake	7
Weight change	9
Length of gestation	10
Litter size	11
Development of the young	12
Birth weight	12
Perinatal survival	13
Neonatal organ weights	14
Postnatal growth and development	16
Postweaning Growth, Development, and Metabolic Efficiency	19
Growth	19
Weight change	19
Body composition	20
Organ development	21
Food consumption	23
Food utilization	26
Brain Development and Behavior	27
Brain weight and cell number	27
Lipid deposition	29
Acetylcholinesterase	31
Behavior	34
Open field exploration	35
Problem solving	36
Discrimination training	37
Operant conditioning	39
Avoidance or escape conditioning	39
Choice of reinforcement	40
Summary	42

	Page
METHODS AND PROCEDURES	44
Animal Selection and Treatment	44
Diets	44
Animal housing and routine care	47
Reproduction	48
Behavioral Training	49
Autopsy Procedure	52
Acetylcholinesterase Determination	53
Statistical Design and Analyses	56
Experimental Plans	59
Experiment I	59
Experiment II	62
Experiment III	64
RESULTS	65
Reproductive Performance	65
Maternal adjustments	69
Food intake	69
Weight change	79
Length of gestation	81
Litter size	81
Development of the young	85
Birth weight	85
Mortality at birth and perinatal survival	85
Neonatal organ weights	87
Postnatal survival, growth, and development	92
Postweaning Growth, Development, and Metabolic Efficiency	102
Growth	102
Body weight	102
Experiment I	102
Experiment II	104
Experiment III	109
Body, carcass, adipose deposit, and organ weights	110
Experiment I	111
Experiment II	113
Experiment III	118

	Page
Relative carcass weights, adipose deposits, and organ weights	122
Experiment I	122
Experiment II	124
Experiment III	128
Food consumption	131
Experiment I	131
Experiment II	131
Experiment III	135
Food utilization	138
Experiment I	138
Experiment II	139
Experiment III	142
 Brain Development and Behavior	 145
Brain weight	145
Newborn females	145
Experiment I	145
Experiment II	145
Experiment III	148
Weanling females	149
Experiment II	149
Experiment III	150
Adult males	150
Experiment I	150
Experiment II	152
Experiment III	157
Brain cholinesterase activity	162
Newborn females	162
Experiment I	162
Experiment II	162
Experiment III	162
Weanling females	163
Experiment II	163
Experiment III	165
Adult males	166
Experiment I	167
Experiment II	167
Experiment III	171
Behavior	175
Experiment I	175
Experiment II	181
Experiment III	195
Correlations	205
Experiment I	205
Experiment II	205
Experiment III	211

	Page
DISCUSSION	216
Reproductive Performance	216
Maternal adjustments	216
Food intake	216
Weight change	219
Development of the young	221
Birth weight	221
Mortality at birth and perinatal survival	221
Neonatal organ weights	223
Postnatal growth, survival, and organ development	224
Postweaning Growth, Development, and Metabolic Efficiency	226
Growth and organ development	226
Food consumption and utilization	229
Brain Development and Behavior	233
Brain weight	233
Newborn and weanling females	233
Adult males	234
Brain cholinesterase activity	235
Newborn and weanling females	235
Adult males	237
Behavior	240
Correlations	246
SUMMARY	247
BIBLIOGRAPHY	253
ACKNOWLEDGMENTS	262a
APPENDIX	263

LIST OF FIGURES

	Page
Figure 1. Interactions of diet, environment, and behavior	1
Figure 2. Diagram of Y maze used for visual discrimination training	50
Figure 3. Diagrams of the dorsal and coronal views of the rat brain	54
Figure 4a. Experimental plan for Experiment I	61
Figure 4b. Experimental plan for Experiments II and III	63
Figure 5. Mean food intake and body weight of female rats in Experiment III from 3 to 20 weeks of age	71
Figure 6. Mean food intake of pregnant rats in Experiment I	73
Figure 7. Mean food intake of pregnant rats in Experiments II and III	74
Figure 8. Mean food intake of lactating dams in Experiments II and III	78
Figure 9. Postweaning growth of male offspring from litter 2 in Experiment II	106
Figure 10. Learning curves of adult male rats during acquisition, reversal, and extinction training in Experiment I	176
Figure 11. Learning curves of adult male rats during acquisition and extinction training in Experiment II	183
Figure 12. Learning curves of adult male rats during acquisition and extinction training in Experiment III	196

LIST OF TABLES

	Page
Table 1. Composition of experimental diets	46
Table 2. Outline of analysis of variance	58
Table 3. Mean maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiment I	66
Table 4. Mean maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiments II and III	67
Table 5. Mean number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiment I	82
Table 6. Mean number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiments II and III	83
Table 7. Mean body and organ weights of newborn female rats in Experiment I	88
Table 8. Mean body and organ weights of newborn female rats in Experiments II and III	89
Table 9. Mean relative carcass and organ weights of newborn female rats in Experiment I	92
Table 10. Mean relative carcass and organ weights of newborn female rats in Experiments II and III	93
Table 11. Mean preweaning body weights of rat pups in Experiments I, II, and III	96
Table 12. Mean body and organ weights of weanling female rats in Experiments II and III	98
Table 13. Mean relative carcass and organ weights of weanling female rats in Experiments II and III	100
Table 14. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment I	103
Table 15. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment II	108

	Page
Table 16. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment III	110
Table 17. Mean body and organ weights of adult male rats in Experiment I	112
Table 18. Mean body, adipose deposit, and organ weights of adult male rats in Experiment II	114
Table 19. Mean body, adipose deposit, and organ weights of adult male rats in Experiment III	119
Table 20. Mean relative carcass and organ weights of adult male rats in Experiment I	123
Table 21. Mean relative carcass, adipose deposit, and organ weights of adult male rats in Experiment II	125
Table 22. Mean relative carcass, adipose deposit, and organ weights of adult male rats in Experiment III	129
Table 23. Mean postweaning food intake of male offspring in Experiment I	132
Table 24. Mean postweaning food intake of male offspring in Experiment II	133
Table 25. Mean postweaning food intake of male offspring in Experiment III	136
Table 26. Mean postweaning food efficiency ratios of male offspring in Experiment I	139
Table 27. Mean postweaning food efficiency ratios of male offspring in Experiment II	140
Table 28. Mean postweaning food efficiency ratios of male offspring in Experiment III	143
Table 29. Mean absolute and relative brain weights of newborn female rats in Experiment I	146
Table 30. Mean absolute and relative brain weights of newborn and weanling female rats in Experiments II and III	147
Table 31. Mean absolute and relative brain weights of adult male rats in Experiment I	151

	Page
Table 32. Mean absolute and relative brain weights of adult male rats in Experiment II	153
Table 33. Mean absolute and relative brain weights of adult male rats in Experiment III	158
Table 34. Mean specific and total brain ChE activities of newborn female rats in Experiment I	163
Table 35. Mean specific and total brain ChE activities of newborn and weanling female rats in Experiments II and III	164
Table 36. Mean brain ChE activities of adult male rats in Experiment I	168
Table 37. Mean brain ChE activities of adult male rats in Experiment II	169
Table 38. Mean brain ChE activities of adult male rats in Experiment III	172
Table 39. Mean trials to criterion during acquisition and reversal training of adult male rats in Experiment I	179
Table 40. Mean latencies and latency reciprocals of adult male rats during acquisition and reversal training in Experiment I	180
Table 41. Mean trials to criterion during acquisition training of adult male rats in Experiments II and III	185
Table 42. Mean latencies of adult male rats during acquisition training in Experiment II	187
Table 43. Mean latency reciprocals of adult male rats during acquisition training in Experiment II	189
Table 44. Mean latencies and latency reciprocals of adult male rats during extinction training in Experiment II	193
Table 45. Mean latencies of adult male rats during acquisition training in Experiment III	198
Table 46. Mean latency reciprocals of adult male rats during acquisition training in Experiment III	200
Table 47. Mean latencies and latency reciprocals of adult male rats during extinction training in Experiment III	203

	Page
Table 48. Correlations of TCA with brain weights and ChE activities of adult male rats in Experiment I	206
Table 49. Correlations of TCA with brain weights of adult male rats in Experiment II	207
Table 50. Correlations of TCA with brain ChE activities of adult male rats in Experiment II	209
Table 51. Correlations of TCA with brain weights of adult male rats in Experiment III	212
Table 52. Correlations of TCA with brain ChE activities of adult male rats in Experiment III	214
Table 53. Mean body weights of male offspring in Experiments I, II, and III at 3 and 20 weeks of age	227
Table 54a. Mean specific cortical, subcortical, and total brain ChE activities of adult male rats in Experiment I	238
Table 54b. Mean specific cortical, subcortical, and total brain ChE activities of adult male rats in Experiments II and III	238
Table 55a. Mean percent weight change of adult male rats when subjected to water deprivation in Experiment I	243
Table 55b. Mean percent weight change of adult male rats when subjected to water deprivation in Experiments II and III	243
Table A1. Composition of Steenbock XV and XVII diets	264
Table A2. Composition of diets for Experiment I _b	265
Table A3. Ranges of maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiments I, II, and III	266
Table A4. Ranges of number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiments I, II, and III	268
Table A5. Ranges of body and organ weights of newborn female rats in Experiments I, II, and III	269
Table A6. Ranges of average preweaning body weights of pups in Experiments I, II, and III	270

	Page
Table A7. Ranges of body and organ weights of weanling female rats in Experiments II and III	271
Table A8. Ranges of body weights of male offspring at intervals from 3 to 20 weeks of age in Experiments I, II, III	272
Table A9. Ranges of body, adipose deposit, and organ weights of adult male rats in Experiments I, II, and III	273
Table A10. Ranges of postweaning food intake of male offspring in Experiments I, II, and III	275
Table A11. Ranges of postweaning food efficiency ratios of male offspring in Experiments I, II, and III	276
Table A12. Ranges of absolute brain weights and specific brain ChE activities of newborn and weanling female rats in Experiments I, II, and III	277
Table A13. Ranges of absolute brain weights and brain ChE activities of adult males in Experiments I, II, and III	278
Table A14. Ranges of trials to criterion and latencies during acquisition and reversal training and percent weight change when subjected to water deprivation of adult male rats in Experiment I	280
Table A15. Ranges of trials to criterion and latencies during acquisition and extinction training and percent weight change when subjected to water deprivation of adult male rats in Experiments II and III	281

LIST OF SYMBOLS AND ABBREVIATIONS

HP = (high protein) 24% casein

HP_p = (high protein) 24% casein for females from weaning (Experiment III)

LP = (low protein) 6% casein for pregnant females
 10% casein for lactating females
 10% casein for males after weaning

LP_p = (low protein) 6% casein for females from weaning through gestation
 (Experiment III)

4_g = 4% protein in gestation

15₁ = 15% protein in lactation

15 = 15% protein

St = stock diet (about 24% protein)

/ = weaning

nt = not trained; rats were not subjected to behavioral training

M = modified; 10% casein fed to LP rats during days 15, 16, 17 of gestation

H = hormone; injections of hydrocortisone and prolactin administered periodically during lactation

Symbols are combined to indicate treatment throughout life, e.g.,
 LP/HP = 6% and 10% casein in gestation and lactation, respectively,
 and 24% casein after weaning
 4_g 15₁/15 = 4 and 15% protein in gestation and lactation, respectively,
 and 15% protein after weaning
 LP_p/LP = dam fed 6% casein from weaning through gestation, 10% casein
 in lactation; offspring fed 10% casein after weaning

C/SC = g cortex/g subcortex

ChE = cholinesterase

C:SC ChE ratio = (ChE/g cortex)/(ChE/g subcortex)

TCA = trials to criterion in acquisition

TCR = trials to criterion in reversal

INTRODUCTION

The relationships governing the interaction of nutrient supply, behavior, and development are complex. A recent position paper published by the Food and Nutrition Board of the National Academy of Sciences-National Research Council suggested a scheme (Figure 1) to describe these interactions (N.A.S.-N.R.C., 1973).

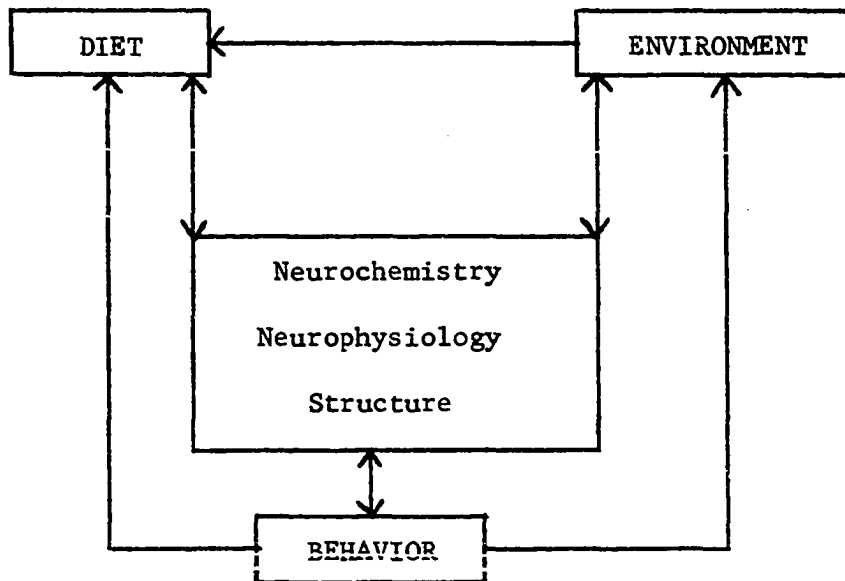


Figure 1. Interactions of diet, environment, and behavior (N.A.S.-N.R.C., 1973)

Diet, which is itself an environmental influence and also affected by other environmental factors such as housing or stimulation, has been shown in animals to influence neurochemistry, neurophysiology, and structure. Other environmental factors also influence these variables, and they determine behavior which in turn influences both diet and environment.

Evidence for the direct influence of nutrient supply on human behavior is difficult to collect. Problems include separation of dietary influence

from those of other environmental influences and the selection of adequate controls. When children from different homes within the same socio-economic classes have been studied, genetic and environmental factors such as maternal intelligence quotient (I.Q.) and similarity of the environment in the home have not been well controlled. The choice of siblings as controls has been criticized because nutritional data were not necessarily similar. In addition, most work with humans has been retrospective in nature with the accompanying disadvantages of inadequate information about the degree and duration of malnutrition or the presence or absence of mitigating factors.

Work with experimental animals has obvious advantages in the study of nutritional, environmental, and behavioral interactions. Greater control of genetic heritage, diet quantity and quality, onset and duration of malnutrition, and social conditions is possible than with human populations. Studies may examine not only the extreme limits of malnutrition but also underlying biochemical mechanisms in various tissues. Work with animals is not without methodological problems, however. Confounding social and environmental influences may change the behavior of the nutritionally deprived dam toward her young. When the nutrient supply is decreased by increasing litter size or limiting access to the mother, environmental as well as nutritional variables are manipulated.

An additional problem in the use of the albino rat as an animal model in the study of protein-calorie malnutrition is the poor neonatal survival rate. Zeman (1967) reported 100% mortality in the progeny of dams fed 6% casein in gestation and lactation, and Turner (1973) reported loss of 73% of the low protein progeny within 4 days of birth. With a high mortality rate, the question of a surviving selected genetic sample is raised. Cer-

tainly the restricted group in such experiments are the progeny of animals able to lactate sufficiently to maintain life under marginal conditions and are composed of animals who were the least affected by deprivation.

Correlation of biochemical and behavioral findings has been missing in the study of the effects of malnutrition. Decreased brain weight, decreased cell number as measured by DNA, decreased rate of myelination, abnormal content of various components including total lipid, cholesterol, various amino acids, enzymes, and neurotransmitter substances have been demonstrated in animals subjected to early malnutrition. These parameters have been measured infrequently in animals which have also undergone behavioral testing. Certainly the lack of explicit knowledge regarding the biochemical basis of the learning process inhibits this type of investigation. Nonetheless, validation of the coexistence of behavioral and biochemical aberrations in the same animals would be valuable.

The present investigations compared neonatal survival from several reproduction paradigms. Reproductive performance was measured for rats fed restricted amounts of protein from weaning, for rats restricted in protein beginning on day 0 of pregnancy, and for those who had successfully weaned one litter on stock ration prior to restriction. The experiments also measured the effects of protein restriction before and/or after weaning on growth, morphological development, metabolic efficiency, learning behavior, and brain cholinesterase (ChE) activity of progeny. Morphological development and brain ChE activity were measured in the progeny neonatally, at weaning, and at approximately 8 months of age. Food consumption and utilization in males were measured from weaning, and learning behavior was assessed at 6 to 8 months of age. Correlations of learning behavior with brain weight and ChE activity were examined because these three parameters were available from the same animals.

REVIEW OF LITERATURE

Restriction of Protein or Calories?

Both protein and energy sources in developing countries may be limited for segments of the populations. Various experimental paradigms have been designed to examine the effects of deprivation of each of these variables on animals with respect to reproduction, growth, development, and ability to learn. In some instances, total maternal food intake has been restricted, and in others only protein has been limited. There is evidence, however, that when total food intake has been restricted, protein has been the limiting nutrient. During gestation and lactation, Chow and Lee (1964) restricted the intake of an adequate diet (laboratory chow) to 50 or 75% of that consumed ad libitum. They observed depressed birth weights and growth rates as well as various physiological anomalies in the progeny. Hsueh et al. (1967), from the same laboratory, manipulated the composition of restricted diets by restoring, in sequence, vitamins, minerals, and energy content to control levels. Neither restoration of vitamins or minerals nor addition of sucrose influenced the effects of dietary restriction. Though protein was not manipulated, these investigators concluded that it must be the critical dietary constituent in food restriction. Some support for this conclusion may be derived from the investigations of Nelson and Evans (1953) and Zeman (1967) who found that animals fed a diet adequate in protein and pair-fed with animals receiving 0 or 6% casein in their diets equaled the reproductive performance of ad libitum controls. On the other hand, when Barton (1973) manipulated diets so that protein, mineral, and vitamin intakes for energy-restricted animals would approach

control amounts, she observed significant differences between the progeny of energy-restricted animals and progeny of those fed a methionine-supplemented casein ration which supplied approximately 15% protein. In fact, neonatal spleen and brain weights were depressed to a greater extent in the energy-restricted progeny than in the progeny of dams fed a 4% protein ration during gestation.

Knittle (1972) distributed newborn male rats in litters of 12 among mothers fed stock diet and then restricted the dams either to 50% of their normal intake or fed the dams a diet containing 3% protein during lactation. A group fed 10% protein from parturition served as controls. All pups were given free access to stock ration after weaning. Weaning weights at 3 weeks were not different among the 3 groups; however, by 5 weeks of age, both the protein-restricted and energy-deficient animals were significantly smaller than the controls. This difference persisted until after 8 weeks when the energy-deficient animals experienced a growth spurt so that at the age of 12 weeks their weights were similar to those of controls. They were significantly heavier than the protein-restricted group. Analysis of cell size and cell number of the epididymal fat pads of these animals showed that both parameters had been depressed by protein restriction of the dams during lactation while only cell size had been decreased by food restriction. Since previous investigators (Mueller and Cox, 1946) had demonstrated that protein restriction of dams decreased total milk production without altering the quality of milk, Knittle suggested that the difference between the 2 groups was related to caloric intake of offspring rather than to a specific effect of protein. He also suggested that since the dams were well-nourished through parturition, deprivation of pups

nursed by the energy-deficient dams may have begun later, at a less critical point in development, when compared to pups of severely protein-restricted females.

Miller (1970) argued in favor of a critical role for protein in neonatal development, however. He established the following experimental groups: 1) a high protein control consisting of dams and progeny fed 25% casein in gestation and lactation, 2) a low protein control fed 8% casein in gestation and lactation, and 3, 4, and 5) groups born to control dams and suckled by restricted animals. In addition to nursing, groups 3, 4, and 5 were fed by intubation 3 times daily a mixture simulating rat's milk and containing 10% protein or an isocaloric protein-free mixture or a mixture isonitrogenous with rat's milk but with less than 50% of its energy value. The animals supplemented with an energy source only were smaller than the low protein controls at the end of 10 days while those pups receiving the supplement simulating rat's milk grew similarly to controls. The group supplemented with the high protein, low calorie formula grew at an intermediate rate.

These reports seem to indicate that variations in protein-energy ratio may influence the symptoms observed in protein-calorie malnutrition. Certainly the effects of severe energy restriction may be modified by a larger proportion of protein or an increased energy supply may spare amino acids from a protein-limited diet for tissue anabolism. Studies in which both factors can be controlled quantitatively and qualitatively are needed to determine the relative importance of the 2 factors.

This review will concern primarily those studies in which only protein was limited but will examine some instances of total food restriction. The

effects of nutritional insult have been found to vary with 1) its extent or severity, 2) its time of onset, and 3) its duration. The results are presented in terms of those quantitative and qualitative changes, at the cellular, tissue, and organ levels, which may explain gross observations in the total organism. Gross observations alone are also reported.

Reproductive Performance

Maternal adjustments

Food intake Nelson and Evans (1953), feeding isocaloric purified diets containing 3 to 30% casein, confirmed earlier findings (Guilbert and Goss, 1932; Macomber, 1933) that total food intake during gestation did not vary significantly with decreased protein in the diet. When a protein-free ration was fed, food intake decreased, however. Subsequent reports by Wang et al. (1966), Berg (1967), Zeman (1967), Chou (1970), Zamenhof et al. (1972), Barton (1973), and Turner (1973) were in agreement. Goettsch (1949), who fed a nonisocaloric, modified stock diet which combined rice, beans, and casein to supply from 7.1 to 19.1% protein, reported that although food intake for all groups increased in the first 2 weeks of gestation, the increase was smaller at the lower protein intakes. She found no further increase in food intake by any group during the last week of gestation when maternal weight gain was most rapid.

Zeman (1967) observed a distinctly different pattern of food consumption between groups fed isocaloric rations of 6 and 24% casein. Dams fed the low protein (LP) diet consumed more food than those fed the high protein (HP) diet during days 0 to 10 but less during the last 10 days of gestation. Daily food intake decreased sharply in both groups as parturition

approached. The phenomenon occurred on day 16 for the LP group and on day 18 for the HP dams. Chou (1970) and Barton (1973) observed similar distribution of intake in their experimental animals fed 4 or 5 and 15% protein.

In a review of lactation studies, Nelson and Evans (1958a) reported that food consumption of lactating rats usually averaged 2 to 3 times that of nonlactating females and varied with both the number of young suckled and the composition and energy value of the diet. In a carefully conducted study, these investigators (Nelson and Evans, 1948) compared lactation performances of normal dams suckling 6 young and fed isocaloric rations of 6 to 48% casein at parturition. The average daily food intake was 13 g at 6% casein and gradually increased to a maximum of 32 to 34 g at 24, 30, 36, and 48% casein. The increased food intake was accompanied by improved lactation as indicated by average weaning weight (17 g at 6% casein and 48 g at 24% casein) and weight change of the mother during lactation (-100 g vs. +18 g for the 6 and 24% casein diets, respectively). No further improvement was observed at casein intakes greater than 24%. Goettsch (1949) and Turner (1973) also noted that lactating dams fed a low protein ration (6 or 8%) consumed amounts with only 1/2 to 2/3 the energy value of that eaten by the controls fed 20% protein rations.

Menaker and Navia (1973) studied appetite regulation in dams fed isocaloric diets containing 8 or 25% protein. They observed 3 distinct phases of appetite regulation. When female rats (250 g) were neither pregnant nor lactating, food intakes for the 2 groups were the same; therefore, energy requirement appeared to be the predominant regulator of appetite. During pregnancy, dams fed the low protein ration consistently consumed approximately 50% more diet than the controls but gained the same amount of

weight. Menaker and Navia (1973) interpreted this finding to reflect the fact that larger amounts of the low protein diet were needed to meet the amino acid requirements of pregnancy while smaller amounts of the high protein ration filled this need. With the onset of lactation, lack of protein in the diet may limit the quantity of milk produced (Mueller and Cox, 1946), and as a result, the energy needed for milk synthesis would be reduced and food intake would be reduced.

Kennedy (1957) reported that the maximum recorded energy intake in any physiologic state was about 45 kcal/100 g body weight/day. This maximum was usually reached during lactation if diet and other conditions were optimal. With a low protein ration, rats produced limited amounts of milk in the study carried out by Menaker and Navia (1973). Consequently, energy needed for milk synthesis was proportionately reduced; observed increases in food intake also were small. At the same time, lactating rats lost approximately 40 g, an indication that their energy needs were not totally fulfilled.

In summary, food intake in the rat normally may be controlled by energy requirements, but it is influenced by the adequacy of protein intake during physiological stress of pregnancy and lactation.

Weight change Net maternal weight change in gestation (postpartum weight minus weight at mating) was generally smaller with lower protein intakes, especially when dietary protein fell below 10% (Goettsch, 1949; Curtiss, 1953; Nelson and Evans, 1953, 1958b; Wang et al., 1966; Zeman, 1967; Tagle and Donoso, 1969; Chou, 1970; Barton, 1973; Turner, 1973). A small weight gain by the pregnant rat fed a low protein diet may represent depletion of maternal tissues (Zeman, 1967). Beaton et al. (1954) sug-

gested that rats fed a commercial ration supplying 20% protein deposited fat during days 0 to 15 of gestation and used this reserve of energy to support increased protein synthesis during the final week of pregnancy.

Maternal weight change in lactation has long been used to judge the adequacy of the diet (Nelson and Evans, 1947a, 1947b). The well nourished dam will maintain her postpartum weight or gain weight during lactation (Nelson and Evans, 1958a, 1958b). As the quantity or quality of protein in the ration is reduced, weight loss of the lactating dam usually increases (Macomber, 1933; Nelson and Evans, 1958a, 1958b; Widdowson and Cowen, 1972; Turner, 1973). Weight change has been correlated with food intake generally, but on occasion this relationship has been confounded by variations in such conditions as litter size or laboratory environment (temperature and humidity).

Length of gestation Occasionally rats fed low protein rations during gestation have delivered litters later than normal, i.e., on day 23 or 24; usually the pups were stillborn. In some cases when females were sacrificed 3 days after expected delivery, they have had a fully grown litter in utero in the process of being resorbed. Turner (1973) speculated that prolonged parturition, i.e., failure to initiate labor, contributed to high perinatal loss among low protein groups. In general, however, investigators have not observed differences in gestation length between dams fed restricted protein diets and those fed optimal diets (Seegers, 1937; Stewart and Sheppard, 1971; Lee, 1973; Turner, 1973). Perhaps observed cases of prolonged gestation are an atypical expression of the interactions of diet and pregnancy in individual rats. Or, the lack of data confirming extended gestation length in restricted dams may be due to difficulty of

obtaining accurate estimations of gestation length when females are not observed on a 24-hour basis.

Litter size Protein restriction initiated at the time of conception or at some time during gestation did not affect the number of pups produced by rats completing pregnancy (Thompson, 1937; Nelson and Evans, 1953; Venkatachalam and Ramanathan, 1964; Wang et al., 1966; Berg, 1967; Zeman, 1967; Kenney, 1969; Tagle and Donoso, 1969; Adeyanju, 1971; Stewart and Sheppard, 1971). An all-or-none phenomenon involving timing as well as degree of protein deprivation occurred. Rats fed a diet free of protein might exhibit reproductive failure in most cases, but if litters were delivered, they contained a normal number of young (Seegers, 1937; Venkatachalam and Ramanathan, 1966; Berg, 1967). The need for protein, according to Berg (1967), was critical at 2 periods in gestation, namely after mating (days 0 to 2) and after implantation of the blastocyst (days 5 to 9); transitory feeding of a diet containing 20% protein during either of these periods to rats fed a protein free diet was sufficient to sustain pregnancy as well as in protein-fed controls. Although litter size was unaffected, fetal growth was retarded when transitory supplementation was given. Seegers (1937), Nelson and Evans (1953), Venkatachalam and Ramanathan (1966), and Zamenhof et al. (1971) also reported that a protein supply during the early period of gestation was critical to the maintenance of pregnancy.

When protein restriction has been initiated at the dam's weaning or one month or more before conception, the number of pups produced per litter was decreased (Macomber, 1933; Goettsch, 1949; Cowley and Griesel, 1963; Gupta and Lacy, 1967; Widdowson and Cowen, 1972; Turner, 1973). Gupta and

Lacy (1967) fed 10 and 5 NDp Cal % to their control and experimental animals, respectively, and counted the corpora lutea, the implantation sites on days 10 to 11 and the live embryos on days 13 to 15 of pregnancy. Experimental feeding began at weaning when the females were approximately 25 days of age and weighed about 35 g. Mating occurred at ages ranging from 51 to 90 days. Ten-day age ranges (e.g., 51-60, 61-70, etc.) were established for comparison of the effects of the diets. Number of ova released by protein-restricted rats was significantly fewer than from rats of the same chronological age on a control diet. As would be expected, number of implantations and live fetuses were fewer due not only to decreased ovulation but to increased preimplantation loss also. These findings, particularly decreased ovulation, offer a reasonable explanation for differences in litter size when protein restriction was initiated 30 days or more prior to mating rather than at the time of conception or later.

Development of the young

Birth weight Weight at birth was significantly lowered in pups born of dams fed less than 10% protein during gestation (Macomber, 1933; Thompson, 1937; Goettsch, 1949; Curtiss, 1953; Nelson and Evans, 1953; Wang et al., 1966; Zeman, 1967; Kenney, 1969; Stewart and Sheppard, 1971; Barton, 1973; Turner, 1973; Younoszai and Ranshaw, 1973). Underweight, nonviable litters occurred among both adequately and poorly nourished animals but made up a larger proportion of the litters from dams fed restricted amounts of protein (Turner, 1973). In some studies, decreased birth weight among pups of protein-deprived females did not occur with the

first litter (Venkatachalam and Ramanathan, 1964; Cowley and Griesel, 1966; Tagle and Donoso, 1969; Widdowson and Cowen, 1972). Such results no doubt reflected better maternal nutritional status at the initiation of pregnancy.

The time of protein restriction as well as amount of protein fed may have particularly significant influences on birth weight. Since fetal weight gain is greatest during the latter third of gestation (Beaton et al., 1954), protein deprivation of the mother during this period would be expected to reduce weight of pups at birth. Venkatachalam and Ramanathan (1966) found birth weight was most severely depressed among progeny of dams deprived of protein during the final 7 days of gestation compared with birth weight of progeny of dams deprived during the first or second 7 days.

Perinatal survival Survival of newborn pups decreased when the protein in the gestation diet was decreased (Macomber, 1933; Thompson, 1937; McCoy, 1940; Goettsch, 1949; Cowley and Griesel, 1959, 1963; Venkatachalam and Ramanathan, 1964; Wang et al., 1966; Zeman, 1967; Kenney, 1969; Adeyanju, 1971; Stewart and Sheppard, 1971; Widdowson and Cowen, 1972; Barton, 1973; Turner, 1973; Younoszai and Ranshaw, 1973). Venkatachalam and Ramanathan (1966) reported that a diet devoid of protein during one week of gestation also resulted in increased perinatal death. Protein deprivation during the final week of gestation resulted in greater mortality (81%) than when dams were deprived during the first or second week (26 and 50%, respectively). Lactation failure has been implicated in this high mortality (Macomber, 1933; Mueller and Cox, 1946; Goettsch, 1949; Zeman, 1967); however, initiation of an adequate diet at parturition or

transfer to well fed foster mothers did not erase differences in perinatal death rates between pups whose mothers were fed optimal and restricted protein during gestation (Wang et al., 1966; Zeman, 1967; Turner, 1973).

Zeman (1967) reported that compared with control progeny, the newborn from mothers fed 6% casein during gestation were less active, darker in color, and showed multiple subcutaneous hematomas at birth. These pups did not survive when nursed by foster mothers fed normal amounts of protein unless control pups were also present. Presumably the more active control pups were necessary to stimulate lactation.

Turner (1973) found that rats fed either 8 (LP) or 25% (HP) casein during gestation produced some litters from which no member survived to weaning (nonviable litters). However, 78% of HP litters were viable while only 33% of the LP litters survived to weaning. When the lactating LP females were transferred to the 25% casein diet at parturition, the weight gain of their offspring was similar to that of the offspring of HP animals; nonetheless, the number of nonviable litters remained unchanged indicating that prenatal changes could not be reversed. Survival to day 4 or 5 indicated a good possibility that the pup would be weaned (Turner, 1973; Barton, 1973).

Neonatal organ weights Growth of the whole animal or of individual organs may be measured by various parameters including weight, length, circumference. Technological advances have enabled investigators to measure growth more precisely in terms of cell number and cell size (Enesco and Leblond, 1962). Winick and Noble (1965) determined that cellular growth in the rat occurred in 3 phases: 1) hyperplasia or cell multiplication, 2) a combination of hyperplasia and hypertrophy or cell growth, and 3) hyper-

trophy alone. Each organ followed its own genetically determined time course of development through each phase. As a result, malnutrition affected various tissues in different ways depending on the phase of development at the onset of the insult (Winick and Noble, 1966). Restriction during hyperplasia or the period of combined hyperplasia and hypertrophy resulted in a permanent decrease in cell number. A transient decrease in cell size alone occurred if restriction was instituted during the phase involving only hypertrophy.

Since all fetal tissues except the placenta undergo cell division throughout gestation, various organs from pups of females fed low protein rations in gestation contained fewer cells than those of controls as measured by DNA on day 16, 18, or 20 of gestation (Zeman and Stanbrough, 1969; Chcu, 1970). Decreased cell number was reflected in decreased weights of carcass, brain, liver, kidneys, and spleen of progeny from dams fed low protein diets in gestation (Zeman, 1967; Kenney, 1969; Barton, 1973). Zamenhof et al. (1968, 1971, 1972) measured and reported decreased weight, DNA, and protein for the brains of pups produced by dams fed restricted amounts of protein in gestation. Although the absolute weights of the carcass and other organs of the protein-restricted pups were smaller than those of the progeny of rats fed adequate protein in gestation, the relative weight (g/100 g body weight) of the brain in these animals was increased, indicating that deprivation had a lesser effect on the brain than on the animal as a whole. On the other hand, relative liver and kidney weights were depressed. The variation in effects may be explained on the basis of the developmental time course for these organs.

The brain contains 90% or more of its adult cell number at birth (McIlwain and Bachelard, 1971) and, therefore, may be well along the path of its developmental potential prior to the time at which the fetus undergoes a marked growth spurt, beginning about day 15 of gestation. As a result, the requirement for protein by fetal tissues is markedly increased then. If supply were limited at that time, organs other than the brain would be affected most severely. Alternatively, brain development may enjoy a biological protection greater than that of other tissues against protein restriction, i.e., nutrient needs of brain tissue may be preferentially met. Protection of brain tissue was demonstrated by Oh and Guy (1971) who retarded intrauterine growth by uterine artery ligation. When ligation was imposed on day 17, the DNA, protein, and weight of both the liver and carcass of neonates from the ligated horn were significantly reduced while weight, DNA, and protein of the brain were comparable with those of control fetuses born from the opposite horn.

Postnatal growth and development Growth and development during suckling reflects the conditions of the prenatal as well as the postnatal period. Restriction during suckling may be accomplished by: 1) continuing or instituting dietary restriction of the dam to limit milk production (Macomber, 1933; Thompsen, 1937; Goettsch, 1949; Nelson and Evans, 1958a, 1958b; Venkatachalam and Ramanathan, 1964; Zeman, 1967; Barnes et al., 1968, 1973; Stewart and Sheppard, 1971; Knittle, 1972; Puar, 1972; Widdowson and Cowen, 1972; Turner, 1973), 2) increasing the litter size to 16 or 18 pups compared with the 6 to 8 that can be adequately nourished by a well fed dam (Barnes et al., 1966; Benton et al., 1966; Winick and Noble, 1966; Chase et al., 1967; Baird et al., 1971), or 3) limiting the period of

access of pups to the mother during each 24 hours (Rajalakshmi et al., 1967). All of these methods have resulted in some degree of increased mortality, depressed growth as measured by body weight, and depressed development of various organs. Various developmental indices were also affected; these indices included the day on which the eyes open, the external ear unfolds, and the righting reflex appears.

Turner (1973), in a typical study, fed dams 8 (LP) or 25% (HP) casein from the time they were weaned; he observed a reduction of more than 50% in weaning weight of the LP offspring at 21 days (HP, 31.2 g; LP, 13.3 g). There was no difference in the weight of viable pups on the first postpartum day (HP, 5.6 g; LP, 5.3 g); however, weight gain of the LP progeny lagged steadily from that point, presumably due to poor milk supply of their dams. Young of mothers changed from LP to HP ration at parturition were weaned at a weight similar to control pups (31.5 g). Barnes et al. (1973) obtained a similar reduction in weaning weight (approximately 50%) when protein was restricted during gestation and lactation or during lactation alone; they also observed that restriction during gestation only did not result in a significantly decreased weaning weight.

In contrast, Venkatachalam and Ramanathan (1964) found that weaning weight and percent body fat compared with controls were decreased by one-half or more in rats whose mothers had consumed 7% wheat protein in either gestation or lactation. Allen and Zeman (1971) also observed a weight deficit of approximately 18% but no decrease in percent body fat among pups born of dams fed 6% casein but foster-nursed in litters of 10 by adequately fed dams. Decreasing litter size to 4 pups on day 7 erased the weight deficit.

Cowley and Griesel (1966) observed postnatal development for several generations in progeny of rats fed simulated Gambian diets containing approximately 14 or 21% protein. A significantly smaller percentage of first generation restricted pups were able to right themselves on days 1, 2, and 3 than of controls. The difference disappeared by day 4. Retardation in righting, unfolding of the external ear, eruption of upper incisors, and eye opening was greater as successive generations were reared on the low protein diet. Allen and Zeman (1971) reported that delayed eye opening in young of animals restricted in gestation was corrected when the litters were reduced in number after the first 7 postpartum days.

Examining cellular growth in the postnatal period, Zeman (1970) found that rats deprived of protein in gestation only had significantly decreased organ weights, DNA, RNA, and total protein at birth, 7, 14, and 21 days; the deficit did not increase with age, however. Increased weight and cell size in the liver, kidneys, and heart were promoted by reducing litter size to 4 at the end of 1 week. Small litters did not affect brain weight or cell size or the cell population deficit in any organ studied, however.

Rats born of adequately nourished dams, then suckled in litters of 18, demonstrated decreased body weights, organ weights, RNA, and DNA (Winick and Noble, 1966); these conditions were corrected within 10 or 12 days if pups were transferred in groups of 3 to well fed foster mothers on or before day 10 (Winick et al., 1968). A more pronounced effect of deprivation was seen when prenatal and postnatal insults were combined (Winick, 1971). Animals subjected to malnutrition during both gestation and lactation exhibited a 60% reduction in total brain cell number at weaning compared to a 15 to 20% deficit when malnutrition was imposed during either

period alone. The time and duration of nutritional deprivation, as well as the severity, are critical to the ultimate impact.

Postweaning Growth, Development, and Metabolic Efficiency

Growth

Weight change Growth and development in the rat are affected by nutritional conditions of the prenatal, postnatal, and postweaning periods. Conflicting data regarding the effect of deprivation in gestation only have been reported. Barnes et al. (1973) found that at 3 and 30 weeks body weights of male rats born of mothers fed 7% casein in gestation and foster-nursed by dams fed 25% casein in gestation and lactation were equal to those of the progeny of dams fed 25% casein in gestation and lactation. Similarly, Adeyanju (1971) found that weights of progeny of females restricted to 7% casein or to 50% of the control ration during gestation only did not differ from controls at 56 days. In addition, female progeny of dams restricted in protein or energy during gestation only weighed the same as controls at 21 and 90 days (Barton, 1973). The marginal ration (15% protein) used as a control in the last study may not have enabled any of the animals to reach their genetic potential, however.

Work in Chow's laboratories (Chow and Lee, 1964; Blackwell et al., 1969; Chow and Stephan, 1971), which examined the growth of progeny whose mothers were restricted to 50% ad libitum intake of laboratory chow, found that with prepartum restriction only, weaning weights were similar to those of controls; a small persistent weight deficit was evident by 6-8 months, however. Perhaps the effectiveness of adequate feeding immediately after

birth in promoting recovery depends on the severity of prepartum restriction.

An inadequate supply of protein or energy for the dam during lactation or throughout gestation and lactation has resulted in body weight deficits of 50% at 3 weeks and 15 to 30% at 6 to 8 months even when the progeny consumed ad libitum amounts of the control ration from weaning (Thompson, 1937; Chow and Lee, 1964; Barnes et al., 1968, 1973; Blackwell et al., 1969; Adeyanju, 1971; Widdowson and Cowen, 1972). Extension of restriction for a short period (4 weeks) after weaning by limiting food or protein intake resulted in further stunting (Barnes et al., 1968, 1973). In contrast, feeding a diet containing 3% casein for 4 weeks after weaning to animals adequately nourished in prenatal and postnatal life did not affect body weight permanently. Catch-up growth followed the reinstatement of the control ration and resulted in normal body size (Barnes et al., 1973). Longer periods of restriction (10 weeks or more) resulted in permanent stunting if begun soon after rats were weaned (Jackson and Stewart, 1920).

Body composition Changes in body weight were reflected in modifications of body composition in rats nutritionally deprived in early life (Barnes et al., 1968, 1973; Adeyanju, 1971). Animals malnourished in the first 7 weeks of life demonstrated decreased absolute and relative amounts of body fat at 32 and 50 weeks of age. Relative amounts of body protein were increased at 32 weeks but not at 50 weeks (Barnes et al., 1968, 1973). Adeyanju (1971) determined moisture, protein, and fat at 56 days in a limited number of animals whose mothers had been deprived of food (undernourished) or protein (malnourished) during gestation, lactation, or both. Relative body fat tended to decrease in progeny of females restricted in

food intake in both gestation and lactation and to increase or remain unchanged in progeny of animals whose total food or protein was limited during gestation or lactation alone. Variations in relative body fat or protein were not statistically significant, however. Relative moisture increased significantly in progeny of animals undernourished in gestation and lactation and decreased significantly when undernourishment occurred in gestation or lactation only. Moisture values for offspring of females restricted in protein rather than in energy during gestation or lactation were similar to those of the control group.

Organ development Changes in organ development, like those in body weight, may be transient or permanent depending on the age of the animal and the severity and duration of nutritional deprivation. Winick and Noble (1966) reported a weight deficit which persisted to 19 weeks for all organs from animals fed in litters of 18 pups during suckling. In the same experiment, restriction to 50% ad libitum food intake from day 21 to day 42 also resulted in persistent weight deficits in all organs examined except the brain and lung. All organs except the thymus from animals restricted from day 65 to day 86, though smaller at the end of restriction, reached normal weight by 19 weeks. Changes in organ weights generally reflected differences in DNA. Cell number was permanently decreased in all organs of rats restricted during suckling and in all organs except the brain and lungs of animals restricted immediately after weaning. In those animals restricted after 9 weeks of age, cell number was low in the thymus only.

Roeder and Chow (1972) reported that organs (liver, kidneys, heart, testes, adrenals) were small through 7 months of age in rats from dams restricted in both gestation and lactation to approximately 50% ad libitum

intake. These differences in organ weights were no longer significant by 19 months of age even though body weight remained about 30% below that of controls.

Effects on organ development of protein restriction after weaning were reported by De Castro and Boyd (1968). Male rats, initially weighing 66 ± 6 g and fed a diet which supplied 8% casein for 4 weeks, had significantly smaller organs than controls fed a 27% casein ration. Cecum, kidney, liver, muscle, skin, spleen, salivary gland, and thymus weights were more severely affected than total body weight; relative weight deficits of adrenals, brain, stomach, heart, and testes were less than that of the body. Growth of skin and muscle, which predominates in this period, was severely depressed (approximately 60%) in restricted animals but was still greater than that of other organs examined; this pattern indicated that the animals were responding to genetic influences as well as to the low protein diet (McCance and Widdowson, 1962).

Dickerson et al. (1972) examined the effects of protein restriction (5% casein) on brain and liver for 4 weeks immediately after weaning. This period was followed by rehabilitation on a ration containing 25% casein to 20 weeks. Controls were fed a 25% casein diet from weaning. The 3 sections of the brain, i.e., forebrain, cerebellum, and brain stem, were in different stages of development at the time of protein restriction. Ultimate results of deprivation and rehabilitation varied with the particular section analyzed. When deprivation ended, cholesterol and DNA concentrations were similar to those of controls only in the forebrain. Following rehabilitation, cerebellum, brain stem, and whole brain but not forebrain weights were lighter than those of controls. Cell number, as indicated by

DNA, was smaller than for controls in the cerebellum but not in the fore-brain. In contrast, cholesterol concentration was lower in the forebrain, brain stem, and whole brain but not in the cerebellum than in controls.

Hepatic weight and DNA were severely depressed after 4 weeks of protein restriction but after 16 weeks of rehabilitation were similar to those for controls (Dickerson et al., 1972). In contrast, Winick and Noble (1966) had observed permanent deficits in hepatic weight and DNA content following 50% food restriction for 3 weeks after weaning. Experimental conditions such as differences in age when restriction was imposed could explain these discrepancies. For example, Winick and Noble's animals were 21 days old when dietary restriction began while Dickerson's were restricted at 24 days; cell multiplication in the liver may have been completed in the additional time prior to deprivation in the later experiment. Strain of rat, method of deprivation, and composition of the diet during rehabilitation also differed between the 2 experiments.

Food consumption

Anomalies in food intake patterns have been observed in offspring of rats deprived of food or protein during the reproductive cycle and in animals deprived shortly after weaning. Barnes et al. (1973) compared food intake in rats deprived during gestation (DG), during lactation (DL), during gestation and lactation (DG + DL), for 4 weeks postweaning (DW), and during lactation and postweaning periods (DL + DW) with that of animals well nourished throughout life. Dams deprived in pregnancy were fed a ration supplying 7% casein; those deprived in lactation, a diet containing 12% casein; and deprived weanling pups, a 3% casein ration. The control

diet during gestation, lactation, or after weaning contained 25% casein. A high food requirement for growth was evident in all animals. When intake was expressed as a function of metabolic body weight ($\text{g/kg BW}^{3/4}/\text{day}$) and groups were compared at equal rates of growth expressed as gain in g/day , 3 groups, DL, DG + DL, and DL + DW, reflected food costs for growth which were substantially higher than those for controls. Food intake for groups DG and DW varied little from that of controls. When the comparison was made at equal ages, consumption for groups DL, DG + DL, DL + DW, and DW increased, particularly during the weeks immediately following weaning. Differences among groups became smaller as the animals aged but persisted until the end of the study when rats were 16 weeks of age. Rats restricted in gestation only (DG) ate amounts of food that were similar to control intake when the comparison was made at equal ages.

Barnes et al. (1973) concluded that the only period during which nutritional deprivation caused a rise in food consumption was lactation. Similar conclusions were reached from earlier work (Barnes et al., 1968) and by other investigators (Hsueh et al., 1970, 1974). These studies introduced deprivation to offspring through 1) food restriction of the pregnant or lactating dam to 50% of ad libitum control intakes or 2) suckling in litters of 18 pups. The latter method produced animals which ate significantly more food/100 g body weight at 31 weeks of age than controls, but the difference disappeared when the comparison was made in terms of metabolic body size (Hsueh et al., 1970). When food supplied to the pregnant or lactating dam was limited, food intake of progeny restricted only in gestation (RN) did not differ significantly from that of controls at 2, 4, 9, or 14 weeks of age (Hsueh et al., 1974). Rats restricted in lacta-

tion alone (NR) or in both gestation and lactation (RR) consumed significantly more food than controls based on body weight or metabolic body size at all ages except 14 weeks, at which time differences between NR and NN (controls) expressed on the basis of metabolic body weight were not significant.

Data for food consumption were conflicting when a low protein diet was fed beginning immediately after weaning or a few weeks later. Barnes et al. (1973) observed very high food intakes in rats fed 3% casein immediately after weaning. Offspring born of adequately nourished females and foster-suckled by dams fed 12% casein consumed approximately $116 \text{ g/kg}^{3/4}/\text{day}$ when fed 3% casein after weaning while those born of and foster-suckled by normally fed females (preweaning controls) consumed $76 \text{ g/kg}^{3/4}/\text{day}$ when fed the 3% casein ration; control weanlings provided a 25% casein ration after weaning ate $60 \text{ g/kg}^{3/4}/\text{day}$. In contrast, when Stead and Brock (1972) fed a 4% protein diet to rats weaned as soon as physiologically possible (25-30 g), the energy value of their rats' intakes on the basis of metabolic body size was about 32% lower than that of controls fed 20% protein. After 1 week on an 8% casein diet, male rats weighing 66 g initially consumed consistently more food than controls fed a diet with 20% casein (De Castro and Boyd, 1968). During the 4th week of restriction, energy consumption of weanlings fed the 8% casein diet was about 29% more than that of the controls. Kirsch et al. (1968) fed diets containing 5, 8, 12, and 20% protein to male rats for 60 days. The rats weighed 100 g when diets were initiated. Rats on 8 and 12% protein regimens ate significantly more food than those fed the control diet, but there was no difference between controls and animals fed the 5% protein diet.

Several factors may have influenced food consumption in the studies described. Houpt and Epstein (1973) determined that early in the rat's life feeding was dominated by signals from the upper gastrointestinal system and that neural response to chemical signals such as glucoprivation did not develop until pups were 4 to 5 weeks old. This report agreed with the findings of Kennedy (1957) that development of hypothalamic control of appetite was delayed until after the period of most rapid growth. Musten et al. (1974) examined the capacity of weanling rats to regulate protein intake when fed diets containing from 0 to 70% protein. When both a protein-free diet and one containing 5, 10, or 20% protein were provided continuously, rats tended to eat almost entirely from the cup with the protein-containing ration. Total food intake decreased as compared with the 0-10 and 0-20% combinations when the 0-5% protein combination was fed, however. When 40 to 70% protein diets were provided, progressively more food was selected from the protein-free ration. From these findings and others from their studies in which protein quality, caloric density, and ambient temperature were varied, the authors concluded that weanling rats possessed the ability to regulate protein intake and that mechanisms controlling protein and energy consumption interacted to control total food intake.

Food utilization

Often food efficiency (g weight gain/g food eaten) has varied inversely with food consumption. Pups from dams deprived of adequate protein in both gestation and lactation or during lactation only or pups suckled by protein-deficient females then fed a low protein ration for 4 weeks after weaning required significantly more of an adequate diet later

in life to achieve the same weight gain as controls (Barnes et al., 1973). Hsueh et al. (1974) examined food efficiency of progeny from mothers fed ad libitum or subjected to approximately 50% food restriction in gestation or lactation or during both periods. When plotted as a function of age, food efficiency decreased, but control and experimental groups did not differ. However, compared on the basis of intake per unit of body weight, controls utilized food significantly more efficiently than groups whose mothers were deprived in lactation or gestation and lactation; control and gestationally deprived groups used food similarly. All of these data indicated that food efficiency in the progeny was affected to a greater degree by restricting the lactating rather than of the pregnant female's diet.

After weaning, food efficiency was significantly reduced by an inadequate protein supply (De Castro and Boyd, 1968; Kirsch et al., 1968; Stead and Brock, 1972; Musten et al., 1974). Since young of rats fed adequate amounts of protein, followed by a postweaning deprivation of 4 weeks, manifested a food efficiency similar to that of controls (Barnes et al., 1973), the effect seen in rats deprived after weaning is probably transitory.

Brain Development and Behavior

Brain weight and cell number

In the rat, as in other species, brain development occurs very early in life. By weaning (21 days), adult DNA content and 80% of the final brain weight are achieved. The maximal rate of weight gain occurs from the 5th through the 15th days postnatally. As indicated by an increase in DNA, cells divide most rapidly from days 6 to 10 after birth (Dobbing, 1968). Consequently, prenatal or preweaning nutritional restriction have caused

permanent deficits in these gross indices of neural development (Winick and Noble, 1966; Culley and Lineberger, 1968; Guthrie and Brown, 1968; Zamenhof et al., 1968, 1971, 1972; Winick, 1970). Restriction after weaning resulted in a reversible decrease in brain weight but no reduction in cell number (Winick and Noble, 1966).

Changes in total brain DNA and weight are the sum of changes in various regions of the brain. Specific areas should be affected preferentially according to the synthetic activity which normally should be occurring at the time nutritional restriction is imposed. With this in mind, Winick (1970) assessed cell division in discrete fetal brain regions by measuring DNA concentration on the 16th day of gestation. In the cerebral white and gray matter of protein-deficient offspring, the decrease of cell division was small, while in the area adjacent to the 3rd ventricle and subiculum, cell division was moderately affected; a marked decrease occurred in the cerebellum and in the area adjacent to the lateral ventricle. Postnatal restriction also induced differences in specific regions and cell types within the brain (Fish and Winick, 1969); however, defects in brain weight and cell number were reversed almost entirely if rehabilitation began several days before weaning (Winick et al., 1968). Quantitatively, the number of cells approached normal; qualitatively, the early deficits in cell number were probably compensated for by cell proliferation in different areas. Although partial rehabilitation may have occurred, specific regions of the brain may have suffered permanently.

Lipid deposition

Like cell division, myelination occurs at different times in various areas of the nervous system. Peak myelin formation in total rat brain takes place from 10 to 21 days postpartum. Accumulation of myelin probably extends to the age of 5 to 6 weeks, however. Since myelin is synthesized by oligodendroglia, formation of the lipid complex depends on proliferation of these cells and thereby is influenced by factors which affect cellular growth (Winick, 1970).

Myelin of rat brain contains approximately 70% of total brain cholesterol, sulphatides, and sphingomyelin. Also, most of the cerebrosides and plasminogen appear to be part of the myelin sheath (Davison and Dobbing, 1966). As a result, analyses for these substances will estimate quantitatively the amount of myelin in brain. Since there is little turnover of brain myelin, serial determination of these components may estimate rates of myelin formation.

Effects of inadequate nutrient supply on brain lipid composition have been demonstrated. Results varied with the lipid component measured and the age at which stress was imposed. Underfeeding during either the prenatal (Stephan, 1971) or the postweaning period (Dobbing and Widdowson, 1965) did not affect cholesterol content. These results supported the belief that the suckling period is the critical time for myelin formation in the rat.

Dobbing and McCance (1964) reared rats in litters of 3 or 15 pups from birth to weaning. Then an adequate diet was fed ad libitum to all rats. Total cholesterol deposition and concentration (mg/g) in the brain were significantly smaller for large than small litters, regardless of sex, at

12 and 21 days postpartum. The difference in total brain cholesterol was present only in females when measured at 35 and 56 days of age, however. Refeeding begun at 21 days of age apparently equalized total cholesterol deposited in males and its concentration in both males and females. This was true although brain was still small at 56 days in both males and females from large litters. Guthrie and Brown (1968), who intensified large litter deprivation by feeding dams 8% protein during lactation, found that total brain cholesterol but not brain weight was restored by 19 weeks when rehabilitation (18% protein) began at weaning. Feeding a 3% protein diet after weaning to extend deprivation to 5, 7, or 9 weeks of age caused permanent decreases in total cholesterol but not concentration. After imposing restriction by suckling 16 to 21 pups to an adequately nourished dam during lactation, Benton et al. (1966) demonstrated complete recovery of brain weight and lipid with refeeding for only 3 weeks after weaning.

Contrary to these reports, Culley and Lineberger (1968) found that total brain lipid in rats nutritionally restricted was not restored by subsequent ad libitum feeding. They limited access to the nursing dam before weaning from 5 to 11 or 17 days or continued the restriction after weaning to 60 days by limiting food intake. All groups were fed ad libitum from the end of the restricted period until the rats were 110 days old. Concentrations of phospholipids, cerebroside, and cholesterol were decreased significantly only in brains of rehabilitated rats whose food intake had been restricted until 60 days of age. When Geison and Waisman (1970) reared rats in large (13 to 16 pups) or small (2 to 4 pups) litters and fed laboratory chow from weaning to 8 weeks, they observed that total brain lipid, phospholipid, cholesterol, galactolipid, and proteolipid protein

were decreased in brains of animals from large litters. Ganglioside N-acetyl neuraminic acid (NANA) increased, suggesting that the effect on synthesis of ganglioside-rich synaptic and neural membranes was less severe than on other components. These investigators concluded that myelin-related lipid classes whose concentration decreased extensively after postpartum undernutrition were those that normally should have been synthesized most rapidly in the weeks following birth. Proportions of lipid components in undernourished rats resembled those of younger, faster growing rats and suggested a delay in the maturation process.

Acetylcholinesterase

Acetylcholine (ACh), a neural transmitter substance operative in the peripheral and central nervous systems, is difficult to assay biochemically. By contrast, acetylcholinesterase (AChE), the enzyme responsible for inactivation of the transmitter through hydrolysis to acetate and choline, is amenable to biochemical or histochemical assay. Concentration of AChE is generally proportional to that of ACh in the central nervous system, though it has been shown to be more concentrated than the substrate in the cerebellum (Koelle, 1969). As a result, AChE has been measured as an index of brain cholinergic activity and as an indicator of neurological metabolism and development.

Most assays do not distinguish between "true" or acetylcholinesterase activity, specific for ACh, and several pseudocholinesterases which are capable of hydrolyzing ACh and a variety of other esters. Therefore, most studies report values for cholinesterase (ChE), the sum of acetyl- and pseudocholinesterase activities. Measurement of independent activities of

the enzymes in the rat brain indicated that AChE is approximately 30 times as active as pseudocholinesterase in this tissue (Ellman et al., 1961); thus it is unlikely that variations in pseudocholinesterase activity were reflected in combined measurements.

Changes in rat brain in ChE have been correlated with behavioral changes in animals subjected to environmental stimulation (Krech et al., 1962). In a preliminary study, Rosenzweig et al. (1962) assigned male weanling littermates to one of two environmental treatments. Rats receiving environmental complexity training (ECT) were housed from weaning in groups of 10 in large cages. A small maze and various wooden toys, e.g., stairs, blocks, etc. which were changed from time to time, were available for their use. For 30 minutes each day, animals in this group explored a Hebb-Williams maze in which barrier patterns were changed; animals were formally trained in additional testing devices also each day. Isolated controls (IC) were housed individually under reduced illumination without contact or sight of other animals. They were handled minimally when weighed and were given no opportunities for exploration or training in testing devices. Food and water were available ad libitum for both groups. Respective environmental conditions were maintained until the rats were sacrificed at 110 days. At that time, ChE concentration was decreased in the cerebral cortex but was elevated in the subcortex of ECT rats; thus cortical-subcortical (CS) ratios for the enzyme were reduced. Cerebral cortex weight and total ChE activity in the subcortex and whole brain were higher in experimental animals than in littermate controls.

Conditions in a succeeding study (Krech et al., 1962) were identical except that the enriched environment did not include formal training and

was designated EC rather than ECT. Each group was exposed to its respective environmental condition for 30 days after weaning. During a second 30-day period, which preceded sacrifice and brain analysis, both groups were deprived of food, pre-trained and tested using reversal visual discrimination problems. Performance of the EC group was significantly superior to that of the IC group on the discrimination tests. Behavioral scores of EC rats correlated directly with CS ratios of specific ChE activity and inversely with CS ratios of brain weight ($P < 0.01$). Corresponding correlations within the IC group were lower, and only the relation between behavioral score and CS ratio of ChE activity was significant. In contrast to the earlier experiment, brain weight and ChE activity differed little between the EC and IC groups. The authors hypothesized that this finding was due to the 30-day period of visual discrimination training which may have increased cortical weight and ChE activity in the IC rats to values similar to those attained earlier by the EC group as a result of their exposure to the enriched environmental treatment. Confirmation of the hypothesis was not possible with data from this experiment since brain weight and ChE measurements were made only at the termination of the study.

Correlation of ChE values with behavior led to investigation of the effect of other environmental variables, specifically nutrition, on this enzyme. Im et al. (1971) restricted protein intake of rats during the first 7 weeks of life and measured brain ChE. Experimental animals were progeny of dams fed 25% casein in gestation then restricted to 12% casein in lactation. Restriction was extended by feeding the pups 3% casein for 4 weeks after weaning. Control animals received the same 25% casein diet after weaning as their mothers had consumed in gestation and lactation.

The activity of ChE was assayed in brains of 9-day, 3-, 7-, and 38-week old males. Brains of the control group were larger and thus had significantly higher total ChE activity at 3 and 7 weeks of age than those of experimental animals. Specific activity was significantly greater, however, for malnourished animals at 9 days, 7 weeks, and 38 weeks than for controls; the authors concluded that protein-energy restriction resulted in long-lasting increases in the concentration of brain ChE in rats.

Conflicting results regarding effects of undernutrition on AChE before weaning were obtained by Sereni et al. (1966). At 6, 8, and 14 days, the AChE activity had decreased significantly in brains of rats suckled in litters of 16 compared with controls reared in groups of 4. This deficit was no longer significant by 21 days and had disappeared entirely by 35 and 45 days even though 50% food deprivation was continued after weaning for the experimental animals. Adlard et al. (1970) also reported lower brain AChE activity at weaning in the progeny of dams undernourished in gestation and lactation. Brain AChE specific activity was 14% below that of controls, compared with deficits of 27% in brain weight and 66% in body weight.

Behavior

Interpretation of animal behavior poses a number of problems. Learning, for example, cannot be measured directly; instead, performance of a particular task must be evaluated. Unfortunately, performance is influenced by such factors in addition to learning as motivation, incentive, and emotional stability of animals. Therefore, it is necessary to demonstrate that these factors are homogeneous among experimental groups or to use

tests which are relatively unaffected by differences in these factors before conclusions regarding learning per se may be drawn. A variety of test situations and apparatus has been designed to evaluate effects of malnutrition on behavior.

Open field exploration Open field exploration has been used to evaluate the emotionality of experimental animals. The open field test apparatus consists of a large enclosed platform with a one-way glass cover. The floor is divided into squares. Animal behavior is evaluated by recording the number of squares entered or times when the animal reacts by raising its head or by standing up; time required to leave the starting platform or enter the open field may be observed as well.

Frankova and Barnes (1968a) assessed for 6-minute periods the exploratory activity of pups suckled by dams fed 12% casein from parturition. At 10, 14, and 21 days, restricted pups entered fewer squares, attempted to raise their heads less frequently, and exhibited longer periods of inactivity than controls. Sex differences in exploratory behavior were not evident during the preweaning period.

At weaning, some restricted pups were given a 5% casein diet ad libitum for 4 weeks; others were fed 50% as much control diet as that consumed ad libitum by a control group, and others were fed a 25% casein diet ad libitum. Beginning with day 50, all groups were fed 25% casein ad libitum. For males deprived of energy or protein after weaning, spontaneous activity on day 50 in the open field was increased as compared with that of controls. Males deprived during suckling only displayed longer periods of inactivity than the controls in the open field test (48.7 vs 5.3 sec.) on day 50, however. After rehabilitation, on days 75 and 85, exploratory drives of all previously malnourished animals were similar but significantly below those of controls. Females displayed similar trends in

exploratory behavior, but differences between restricted and control groups were less evident than in males.

Hsueh et al. (1973) measured open field behavior at 19 months of age in male rats whose mothers and foster mothers had been restricted to approximately 50% of controls' food intake during gestation, lactation, or both. In agreement with Simonson et al. (1971), restricted offspring displayed delayed reaction times, entered fewer squares, and passed more fecal boli than controls. Behavior of doubly-deprived animals deviated most from that of controls, followed by that of the group deprived in utero only.

Problem solving The Hebb-Williams maze, which has been used frequently to measure learning ability, is a square box with barriers which are moved so that the animal must cross the field diagonally to a reward on the other side of the box.

Cowley and Griesel (1959) found that male rats whose mothers had been fed a low protein diet from weaning made more errors and took longer to reach the goal box than controls. Females demonstrated a similar trend, but effects were less pronounced. Differences observed were of particular significance because of the experimental design. Protein-restricted animals were maintained on an inadequate ration, and the control ration was used as the reward; therefore, the restricted group should have had added incentive to reach the goal (Levitsky and Barnes, 1973).

Baird et al. (1971) examined performance in the Hebb-Williams maze of rats exposed to increasingly severe conditions: 1) born of control mothers, suckled by control mothers, then restricted in either protein or energy from 4 through 14 weeks of age, 2) born of control mothers, suckled in large litters, then restricted in either protein or energy from 4

through 14 weeks of age, or 3) born of and suckled by mothers restricted in either protein or energy from weaning through lactation, then restricted in either protein or energy from 4 through 14 weeks of age. An adequate diet was given to all rats from the 14th week; behavior was tested between 11 and 14 weeks of age and again at 18 weeks of age after 4 weeks of rehabilitation. Rats were deprived of food for 24 hours prior to testing, and food was placed in the goal box as a reward. All previously malnourished animals made more errors than controls both at the end of restriction and following rehabilitation. Generally, scores for animals deprived of either energy or protein did not differ significantly from one another. These results might have been unexpected because an adequate ration was used as a reward and should have enhanced motivation for the restricted groups, especially during the first test period when they were consuming the restricted rations prior to rehabilitation.

In contrast to Baird et al.'s findings, Smart et al. (1973) found that males suckled by dams restricted to about half the ad libitum food intake of controls made fewer errors and took less time on a Hebb-Williams maze problem than animals nursed by dams receiving an adequate intake. Rats were tested in the maze at 15 weeks of age following rehabilitation from weaning with ad libitum supplies of an adequate diet. For testing, all rats were reduced to and maintained at 80% of their weights at 15 weeks of age; food served as the reward.

Discrimination training In discrimination situations, which generally involve a Y or T maze, animals obtain a reward or escape an unpleasant situation by making a correct choice based upon a visual or spatial (left or right choices always correct) cue at one or more decision points.

Barnes et al. (1966) observed the ability to solve a visual discrimination problem and escape from a water maze of 6- to 9-month old rats nursed in large litters (14 to 16 pups) then fed 3 or 4% protein for 8 weeks after weaning. Additional experimental groups were restricted only during lactation or for 8 weeks after weaning. Controls were nursed in groups of 8 and fed a 25% casein ration after weaning. Male rats deprived both before and after weaning made significantly more errors than controls, while scores for animals deprived during either period alone were intermediate. Differences were not apparent among female rats subjected to the same treatments.

Rajalakshmi et al. (1965) used food reinforcement when they measured visual discrimination performance of rats fed a 9 or 11% protein diet for 4 to 6 months, beginning at 1, 6, or 12 months of age. All restricted groups required significantly more trials to reach the performance criterion of 18 of 20 correct choices on 2 consecutive days. Since the deficient animals were not rehabilitated before testing and the low protein ration was used as reinforcement for them, such results might have been expected. On the same test problem, rats given limited access to lactating rats during the suckling period (weaning weights were about 50% of controls) demonstrated no impairment in discrimination ability following rehabilitation with a stock ration (Rajalakshmi et al., 1967).

Simonson and Chow (1970) used an elevated T maze to test spatial discrimination of male offspring from females restricted to about half normal food intake in gestation and lactation. Water was chosen as the reward. At 10 weeks of age, progeny of malnourished animals demonstrated significant increases in starting time, running time, and total errors. During

extinction (when reward was withdrawn), the previously malnourished group continued to make more errors and run more trials than the control group. In an extension of these studies, Hsueh et al. (1974) studied performance of animals whose mothers were restricted in either gestation or lactation. Food restriction during gestation increased starting times and total errors, while performance of animals restricted during the nursing period only was not significantly different from that of controls.

Operant conditioning A Skinner box or operant conditioning apparatus is a compartment equipped with a lever which the animal may operate to obtain a reward or to avoid punishment.

Smart et al. (1973) examined motivation expressed by pressing a bar for a food reward. Rats whose mothers or foster mothers were restricted in food intake during gestation or lactation or both were maintained at 80% of their rehabilitated weights by food restriction during testing at approximately 18 weeks of age. In contrast to the performance of these animals in the Hebb-Williams maze, when deprivation during the suckling period resulted in fewer errors than deprivation during gestation, i.e., better performance, animals deprived during gestation pressed the bar for a food reward more often than those deprived after birth.

Avoidance conditioning A compartment with one or more subdivisions into which an animal may escape from an aversive stimulus such as electric shock (unconditioned stimulus, UCS) is utilized for avoidance training. The stimulus usually is administered following a conditioning cue such as a buzzer, tone, or change in light (conditioned stimulus, CS).

Frankova and Barnes (1968b) rehabilitated male rats subjected to protein or energy restriction to 3 or 7 weeks of age by feeding them a ration

containing 25% casein. When the animals were 95 days old, avoidance conditioning was instituted. Ten seconds after presentation of a tone (CS), an electric current (UCS) passed through the electric floor grids. To avoid the electric shock, rats learned to jump to a raised screen after hearing the tone. Latency, i.e., time elapsed between CS and movement to the screen, and spontaneous activity were observed. Six tests and one extinction experiment, each consisting of 6 trials, were conducted. Latency times of rats malnourished in early life and of controls were the same. Rats malnourished both before and after weaning spent significantly more time on the escape screen than did controls or those restricted in the lactation period only. The doubly deprived animals also were slower to extinguish the conditioned response and jumped significantly more times to the escape screen, perhaps indicating greater excitability or sensitivity to the aversive stimulus.

In passive avoidance, the animal is trained to remain in an original compartment to avoid punishment. Smart et al. (1973) employed such a test with animals whose dams or foster dams were deprived of food in gestation, lactation, or both. Adequate nutrition in lactation was associated with general disregard for the electric shock felt upon leaving the original compartment. These animals entered the shock compartment sooner than those malnourished in lactation.

Choice of reinforcement Most behavioral test situations are based on a reward system; this characteristic makes interpretation of behavioral investigations involving malnutrition more difficult, particularly when food is chosen for reinforcement. Bronfenbrenner (1968) cited studies in which restricted food intake early in life led to such manifestations of

feeding frustration as hoarding and increased competition for food. Feeding behavior of adult rats which had been restricted in protein and energy during suckling and for 4 weeks after weaning then fed 25% casein ad libitum was abnormal at 6 months of age (Barnes et al., 1968). Males suckled by inadequately fed dams and maintained on 5% casein for 4 weeks after weaning ate significantly more food than controls, on the basis of relative or metabolic body weight, during both ad libitum periods and periods when feeding for all rats was restricted to 1 hour per day. Food spillage increased significantly when food was available for a short time only among male rats restricted in protein before and after weaning. Males treated identically before weaning, then fed limited amounts of a 25% casein diet to prevent further growth for 4 weeks, also ate and spilled more food than controls, but differences for this group were less marked than those for the protein-restricted group. At the beginning of the hour when food was available, all animals ate voraciously. In a short time, however, controls appeared satisfied and left the feeder to lie down; experimental males generally remained at the feeder throughout the hour. Amounts of food eaten and spilled did not differ between control and experimental females.

An additional problem occurs when a nutritionally adequate ration is used to reinforce behavior of animals maintained concurrently on a deficient diet. When Griffiths and Senter (1954) used a multiple Y maze to test 60-day old rats maintained on a low protein diet, the restricted animals made fewer errors when reinforced with the control ration than when reinforced with the low protein ration. In fact, they performed better than controls when both groups were rewarded with adequate diet.

To avoid food as a reinforcer, some studies have used escape or avoidance tests. Such situations as escape from a cold water maze or avoidance of electric shock involve considerable stress, however. Levitsky and Barnes (1970) found that one behavioral effect of early malnutrition was an increase in the sensitivity of rats to aversive stimulation. In an open field, previously malnourished rats urinated and defecated more frequently following a loud noise, passively avoided electric shock (remained on a "safe" platform over an electric grid for long periods following initial exposure), and pressed a bar more frequently than controls to avoid a shock. Such results indicate that motivational and emotional variables may confound learning behavior in avoidance and escape situations also.

Summary From investigations cited, the following generalizations describe behavior of protein-energy malnourished rats:

1. Animals deprived of protein or energy after reaching maturity (growth plateau), then rehabilitated prior to behavioral testing, are not different from the controls.
2. Male rats deprived either through restriction of food or protein intake early in life exhibit decreased exploratory activity and increased sensitivity to aversive stimuli.
3. A sex difference in behavioral response to malnutrition exists. Because female behavior is more variable than male, behaviors of control and previously malnourished females often are not significantly different.
4. Problem solving and visual discrimination by deprived males vary from one study to another and probably are confounded by severity of nutritional restriction, motivational, and environmental factors.

5. Results from various laboratories attempting to define a critical period of deprivation for production of permanent behavior anomalies are inconclusive. Deprivations initiated during gestation, neonatally, and immediately following weaning have altered behavior in some instances. Whether any one of these periods or a particular combination is of most importance is not definitely known. Differences in strain, experimental design, and laboratory procedures may account for some of the variations among laboratories.

METHODS AND PROCEDURES

The primary purpose of these studies was to investigate the effects of protein restriction upon reproductive performance, growth, development, and behavior in the albino rat. The major variation among the 3 experiments was in the origin and treatment of the dams prior to mating. These differences and other minor variations in animal treatment are outlined in the detailed descriptions of each experiment. The general experimental plan consisted of 1) a pre-experimental period in which the dams were selected for and/or adjusted to the experimental diets, 2) gestation and lactation in which the dams were randomly assigned to one of 2 isocaloric diets, and 3) the postweaning period in which offspring were assigned to isocaloric diets differing only in protein content. These diets contained a) 6% casein during gestation increased to 10% casein for lactation and postweaning treatment (LP) or 24% casein for both gestation and lactation and after weaning (HP). Male offspring were subjected to behavioral testing involving a single-choice visual discrimination problem at approximately 6 months of age. Shortly after completing the testing regimen, the animals were sacrificed.

Animal Selection and Treatment

Diets

The following diets were utilized:

a. for reproduction:

24% casein pregnancy and lactation (24 CPL)

10% casein pregnancy and lactation (10 CPL)

6% casein pregnancy and lactation (6 CPL)

b. for growth and maintenance:

24% casein weanling (24 CW)

10% casein weanling (10 CW)

6% casein weanling (6 CW)

Composition of these diets is given in Table 1. Nelson and Evans (1958b) obtained optimal reproductive performance by supplementing 24% casein with 0.2% DL-methionine in diets containing all other nutrients in recommended amounts. The ratio of methionine to casein in their diet was 0.0083, and this ratio was used in all diets fed in the present experiments. The diets for each period were isocaloric in that cornstarch replaced the casein and methionine deleted from the protein-restricted diets.

All diets were calculated to meet or exceed the N.R.C. requirements (N.A.S.-N.R.C., 1962) for minerals and vitamins for growth and reproduction except for thiamin and riboflavin during the final days of gestation and through lactation. Barrett and Everson (1951) demonstrated a marked increase in the need for these vitamins during the final 2 to 3 days of gestation only. To meet this need, a liquid vitamin supplement supplying 0.1 mg thiamin and 0.05 mg riboflavin in 0.1 ml of 20% ethanol was pipetted into a separate glass container placed in the cage daily beginning on the 18th day of gestation and continuing throughout lactation. One percent NaCl and 36.1 mg additional retinol palmitate were included in the PL diets to meet the increased need for these nutrients during reproduction.

All diets were prepared in 10 kg quantities. The amount needed for a week or less was refrigerated at 4°C and the remainder stored at -20°C until needed. The vitamin mix was prepared in quantities adequate for a

Table 1. Composition of experimental diets

Component	% diet					
	24 GPL	10 GPL	6 GPL	24 CW	10 CW	6 CW
Casein, vitamin free, test ^a	24.0	10.0	6.0	24.0	10.0	6.0
DL-methionine ^a	0.2	0.083	0.05	0.2	0.083	0.05
Corn oil ^b	5.0	5.0	5.0	5.0	5.0	5.0
Hawk-Oser mineral mix ^{a,c}	3.0	3.0	3.0	3.0	3.0	3.0
CaHPO ₄ ^c	1.0	1.0	1.0	1.0	1.0	1.0
NaCl	1.0	1.0	1.0	--	--	--
Vitamin mix ^d	5.0	5.0	5.0	5.0	5.0	5.0
Nonnutritive fiber ^a	2.0	2.0	2.0	2.0	2.0	2.0
Cornstarch	58.8	72.9	76.95	59.8	73.9	77.95

^aGeneral Biochemicals Incorporated, Chargin Falls, Ohio.

^bMazola, Best Foods, Englewood Cliffs, New Jersey.

^cHawk-Oser formulation plus added CaHPO₄ with sulfates of Mn, Zn, and Cu provided in mg/kg diet: Ca, 6200; P, 4250; Na, 920; K, 4900; Mg, 500; Mn, 50; Fe, 127; I, 0.9; F, 7.0; Cu, 5.0; Zn, 0.04.

^dVitamins B and K were ground in a mortar and diluted with cornstarch. Vitamins A and E were added immediately before incorporation of the mix into the diet. The mix provided in mg/kg diet: thiamin-HCl, 1.88; riboflavin, 3.75; pyridoxine-HCl, 1.80; niacin, 22.5; Ca pantothenate, 12.0; choline Cl, 1125; vitamin B₁₂, 0.075; biotin, 0.30; folic acid, 1.5; ascorbic acid, 750; para-aminobenzoic acid, 30.0; menadione, 0.15; dl- α -tocopherol acetate powder (250 I.U./g), 360; retinol palmitate (water dispersible beadlets 0.41 I.U./ μ g), 43.3 for PL mixes and 7.2 for W mixes.

1- to 2-month supply and stored at -20°C until used. Vitamins A and E were stored at 4°C until just prior to diet incorporation.

Animal housing and routine care

Wistar rats for the experiments were obtained from the stock colony, Department of Food and Nutrition, Iowa State University.¹ All animals except for mating pairs and lactating dams were caged singly in suspended galvanized wire mesh cages in a temperature and humidity controlled laboratory. Except for the period of water deprivation during behavioral training of adult males, food and distilled water were available ad libitum. Food intake was recorded over 2-day intervals for dams during gestation and lactation and weekly for progeny during growth and maintenance. Females were weighed daily when vaginal smears were made and during gestation and lactation; their offspring were weighed daily as a group throughout the suckling period. At 1 week of age, the pups' ears were clipped for identification; then they were weighed individually each week until sacrificed at weaning or as adults following behavioral testing.

Cages and water bottles were changed weekly for all animals. Food jars were replaced on alternate days for reproducing females and weekly for weanling pups. A large number of weanling and adult males in all groups developed dermal lesions on the ventral neck area which were diagnosed as suppurative dermatitis and suppurative folliculitis.² Bacterial cultures

¹The rat colony was established in 1962 and is maintained with supplemental breeding stock from Thorpe Laboratories, White Bear Lake, Minnesota.

²Small Animal Clinic, College of Veterinary Medicine, Iowa State University.

of the skin were negative. Topical applications of 95% ethanol were administered with some success.

Reproduction

The female rat's readiness to accept copulation was determined by daily microscopic examination of a vaginal smear as described by Long and Evans (1922). When the appropriate estrous period approached, a normal, nonsibling male of similar age was introduced into the cage.¹ Presence of sperm or a vaginal plug the following morning indicated positive mating (day 0 of gestation). Dams were assigned immediately to the 24 CPL (HP) or 6 CPL (LP) diet. Casein was increased to 10% in lactation for the LP group. At parturition, pups were weighed as a group then litter size was reduced to 10 for Experiment I, litter 1 and 8 for Experiment I, litter 2 and for the remaining experiments. The maximum number of male pups was retained; females were randomly chosen to complete the litter. Any additional males and 2 to 3 females from litter 2, Experiment I and both experimental litters in Experiments II and III as available were sacrificed by decapitation. No neonates from litter 1, Experiment I were sacrificed. Live weight was recorded then carcass, brain, liver, kidney, and spleen were removed and weighed following sacrifice. The brain and other organs were wrapped individually in aluminum foil, frozen in liquid nitrogen, then stored at -20°C .

Nursing litters were weighed as a group daily and individually on days 7, 14, and at weaning on day 21. Signs of development such as furring and

¹Male rats for breeding were obtained from the stock colony, Department of Food and Nutrition, Iowa State University and were fed the Steenbock XVII stock diet described in Table A1 in the Appendix.

eye opening were noted. At weaning, male pups from all experiments and female pups from Experiment I were assigned to postweaning treatment groups. Nine surviving female weanlings from Experiment I were maintained on postweaning regimens identical to those of their male littermates then sacrificed at about 35 weeks of age. They were not subjected to water deprivation nor behavioral training. Growth and food intake for females were similar to that of males receiving the same treatment though group differences were less marked. Due to the limited sample size, the data will not be reviewed in this report. Female weanlings from Experiments II and III were weighed then sacrificed on day 21. Data for weights of carcass, brain, liver, kidney, and spleen were collected; then these tissues were frozen and stored as previously described.

After weaning or expiration of one experimental litter, dams were maintained on the lactation diet for a minimum of 1 week; then vaginal smears were begun. After the dam had mated, she was placed on the same diet she was fed during her first experimental gestation.

Behavioral Training

At approximately 6 months of age, male progeny were adjusted to a water deprivation regimen in which they were without water $23\frac{1}{2}$ hours per 24-hour period. Deprivation was continued throughout an 8- to 12-day adjustment and shaping period plus the total test period. On the 6th to 8th day of adjustment, animals were introduced to the testing device, a simple Y maze diagrammed in Figure 2. Entrance and exit from the maze were through hinged openings in the clear polyethylene cover. Sliding doors at the choice point prevented retracing once the animal entered either arm of

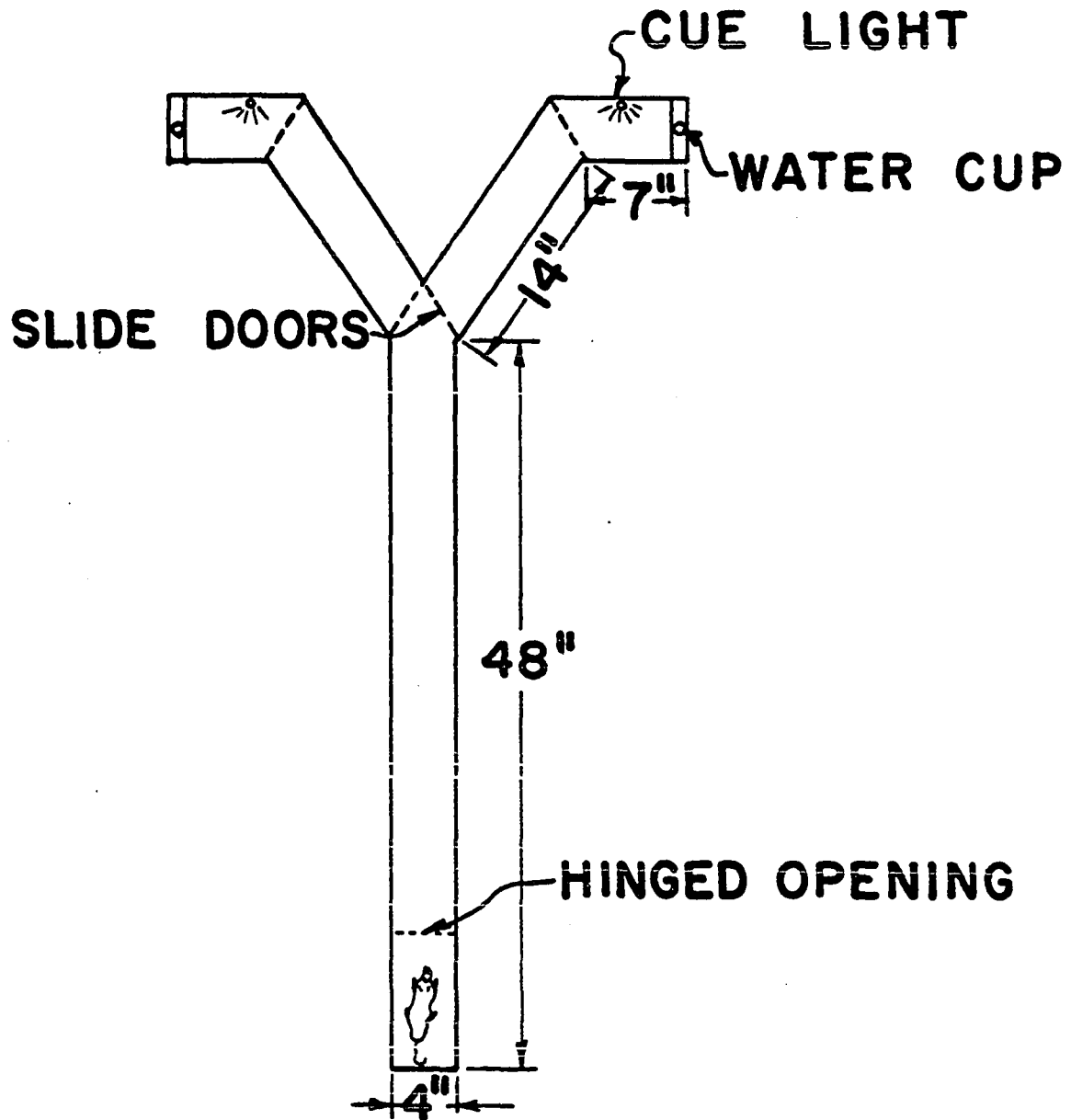


Figure 2. Diagram of Y maze used for visual discrimination training

the Y. Initially, naive subjects (Ss) were placed in the maze in groups of 4 to 5 for 5 minutes each. Next, each animal was placed singly in the goal box or in the start box and allowed to enter either goal box; he was confined there until he drank from the water cup. This procedure was repeated on the succeeding 2 to 5 days. No visual cues were presented during this period. Each animal was given free access to water for one-half hour following the shaping exercise and following the final trial during daily training.

The training procedure which was conducted in a dark room required that the S choose the lighted or dark arm of the Y maze in order to obtain a reward of distilled water. The 0.1 ml reward given to the initial Ss (Experiment I_{a,b}) was increased to 0.2 ml for the remaining studies. Rats were counterbalanced for light and dark correct choices by treatment groups. Rats from Experiment I were given 12 consecutive trials daily while 10 spaced trials were administered to rats from Experiments II and III. In these 2 experiments, Ss were assigned to training groups of 3 to 5 members which whenever possible consisted of at least 1 animal from each treatment group. Individual trials for Ss in each training group were run in sequence so that intertrial intervals for each animal were determined by the total time required for the other group members to run one trial.

The behavioral training period was divided into 3 phases. In the initial or acquisition phase, correct choices of light or dark were rewarded. In the second or reversal period, the correct choice during acquisition became the incorrect choice, i.e., if the lighted arm held the reward in acquisition, the dark arm held the reward in reversal. During the final or extinction phase, no reward was given regardless of choice; for scoring

purposes, however, the correct choice from reversal training was considered correct. The Ss in Experiment I were given acquisition training until they achieved criterion, an average of 10 correct choices out of 12 trials on 2 consecutive days; reversal training was begun the next day after a rat reached criterion in acquisition. After achieving the same criterion on the reversal problem, Ss were placed on the extinction regimen. Results from Experiment I demonstrated that about 80% of the Ss approached criterion by the 15th day of acquisition training; about the same number reached a chance level of performance in extinction training by day 10. Reversal training did not appear to yield additional or significant information in Experiment I. Therefore, for Experiments II and III acquisition was limited to 15 days for all Ss; reversal was omitted and extinction was limited to 10 days. Criterion for Experiments II and III was a minimum of 9 out of 10 trials correct on 2 successive days. Results of individual trials were recorded, and latencies, the time in seconds required from entry into the maze to completion of choice, were measured for each trial on selected days in the 3 experiments. In Experiment I, latency measurements were obtained on 1 of the first 2 days of acquisition training and on 1 of the final 2 days of acquisition and reversal training. In Experiments II and III, latencies were measured on days 1, 5, 10, and 15 in acquisition and on days 5 and 10 in extinction.

Autopsy Procedure

Rats were sacrificed by decapitation upon completion of behavioral training. Ss from Experiments I_a and I_b were held for a minimum of 30 days after training until autopsy procedures could be standardized. These rats

remained on water deprivation schedules for varying lengths of time during the holding period but were allowed free access to water for at least the final week before sacrifice in order that all animals would be in a similar state of hydration when autopsied. Rats from Experiments I_c, II, and III were given water ad libitum for 2 days following the final extinction trial then sacrificed on the 3rd day. Live weight was determined; then weights of carcass, total brain, cortex, subcortex, liver, kidney, spleen, and perirenal and epididymal fat deposits were recorded following autopsy. Organs were stored in the manner described previously. The perirenal and epididymal fat deposits were excised as an estimate of the animal's energy stores. Separation of the cortical and subcortical sections of the brain was made by the method of Rosenzweig et al. (1962), Figure 3.

Acetylcholinesterase Determination

Acetylcholinesterase (AChE) activity was determined in brains of newborn and weanling females and adult males according to the colorimetric method of Ellman et al. (1961). AChE activity was measured by monitoring the rate of formation of the yellow color produced by thiocholine, the AChE hydrolysis product of acetylthiocholine (ASCh) and dithiobis (2-nitrobenzoic) acid (DTNB). The assay is not specific for AChE nor for various pseudocholinesterases, however; therefore, results were expressed as ChE activity.

Tissues were homogenized in 0.1 M potassium phosphate buffer (pH 8.0) in a glass tissue grinder with a teflon pestle. The homogenate was placed

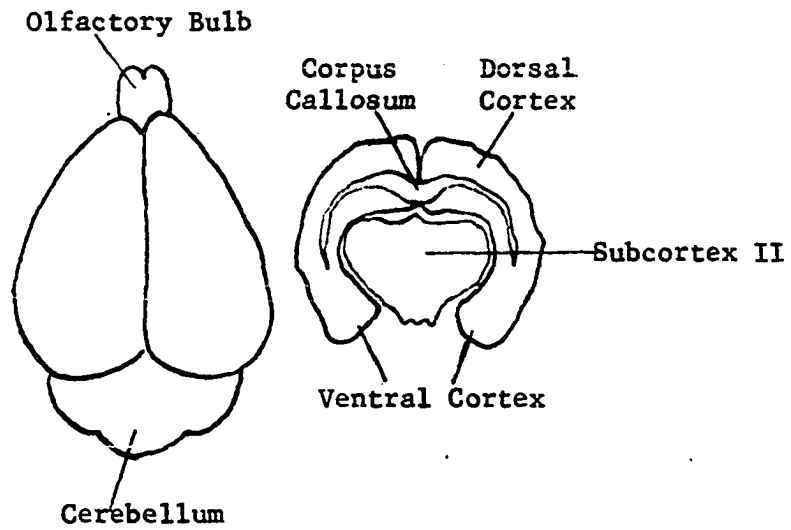


Figure 3. Diagrams of the dorsal and coronal views of the rat brain

Total cortex consists of the dorsal cortex plus the ventral cortex. Subcortex includes subcortex II plus the cerebellum and the olfactory bulbs (Rosenzweig et al., 1962).

in an appropriately sized volumetric flask, the tissue grinder rinsed, and the homogenized mixture diluted to volume with phosphate buffer. Homogenates were diluted to give a standard concentration range for each type of tissue. Dilution ratios were dependent on expected specific activity and total weight of the various tissues. Concentration ranges for the tissues were:

	mg tissue/ml homogenate
Newborns (total brain)	7.5-11.5
Weanlings (total brain)	4.8- 6.5
Adults (cortex or subcortex)	4.3- 6.2

A 0.4 ml aliquot of the homogenate was added to a standard 5 cm spectrophotometer cell containing 2.6 ml 0.1 M phosphate buffer (pH 8.0). After adding 0.1 ml 0.1 M DTNB,¹ the solution was allowed to equilibrate for 5 minutes, then 0.02 ml of 0.075 M ASCh¹ was added to the cuvette. The contents were mixed to insure homogeneity of the reaction mixture and the cuvette placed in a Gilford Spectrophotometer 240.² Absorbance was read at 412 m μ at 1 minute intervals for at least 10 minutes. Change in absorbance per minute was calculated over the final 5 minutes during which variation from linearity was negligible. Two blanks, one containing 2.6 ml phosphate buffer, 0.1 ml DTNB, and 0.02 ml ASCh and the other containing 2.6 ml phosphate buffer, 0.4 ml homogenate, and 0.1 ml DTNB, were read simultaneously with duplicate analyses of each homogenate.

¹Sigma Chemical Company, St. Louis, Missouri.

²Gilford Instrument Laboratories Inc., Oberlin, Ohio.

The brain tissue analyzed in these studies had been stored at -20°C for 18 to 24 months.¹ To test the stability of AChE in these stored samples, 4 newborn stock pups were sacrificed, and brains from these animals were analyzed simultaneously with 4 stock samples which had been stored approximately 24 months. Average specific activity for the stored samples was 1.57 moles ASCh hydrolyzed per minute $\times 10^6$ compared with an average of 1.51 for the fresh samples. Therefore, it was concluded that the enzyme was stable over extended storage.

Statistical Design and Analyses

The dietary treatments were applied to dams individually then to their pups in 2 ways. First, pups were influenced by the diet fed their mother during gestation and lactation. Second, the diets were fed directly to individual pups after weaning. Thus the treatments defined two distinct experimental units: 1) a dam or a whole litter which received through its dam one of the 2 dietary treatments during gestation and lactation and 2) a group of pups within a litter which received one of the 2 postweaning treatments. Such an experimental design is called a split plot since there exist whole plots, dams or litters, to which one set of treatments was applied then subplots or split plots, parts of litters, to which another set of treatments was applied.

Each dam was bred to produce 2 litters. Due to the poor survival rate among pups from the LP treatment, it was randomly assigned to more dams than the HP treatment. Differences in litter size at birth, preferential

¹Samples were partially thawed one time during this period due to freezer malfunction.

retention of male pups when limiting litter size for lactation, and different survival rates among litters resulted in varying numbers of pups being measured at birth, weaning, and after weaning. In Experiment I, all pups in a litter were assigned to the same postweaning treatment. In Experiments II and III, the male pups in each litter were randomly assigned to 1 of the 2 postweaning treatments when removed from the lactating dam.

Table 2 is a sketch of the sources of variation, degrees of freedom, and nature of the F tests used in the analyses of variance for measurements on pups subjected to treatment both before and after weaning. A separate analysis on the data from each of the litters was performed routinely. For adult progeny, the analysis for individual litters consisted of lines 1, 2, 6, 7, and 8 of Table 2 plus a term for animals treated alike. For those measurements made on dams, newborns, and weanling pups, only the gestation and lactation diets were relevant. In these analyses, the first 5 lines of the ANOV outline plus a term for animals treated alike were used. For dams, newborns, and weanlings to which the postweaning diet did not apply, a simple nested design was used for the single litter analysis. The nested analysis was used also for adults from Experiments I_{a,b,c} where comparisons were made on single litters and between only 2 groups treated differently before or before and after weaning.

The nature of the experiments was such that a balanced design was not expected to be achieved, and indeed many missing values occurred due to failure in reproduction, unbalanced sex ratios, etc. beyond the control of the experimenter. The analyses were carried out by using the method of least squares on the unbalanced data in a complete regression approach rather than using the usual calculating formulas for the analysis of vari-

Table 2. Outline for analysis of variance

Sources of variation ^{a,b}	d.f. ^c	F test
1. Diet before weaning	1	
2. Dams within diet before weaning	error (a) n-2	
3. Litter	1	
4. Diet before weaning by litter	1	
5. Litter by dams within diet before weaning	error (b) n-2	
6. Diet after weaning	1	
7. Diet before weaning by diet after weaning	1	
8. Dams within diet before weaning by diet after weaning	error (c) n-2	
9. Diet after weaning by litter	1	
10. Diet before weaning by diet after weaning by litter	1	
11. Residual ^d	error (d) n-2	
12. Among pups treated alike	4n	

^aFor traits when diet after weaning was not applicable, such as measures on the newborn, only the first 5 lines of the analysis plus a term for animals treated alike were used.

^bFor analysis of a single litter when the diet after weaning did apply, lines 1, 2, 6, 7, and 8 plus a term for animals treated alike were used.

^cd.f. given as if a balanced experiment was achieved with $n/2$ dams on each preweaning (reproduction) diet producing 2 litters each with 4 pups from each litter being assigned to each diet after weaning. n = number of dams.

^dResidual = dams within diet before weaning by diet after weaning by litter.

ance. Values of F where $P < 0.05$ were considered significant, and those in which $0.05 < P < 0.10$ were considered to have approached significance.

Experimental Plans

Experiment I

Experiment I examined the effects of 2 treatments: 1) a transitory increase in protein supply during days 15, 16, and 17 of gestation and 2) postpartum hormone injections on the reproductive performance and consequent survival of progeny of dams fed a low protein ration during gestation and lactation. Zeman (1967), Chou (1970), and Barton (1973) have shown that rats fed a low protein diet in gestation demonstrate a dramatic decrease in food intake beginning about day 16 to 18 of gestation. Decreased food intake for controls is seen later. In an attempt to delay the onset of decreased food intake among dams fed 6% casein, 10% casein was fed to one group (LP_M) for days 15, 16, and 17 of gestation. Prolactin and cortisone in combination were superior to other hormones in supporting lactation in rats hypophysectomized on day 12 of pregnancy according to Lyons et al. (1958). In the present experiment, injections of 0.55 I.U. prolactin¹ in 0.2 ml of 0.9% saline and 0.2 mg hydrocortisone¹ in 0.1 ml solution of 0.9% saline² were given to one group (LP_H) on days 0, 1, and 2 of lactation for litter 1 and when indicated by lactation failure (pups not fed) for litter 2.

¹Sigma Chemical Company, St. Louis, Missouri.

²Two drops Tween 80 and 0.09 ml butyl alcohol mixed with 10 ml 0.9% saline and 20 mg. hydrocortisone.

The general experimental plan for Experiment I is shown in Figure 4a. Virgin Wistar females of approximately 80 days of age from the stock colony were mated and randomly assigned to one of the 4 experimental groups, 1) HP (24 CPL), 2) LP (6 CPL), 3) LP_M (6 CPL with transitory 10 CPL on days 15, 16, 17 of gestation), and 4) LP_H (6 CPL with postpartum injections of prolactin and hydrocortisone). All LP rations given during lactation contained 10% casein. Procedures for reproduction, routine care, behavioral training, and autopsy have been described previously. Survival among all groups was poor. From groups of 6 dams, only 1 litter each was weaned from gestation 1 for HP,¹ LP, and LP_M treatments, none from LP_H. From gestation 2, 3 litters were weaned by HP dams, 1 litter each by LP and LP_H females, and none by LP_M rats. Due to the small numbers, the pups from the various LP groups were pooled for postweaning treatment. Pups from litter 1 were assigned to a casein level identical to that of the lactation diet of their mothers. Thus the 2 groups generated for postweaning measurement in Experiment I_a were HP/HP and LP/LP.

Two additional groups were added to the adult treatment design. A group of 7 males whose mothers had received a low protein diet (approximately 4%) in gestation and marginally adequate protein diet (15%) in lactation were obtained from the study conducted by Barton (1973). These pups (4 15_g¹/15) were fed 15% protein after weaning; a detailed description of the diets may be found in the Appendix, Table A2. Six males of similar age

¹One additional female whose pregnancy was not detected until she was well into gestation weaned 1 litter while being fed Steenbock XV ration described in Table A1 in the Appendix. Her pups were pooled with HP pups from litter 1 for postweaning treatment.

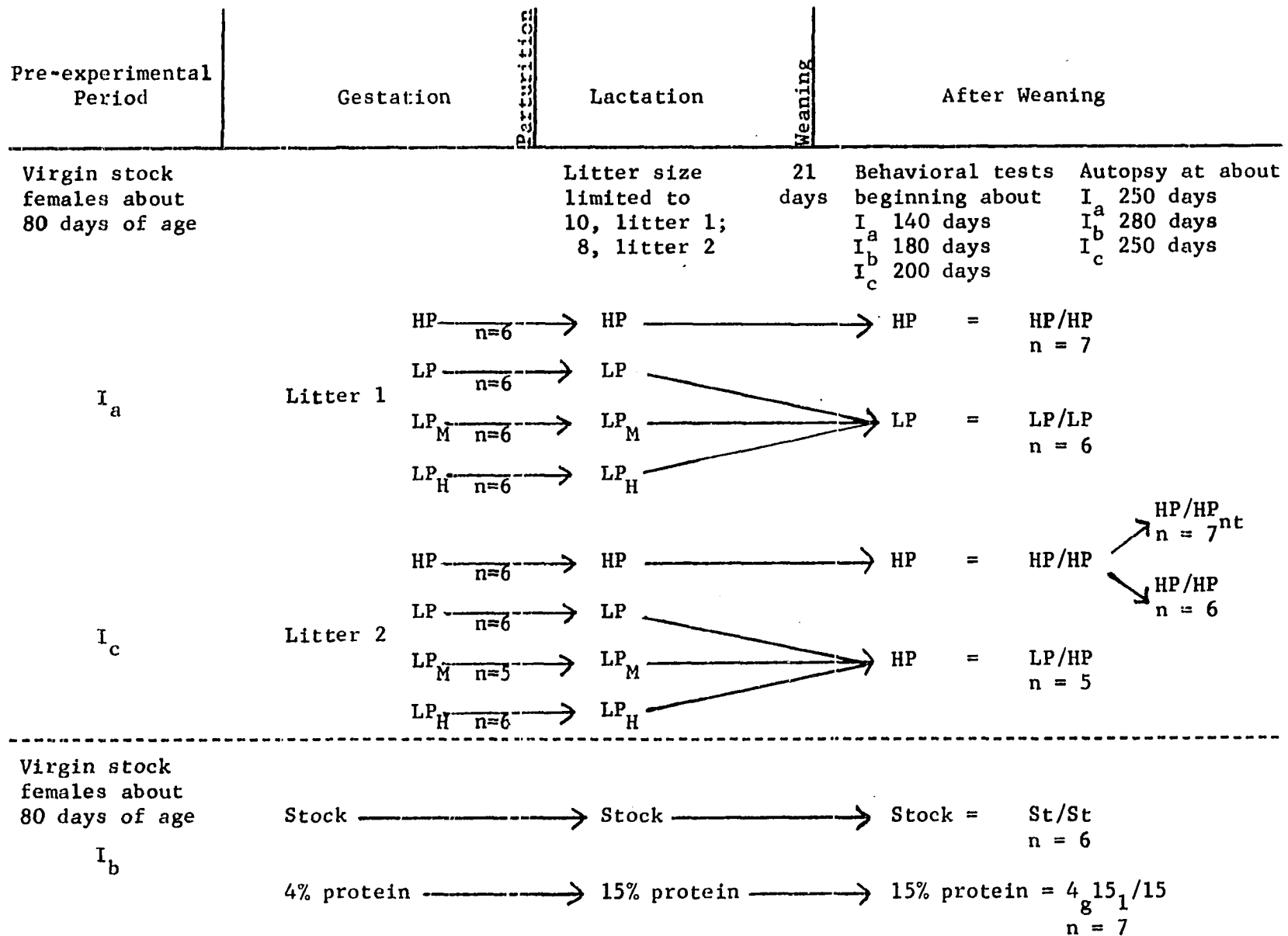


Figure 4a. Experimental plan for Experiment I

were obtained from the stock colony (St/St) to serve as controls for the $4\frac{15}{g}1/15$ group. The stock animals were produced by dams consuming the Steenbock XV ration described in Table A1 in the Appendix. After weaning, they were fed the Steenbock XVII diet also described in Table A1. These 2 groups, St/St and $4\frac{15}{g}1/15$, were compared in Experiment I_b.

All offspring in second litters from Experiment I were assigned to 24 CW at weaning (Experiment I_c). Two groups were thus formed, HP/HP (n = 13) and LP/HP (n = 5). Because Rosenweig et al. (1962) indicated that environmental enrichment, i.e., maze training, altered acetylcholinesterase levels in the brains of their Ss, littermates from the HP/HP group were assigned randomly to a training (HP/HP) or a nontraining group (HP/HP_{nt}). The treatment of these groups was identical in every way including water deprivation except that the HP/HP_{nt} group was never introduced to the maze. The HP/HP group was compared with both the HP/HP_{nt} and LP/HP groups when appropriate.

Experiment II

Because of poor lactation performance among both control and experimental animals in Experiment I, Experiment II was designed to increase pup survival by studying the effects of the protein restriction on dams who had demonstrated the ability to lactate on stock ration. Animals which had successfully weaned their first litter (at least 4 pups) while being fed stock ration (Steenbock XV, Table A1) were mated after a minimum interval of 8 days.¹ The general experimental plan is shown in Figure 4b. Each dam

¹Two females weaned no young from their first litter.

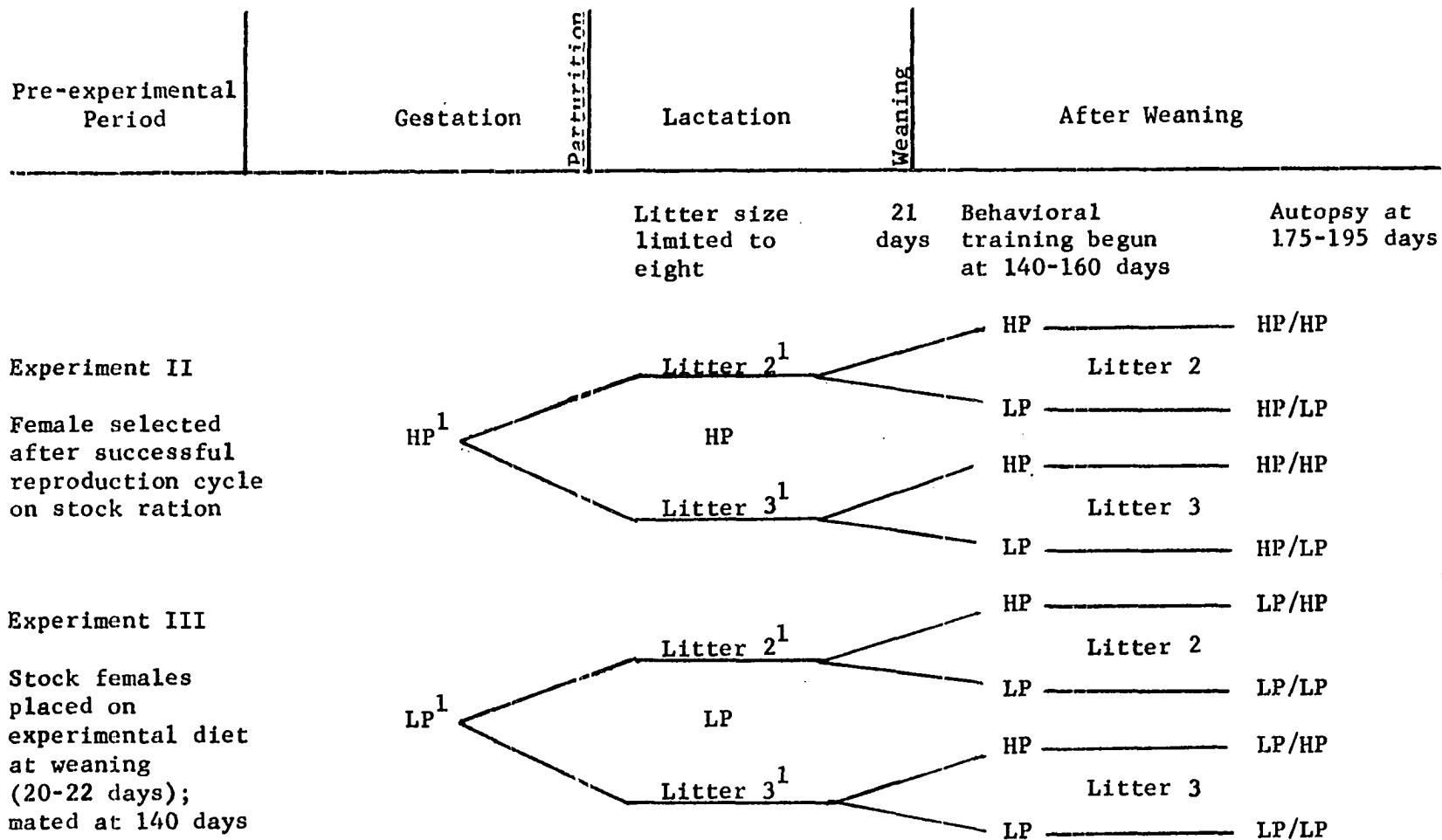


Figure 4b. Experimental plan for Experiments II and III

¹Group designations for Experiment III are HP_p and LP_p; litters are litters 1 and 2

was bred for 2 experimental litters. Male progeny were assigned to HP and LP rations at random upon weaning thus generating 4 groups, HP/HP, HP/LP, LP/HP, and LP/LP for postweaning measurements. Rats in the HP/HP and HP/LP groups were littermates as were LP/HP and LP/LP animals.

Experiment III

Khanam (1965) reported weaning survival rates of 40% or more for pups from females fed a 5% protein ration for 3 to 6 months prior to mating and during gestation and lactation. Such rates were remarkably better than the 5 to 8% survival to weaning observed in Experiment I and rates of less than 25% observed by other investigators (Zeman, 1967; Barton, 1973; Turner, 1973). Presumably long adjustment to the low protein ration resulted in altered feeding and/or metabolic mechanisms which enabled more of Khanam's females to withstand the stress of reproduction while consuming the restricted protein diet. Experiment III was designed to test this hypothesis. Stock females (20 to 22 days of age) were placed on the 6 CW and 24 CW rations at weaning. These rats were designated LP_p and HP_p , respectively. At approximately 140 days of age when the LP_p animals had attained a minimum weight of about 250 g, the rats were bred for their initial litter. Procedures for treatment of the females and their progeny through 2 reproduction cycles and the assignment of male progeny to postweaning treatments were identical to those employed in Experiment II (Figure 4b). The 4 groups of adult male offspring on which food intake and utilization, morphological, and behavioral measurements were made were HP_p/HP , HP_p/LP , LP_p/HP , and LP_p/LP .

RESULTS

Results will be presented in relation to the effects of experimental treatments on 1) reproductive performance including postnatal growth and organ development, 2) postweaning growth, development, and metabolic efficiency, and 3) brain development and behavior. The last section will include the correlation of brain weight and acetylcholinesterase activity with performance on a visual discrimination problem.

Data for pregnant and lactating females, neonates, and weanling pups were subjected to regression analysis to test the effect of diet (HP vs LP) during gestation and lactation and the effect of parity on reproductive performance. The effects of the diets during gestation and lactation and diets fed after weaning as well as the effect of parity were compared for adult progeny. Rats fed 24% casein in gestation and lactation and their offspring fed that ration after weaning were considered to be adequately nourished. Females or progeny fed 6 or 10% casein at any time were evaluated for the effects of protein restriction by comparing their performances with those of adequately nourished animals. Values of F for which $P < 0.05$ were considered significant; those for which $0.05 < P < 0.10$ were considered to have approached significance.

Reproductive Performance

Average ages and weights at mating for females in Experiment I are listed in Table 3. Similar data for Experiments II and III may be found in Table 4. When initially mated, neither weight nor age differed significantly among groups in Experiments I or II. Ages were similar for all females in Experiment III, but the mean weight at 20 weeks of the group

Table 3. Mean maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiment I

Experi- mental group	Age at mating (days)	Wt. at mating (g)	Total food intake			Net wt. change		Length of gestation (days)
			Gestation (g)	Lactation (g)	Lactation/No. pups weaned (g)	Gestation ¹ (g)	Lactation ² (g)	
HP ³			375 ^{a*} (12) ⁴	633 ^a (4)	85 ^a (4)	57 ^a (12)	-32 ^a (4)	21.5 ^a (12)
Litter 1	81(6)	237(6)	387 (6)	575 (1)	72 (1)	63 (6)	-37 (1)	21.5 (6)
Litter 2	126(6)	308(6)	362 (6)	652 (3)	89 (3)	51 (6)	-31 (3)	21.4 (6)
LP ³			392 ^a (12)	534 ^a (2)	134 ^b (2)	19 ^b (12)	-1 ^b (2)	21.7 ^a (12)
Litter 1	78(6)	247(6)	412 (6)	567 (1)	142 (1)	21 (6)	5 (1)	21.5 (6)
Litter 2	124(6)	292(6)	371 (6)	502 (1)	125 (1)	18 (6)	-7 (1)	21.8 (6)
LP _M ³			395 ^a (11)	544 [#] (1)	109 [#] (1)	18 ^b (10)	-18 [#] (1)	21.6 ^a 10
Litter 1	78(6)	255(6)	431 (6)	544 (1)	109 (1)	22 (5)	-18 (1)	21.5 (5)
Litter 2	121(5)	313(5)	351 (5)	-- ₅	-- ₅	14 (5)	-- ₅	21.8 (5)
LP _H ³			397 ^a (12)	406 [#] (1)	101 [#] (1)	21 ^b (12)	-15 [#] (1)	21.5 ^a 12
Litter 1	80(6)	265(6)	427 (6)	-- ₅	-- ₅	28 (6)	-- ₅	21.6 (6)
Litter 2	129(6)	312(6)	366 (6)	406 (1)	101 (1)	14 (6)	-15 (1)	21.5 (6)

¹Net wt. change mating to postpartum.

²Net wt. change postpartum to pups' weaning.

³Arithmetic mean for litters 1 and 2.

⁴No. of observations.

⁵No group member completed lactation.

[#]Insufficient number for statistical analyses.

*Means with the same superscripts are not different (P>0.05).

Table 4. Mean maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiments II and III

Experiment	Group	Age at mating (days)	Wt. at mating (g)	Total food intake			Net wt. change		Length of gestation (days)
				Gestation (g)	Lactation (g)	No. pups weaned (g)	Gestation ^a (g)	Lactation ^b (g)	
II	HP ^c			403(20) ^d	669(15)	106(15)	60(19)	-37(15)	21.7(19)
	Litter 2	149(1.0)	328(10)	413(10)	689(10)	98(10)	58(10)	-40(10)	21.6(10)
	Litter 3	217(1.0)	347(10)	393(10)	627 (5)	123 (5)	62 (9)	-32 (5)	21.8 (9)
	LP ^c			396(39)	528(19)	103(19)	12(36)	-45(19)	21.7(36)
	Litter 2	148(20)	330(20)	410(20)	535(13)	109(13)	10(20)	-40(13)	21.5(20)
	Litter 3	212(1.9)	336(19)	381(19)	513 (6)	90 (6)	14(16)	-56 (6)	21.9(16)
Statistical evaluation									
Diet				NS ^e	-- **	NS	-- **	NS *	NS **
Litter				-- **	-- **	NS	NS	-- *	-- **
Interaction				NS	NS	NS	NS	NS	NS

^aNet wt. change mating to postpartum.

^bNet wt. change postpartum to pups' weaning.

^cArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^dNo. of observations.

^eNS = not significant at least at 0.10 level.

*P<0.05.

**P<0.01.

Table 4. (Continued)

Experiment	Group	Age at mating (days)	Wt. at mating (g)	Total food intake			Net wt. change		Length of gestation (days)
				Gestation (g)	Lactation (g)	Lactation/No. pups weaned (g)	Gestation ^a (g)	Lactation ^b (g)	
III	HP _p ^c			382(15)	611 (9)	108 (9)	64(12)	-34 (9)	21.5(12)
	Litter 1	152 (8)	315 (8)	386 (8)	618 (5)	109 (5)	62 (7)	-37 (5)	21.6 (7)
	Litter 2	212 (7)	333 (7)	376 (7)	604 (4)	106 (4)	68 (5)	-30 (4)	21.5 (5)
	LP _p ^c			318(23)	423 (5)	151 (5)	14(23)	-5 (5)	21.6(23)
	Litter 1	157(12)	247(12)	309(12)	400 (3)	210 (3)	16(12)	7 (3)	21.6(12)
	Litter 2	207(11)	282(11)	327(11)	458 (2)	62 (2)	12(11)	-22 (2)	21.6(11)
Statistical evaluation									
Diet				--**	--*	NS	--**	NS	NS
Litter				NS	NS*	NS	NS	NS	NS
Interaction				NS	--	NS	NS	NS	NS

which has been fed 6% casein since weaning was 68 g below that of the group reared on 24% casein. Females in Experiment I were about 80 days of age when first mated. Dams in Experiment II had reared one litter successfully in the stock colony before they were assigned to this study, and those in Experiment III were not mated until the LP_p group weighed about 250 g. Therefore, females in both of these experiments were about 150 days old when their first experimental litter was conceived.

Maternal adjustments

Food intake Young females fed the low protein diet in Experiment I tended to eat more food than controls during their first pregnancy (Table 3). Dams given 10% casein on days 15, 16, and 17 of gestation consumed an average of 431 g during their first pregnancy compared with 387 g for those fed 24% casein throughout gestation. Groups fed 6% casein throughout pregnancy ate intermediate amounts. Total gestational food intake among experimental groups during the first pregnancy was not significantly different, however. During the second gestation, all groups consumed significantly less total food than during the first pregnancy, but no significant differences occurred among treatment groups.

In Experiment II, animals were older because they had been permitted to rear one litter on stock colony ration (Steenbock XV, Table A1) which supplied about 24% protein from natural sources before being placed on the experimental diets. Their total food intakes during gestation were similar regardless of dietary treatment during their second and during their third pregnancies (Table 4). However, all animals consumed significantly less total food during their third pregnancy than during their second (413 vs

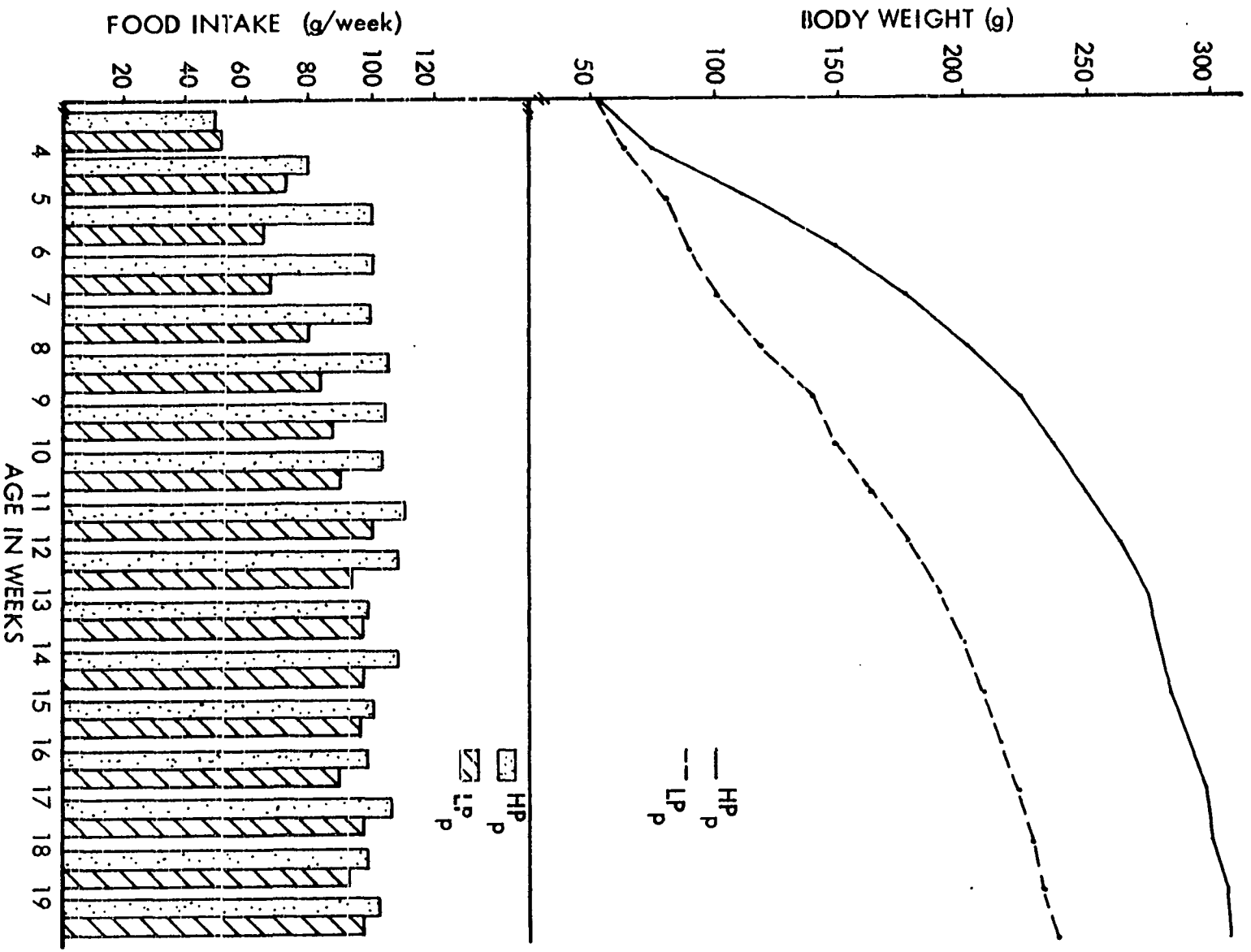
393 g for HP gestations 2 and 3, respectively, and 410 vs 381 g for LP pregnancies).

Young females fed 6% casein in Experiment III ate an average of 51 g of food during the first week after weaning while those fed 24% casein ate 49 g on the average (Figure 5). From week 5 through week 20, the absolute food intake for the HP_p group was consistently higher than that of protein-restricted females (LP_p). Relative to body weight, the weekly intakes of nonpregnant females were similar for both groups, however.

After mating, HP_p females in Experiment III ate an average of 382 g of food per pregnancy, an amount which was significantly more than the 318 g eaten by LP_p dams (Table 4). Compared on the basis of body weight at conception, total intake was approximately 125 g/100 g body weight for all dams in gestation 1 and about 116 and 121 g/100 g body weight for LP_p and HP_p rats, respectively, during gestation 2. From these observations, it was concluded that total food intake during pregnancy did not differ with parity for rats in Experiment III as it had for those under the experimental conditions imposed in Experiments I and II.

When mean food intake per 2-day period was plotted against day of gestation, patterns of food intake were distinctly different for females fed 6 and 24% casein in each of the 3 experiments (Figures 6 and 7). Intakes of protein-restricted dams were higher than those of adequately fed pregnant females through day 15. After day 17, the intakes of both groups decreased, but the decrease for protein-restricted females was more marked than that for adequately fed animals. By days 20-21, protein-restricted animals were eating about 50% as much food as they had eaten on days 0-1; adequately fed

Figure 5. Mean food intake and body weight of female rats in Experiment III from 3 to 20 weeks of age



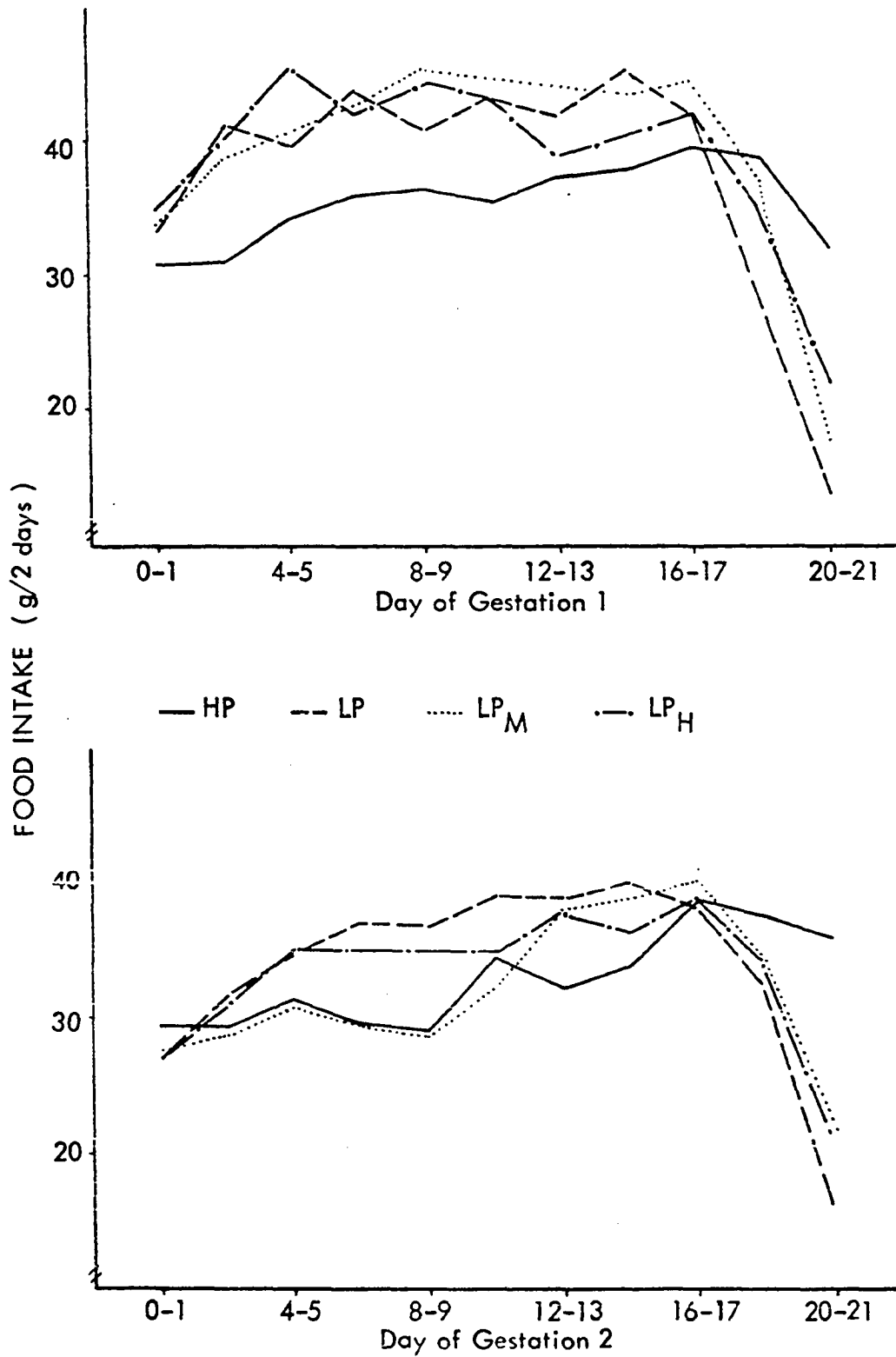


Figure 6. Mean food intake of pregnant rats in Experiment I

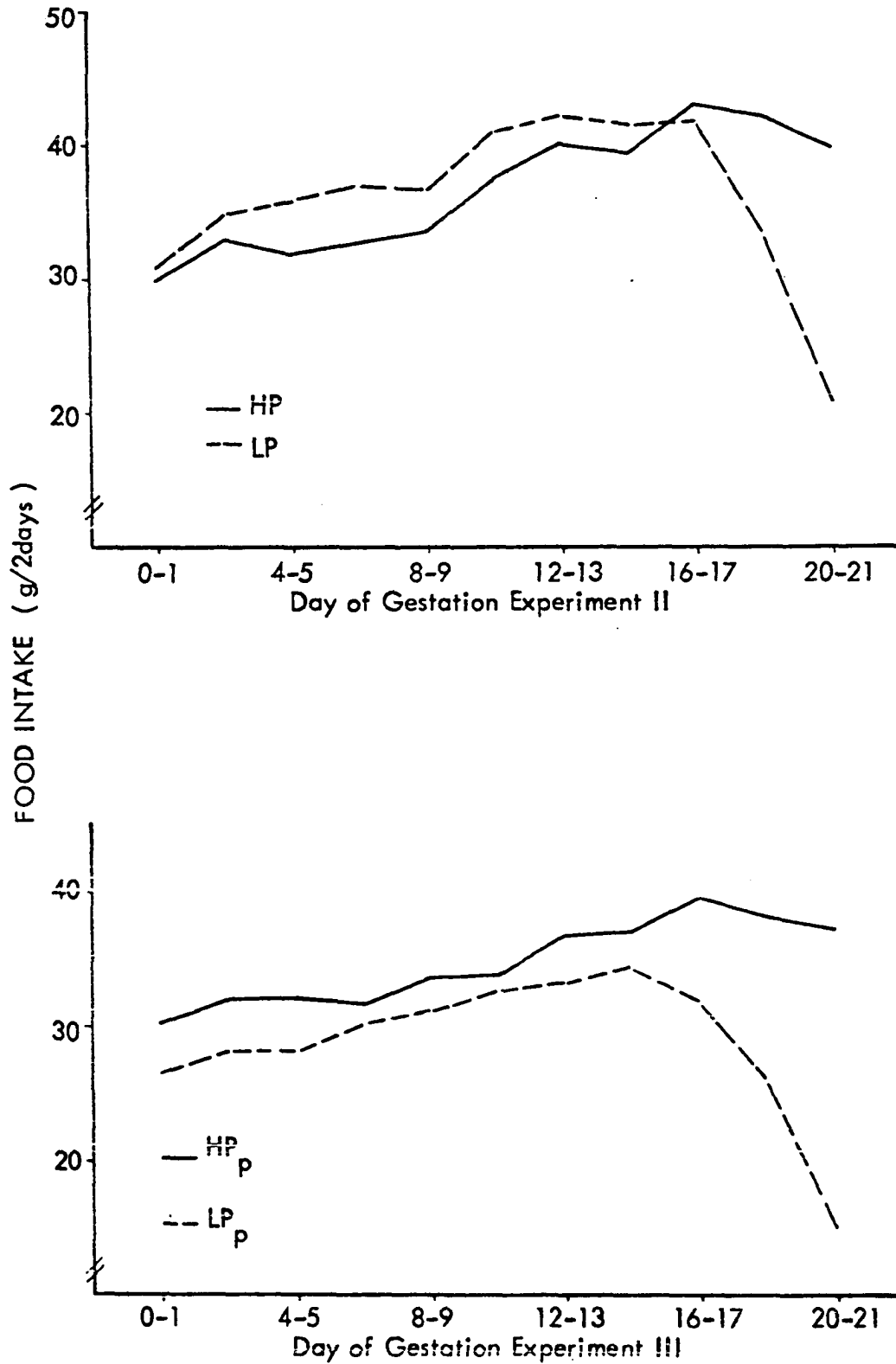


Figure 7. Mean food intake of pregnant rats in Experiments II and III

dams ate about the same amount of food on days 20-21 as they had during the first 2 days of gestation.

Food intakes prior to day 15 among the 3 protein-restricted groups in Experiment I were not significantly different (Figure 6). Transitory feeding of 10% casein on days 15, 16, and 17 did not alter the eating pattern on these days or later.

Gestational eating pattern did not differ significantly between gestations 2 and 3 in Experiment II; therefore, food intakes throughout pregnancy for both gestation periods were combined in Figure 7. Since total gestational food intakes for adequately nourished and protein-restricted pregnant females in Experiment II were similar, variations in the pattern or distribution of food consumption in this study as well as in Experiments I and III were not attributed to the influence of protein restriction only but to the influence of time (day of gestation) and the interaction of protein restriction and time. During early pregnancy, protein-restricted animals consumed more food than adequately nourished rats; however, during the last third of gestation, protein-restricted rats ate markedly less than adequately nourished animals.

Patterns and amounts of food eaten during gestation did not differ between gestations 1 and 2 in Experiment III; consequently, data for both pregnancies are combined in Figure 7. Females fed a 6% casein diet from weaning (LP_p) ate less during pregnancy than those fed 24% casein (HP_p). Relative to body weight, LP_p intakes were 1 or 2 g larger per 2-day period through day 15 and significantly decreased during the last third of pregnancy as compared with those of the HP_p treatment, however.

Previous studies in this and other laboratories (Kenney, 1969; Wang et al., 1966; Zeman, 1967) have shown that the mortality rates for pups suckled by dams fed 6% casein or less were near 100%. Therefore, dietary casein was increased to 10% during lactation in the present studies. One group in Experiment I, LP_H, was also given injections of hydrocortisone and prolactin in an attempt to enhance lactation.

Observations of food intake during lactation were limited in Experiment I by the small number of litters successfully weaned. Young from only 4 of 17 litters cast by protein-restricted dams and 4 of 12 litters produced by adequately nourished dams were weaned. One dam each from the HP, LP, and LP_M groups weaned a first litter; 3 HP, 1 LP, and 1 LP_H females weaned second litters. Because only 1 litter survived to weaning in the LP_M group and in the LP_H group, statistical comparisons involving lactation performance (food intake and weight change during lactation) were limited to data from HP and LP groups in Experiment I. Adequately nourished females (HP) ate an average of 633 g of food during each lactation period while the range for the protein-restricted groups was 406 to 544 g. Seven or 8 pups were weaned from HP litters; therefore, when food intake was calculated on the basis of number of pups weaned, HP dams averaged 85 g per pup which was significantly less than the 134 g per pup averaged by LP animals who weaned only 4 or 5 pups per litter.

Dams fed 24% casein in Experiment II ate an average of 669 g of food during lactation which was significantly more than the average of 528 g consumed by protein-restricted animals while lactating. Dams from both HP and LP groups ate significantly more food while suckling litter 2 than while nursing litter 3. Differences in total lactation intake were influ-

enced more by number of young nursed than by diet or parity, however, because food intake per pup weaned did not differ significantly between HP and LP dams or between dams while they nursed litter 2 and litter 3 in Experiment II.

Food intake per 2-day period during lactation for Experiments II and III is presented in Figure 8. Because food intake measurements during the final days of lactation are confounded with consumption of the food supply by the maturing pups, results were graphed through day 19 only. Analysis of the pattern of food intake in Experiment II revealed that both adequately nourished and protein-restricted females ate significantly more food during the latter part of the lactation period than they had at the beginning; the increase in food eaten as lactation progressed was significantly larger for HP females than for LP females, however. HP animals had increased their intakes by about 150% on day 18-19 while LP animals had increased theirs by only about 100%.

Adequately fed females in Experiment III ate an average of 611 g food during lactation which was significantly more than the average of 423 g eaten by protein-restricted dams (Table 4). Average food intake per pup weaned was 109 g for HP_p rats compared with 210 g for the LP_p group. Two of the 3 litters weaned by LP_p dams consisted of 1 or 2 pups, however. Since the dam's basic need for food in addition to the demands of lactation are reflected in the calculation of food eaten per pup weaned, averages for such small litters cannot be compared readily with those for litters containing 4 or more pups. The two LP_p litters weaned following the second lactation period consisted of 7 and 8 pups, and average food intake per pup weaned for their mothers was 62 g compared with 106 g for HP_p animals.

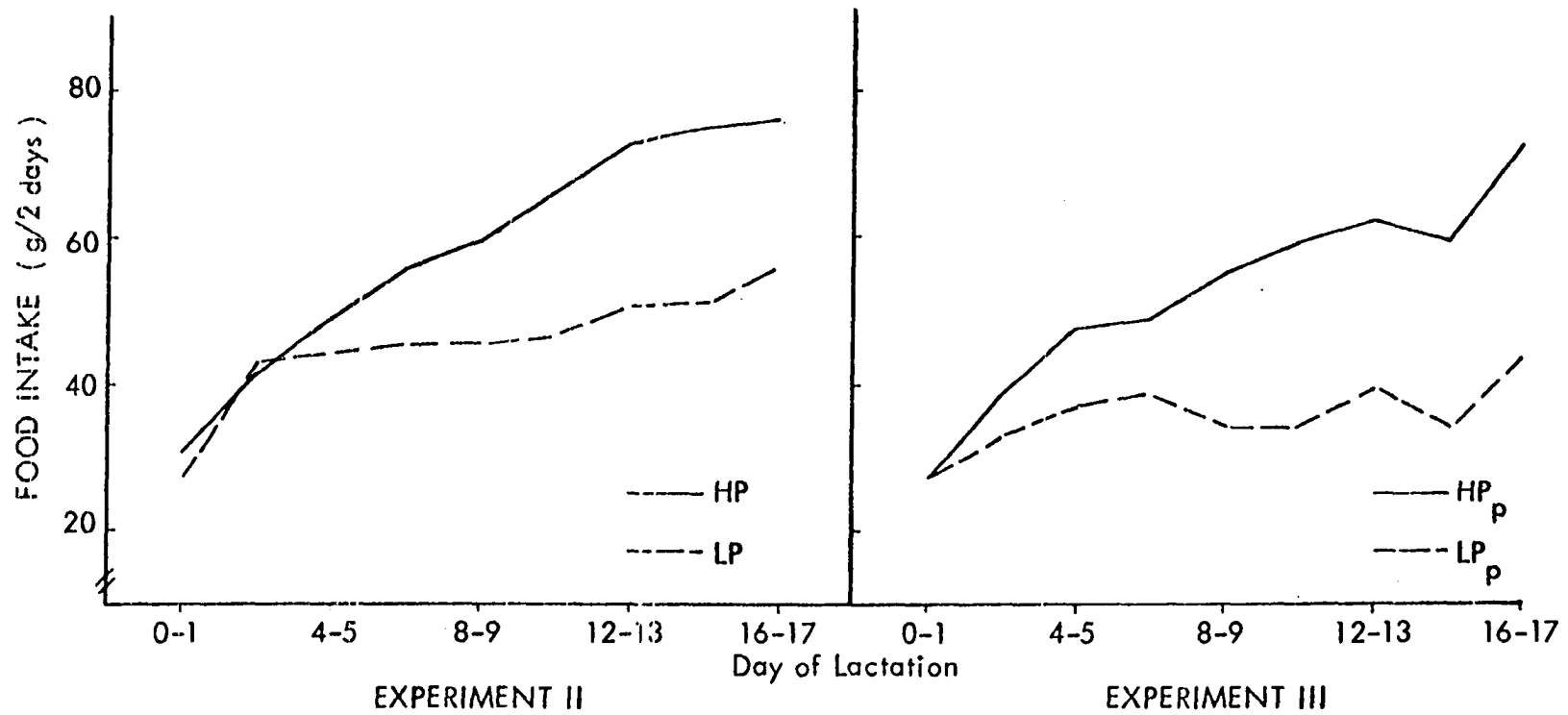


Figure 8. Mean food intake of lactating dams in Experiments II and III

Food intake measured over 2-day periods in lactation in Experiment III revealed a pattern similar to that observed in Experiment II. Adequately nourished and protein-restricted rats ate more food as lactation progressed, but the increase for HP_p animals was much larger than that for LP_p rats.

Weight change Net weight change in gestation was determined by subtracting the weight at mating from the postpartum weight. Net weight gain for rats fed 24% casein during gestation was 2 to 5 times that for protein-restricted animals in the present studies (Tables 3 and 4). Average gestational net gain for adequately nourished females in Experiment I was 57 g; net gains for protein-restricted animals were significantly smaller ranging from 18 to 21 g (Table 3). Average net weight gain during gestation for the LP_M group was 18 g while that for LP animals was 19 g; therefore, feeding 10% casein on days 15, 16, and 17 did not alter weight change.

Adequately nourished animals in Experiment II (HP) were 60 g heavier on the average following parturition than they had been at mating (Table 4). Average net gain for protein-restricted rats was 12 g, a significantly smaller gain.

Growth of females in Experiment III from 3 to 20 weeks is shown in Figure 5. The inadequate protein supply in the 6% casein diet resulted in severely inhibited growth from 3 to 9 weeks of age. Weanling females fed a diet low in protein averaged a gain of 89 g during this period compared with 170 g for the adequately fed animals. From 9 to 20 weeks, growth for the 2 groups was similar. LP_p females gained an average of 97 g compared with 86 g for the HP_p group. Net weight gain during pregnancy for HP_p rats in Experiment III was significantly larger (64 g) than for LP_p rats (14 g; Table 4).

Most lactating females suffered a net weight loss between parturition and the time they weaned their pups (Tables 3 and 4). Dams fed 24% casein in Experiment I lost an average of 32 g during lactation; losses for those fed 10% casein were 1, 18, and 15 g, respectively, for LP, LP_M, and LP_H groups. Well nourished rats weaned litters of 7 and 8 pups compared with 4 and 5 pups for protein-restricted females; thus the larger weight loss of the HP dams was probably related to the larger number of pups fed.

Adequately fed and protein-restricted rats both lost 40 g on the average during their first experimental lactation period in Experiment II (Table 4). However, rats fed 10% casein lost more weight during the second experimental lactation (56 g) than those fed 24% casein (32 g). The weight loss between lactations 2 and 3 differed significantly. Number of pups weaned per litter in Experiment II also varied with parity. Adequately nourished rats weaned an average of 7.1 pups from litter 2 and 5.4 from litter 3 while LP dams weaned an average of 5.4 and 6.2 pups from litters 2 and 3, respectively. Therefore, in Experiment II as well as in Experiment I, number of pups weaned exerted an important influence on weight change during lactation.

Dams fed 24% casein lost an average of 34 g during 2 lactation periods in Experiment III while those fed 10% casein averaged a loss of 5 g (Table 4). Protein-restricted females (LP_p) gained 7 g on the average during their first lactation but lost 22 g on the average during their second. The number of young per litter was 1, 2, and 5 for the first lactation but was 7 or 8 for the second. Average lactational weight loss for HP_p dams was 37 g for the first lactation period and 30 g for the second. These rats weaned an average of about 6 rats from each litter. Weight change

during lactation in Experiment III did not differ significantly with protein restriction or parity, but the findings tended to agree with those of Experiments I and II that number of rats suckled was a dominant factor in maternal weight change during lactation.

Length of gestation No consistent trends concerning the influence of diet or parity on gestation length were apparent in Experiments I or III (Tables 3 and 4). In Experiment II, however, the second experimental pregnancy (gestation 3) was significantly longer for all females than the first (Table 4). Gestation 2 averaged 21.6 days for HP rats and 21.5 days for LP rats while gestation 3 averaged 21.8 and 21.9 days for the respective treatments. As in Experiments I and III, values for the adequately nourished and protein-restricted groups did not differ in Experiment II.

Litter size Number of pups born did not vary with dietary treatment in Experiment I (Table 5). Consistently, an average of fewer pups were born in litter 2 than in litter 1, but mean litter sizes for litter 1 vs litter 2 differed significantly only when the LP_M group was compared with the HP, LP, or LP_H treatment.

An average of 12.0 pups was delivered by HP mothers at the termination of their initial experimental pregnancy (litter 2) in Experiment II while LP females averaged 12.6 pups (Table 6). At the end of gestation 3, HP females delivered an average of 9.3 pups, and LP dams delivered an average of 9.8 pups. Litter size was not significantly changed by protein restriction in Experiment II, but significantly fewer pups per litter were born following gestation 3 than gestation 2 of both HP and LP dams.

Females restricted in protein from weaning in Experiment III (LP_p) bore an average of 8.6 pups in their first litters; HP_p dams averaged 10.5

Table 5. Mean number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiment I

Experi- mental group	No. of observations	No. in litter (pups)	Average birth wt. (g/pup)	Still- births ¹ (%)	No. pups selected to nurse	Survival rate ² day 4 ² (%)	Survival rate at weaning ³ (%)
HP ⁴	12	12.0 ^{a*}	6.22 ^a	1 ^a	8.7	79 ^a	30 ^a
Litter 1	6	12.2	6.18	0	9.3	82	13
Litter 2	6	11.8	6.25	1	8.0	77	46
LP ⁴	12	10.7 ^a	5.53 ^b	14 ^a	7.4	34 ^a	8 ^a
Litter 1	6	11.3	5.52	15	8.8	37	7
Litter 2	6	10.0	5.54	12	6.0	31	8
LP _M ⁴	9	12.0 ^a (10) ⁵	5.69 ^b	14 ^a (10)	8.4	41 ^a	6 ^a
Litter 1	5	14.2	5.58	0	10.0	48	10
Litter 2	4	9.8 (5)	5.82	27 (5)	6.5	31	0
LP _H ⁴	12	11.0 ^a	5.82 ^{ab}	2 ^a	8.3	39 ^a	5 ^a
Litter 1	6	12.3	5.68	4	10.0	37	0
Litter 2	6	9.7	5.97	0	6.7	42	9

¹Number pups stillborn/total number born.

²Number pups alive day 4/number pups selected to nurse.

³Number pups weaned/number pups selected to nurse.

⁴Arithmetic mean for litters 1 and 2.

⁵Number of observations when different from column 1.

* Means with the same superscripts are not different ($P > 0.05$).

Table 6. Mean number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiments II and III

Experiment	Group	No. of observations	No. in litter (pups)	Average birth wt. (g/pup)	Stillbirths ^a (%)	No. selected to nurse/litter	Survival rate, day 4 ^b (%)	Survival rate at weaning ^c (%)
II	HP ^d	19	10.7	6.61	5	7.2	81	66
	Litter 2	10	12.0	6.69	2	7.9	96	90
	Litter 3	9	9.3	6.52	8	6.4	64	39
	LP ^d	36	11.3	5.92(34) ^e	14	7.1	58(34)	39(34)
	Litter 2	20	12.6	5.87	2	8.0	68	44
	Litter 3	16	9.8	6.01(14)	29	6.0	43(14)	33(14)
Statistical Evaluation								
	Diet		NS ^f	--**	NS*	<0.10 ^g	--*	
	Litter		--**	NS	--*	--**	--**	
	Diet x Litter		NS	NS	NS	NS	<0.10	

^aNumber pups stillborn/total number born.

^bNumber pups alive day 4/number selected to nurse.

^cNumber pups weaned/number selected to nurse.

^dArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^eNumber of observations when different from column 1.

^fNS = not significant at least at 0.10 level.

^g0.05 < P < 0.10.

* P < 0.05.

** P < 0.01.

Table 6. (Continued)

Experiment	Group	No. of observations	No. in litter (pups)	Average birth wt. (g/pup)	Still-births ^a (%)	No. selected to nurse/litter	Survival rate ^b day 4 (%)	Survival rate at weaning ^c (%)
III	HP _p ^d	12	10.5	6.45	2	7.6	71	56
	Litter 1	7	10.6	6.47	4	7.4	75	55
	Litter 2	5	10.4	6.43	0	7.8	65	58
	LP _p ^d	23	9.0	5.97(22)	16	6.9(22)	41(22)	14(22)
	Litter 1	12	8.6	5.95(11)	20	6.9(11)	47(11)	11(11)
	Litter 2	11	9.5	6.00	12	6.9	36	17
Statistical Evaluation								
	Diet		NS	--*	<0.10		NS*	--*
	Litter		NS	NS	NS		--	NS
	Diet x Litter		NS	NS	NS		NS	NS

births in litter 1. Second litters averaged 10.4 and 9.5 pups for HP_p and LP_p groups, respectively. None of these values were significantly different; neither parity nor protein restriction affected litter size in this experiment in contrast to Experiments I and II where litter size was decreased with increased parity.

Development of the young

Birth weight Neonates from HP females weighed, on the average, 6.22 g in Experiment I and were significantly heavier than LP newborns which averaged 5.53 g and LP_M newborns which averaged 5.69 g (Table 5). Neonates from the LP_H group weighed 5.82 g on the average and did not differ significantly from HP progeny nor from LP or LP_M offspring. Birth weights for litter 1 pups were not different from those of litter 2 for any group.

Average birth weight of pups born to adequately nourished females in Experiment II was 6.61 g which was significantly heavier than that of protein-restricted offspring which averaged 5.92 g at birth (Table 6). In Experiment III, HP_p pups weighed 6.45 g on the average at birth and were also significantly heavier than LP_p neonates which weighed an average of 5.97 g at birth. As also observed in Experiment I, birth weight did not vary significantly with parity in either Experiment II or III.

Mortality at birth and perinatal survival Mortality at birth averaged 1, 5, and 2% for HP groups in Experiments I, II, and III, respectively (Tables 5 and 6). In Experiment I, birth mortality for the LP and LP_M groups was 14% on the average while that for the LP_H group which was treated identically with the LP group before parturition was only 2%. Mor-

tality for protein-restricted rats in Experiments II and III was 14 and 16%, respectively. Only the values for HP vs LP in Experiment I and for HP_p vs LP_p in Experiment III approached being significantly different ($0.05 < P < 0.10$).

Dams fed 10% casein on days 15, 16, and 17 (LP_M) in Experiment I had no stillbirths in litter 1; one LP_M dam's entire second litter was stillborn, however, so that litter 2 birth mortality for this group was 27%. Discounting this stillborn litter, LP_M birth mortality in litter 2 was 9%, a value similar to the average of 14% observed among LP rats. Mortality at birth increased significantly with parity in Experiment II but not Experiments I or III. In Experiment II, birth mortality was only 2% in litter 2 for both HP and LP pups but was 8 and 29% for HP and LP groups, respectively, in litter 3. The interaction of diet and parity on birth mortality was not significant in any of the 3 experiments.

Percent survival to day 4 was 79, 81, and 71% for HP pups in Experiments I, II, and III, respectively. Only 34, 41, and 39% of the LP, LP_M , and LP_H pups, respectively, in Experiment I survived to day 4. Perinatal survival rates for protein-restricted pups in Experiments II and III were 58 and 41%, respectively. Because of variation within experimental groups, the values for malnourished and adequately fed rats did not differ significantly in Experiment III and only approached significant difference for HP and LP_H groups in Experiment I and HP and LP groups in Experiment II ($0.05 < P < 0.10$).

Day 4 survival declined with parity for every group in Experiment I except LP_H . None of the values in Experiment I differed significantly due to litter effect, however. Increased parity did significantly decrease

perinatal survival in Experiments II and III. Survival rate to day 4 was 96% on the average for HP and 68% for LP pups in litter 2 compared with 64 and 43% for litter 3 pups from these respective groups in Experiment II. In Experiment III, day 4 survival was 75 and 47% for HP_p and LP_p pups, respectively, from litter 1 compared with 65 and 36% for pups from litter 2. The interaction of diet and parity did not significantly influence perinatal survival in Experiments I, II, or III.

Neonatal organ weights No newborn pups from litter 1 in Experiment I were sacrificed; therefore, only data for litter 2 are represented in Tables 7 and 9. Adequately nourished neonates selected at random for autopsy from litters of more than 8 in Experiment I weighed 5.98 g on the average and were significantly heavier than LP progeny which averaged 5.30 g (Table 7). Mean body weights for newborn pups in the LP_M (5.41 g) and LP_H (5.60 g) groups did not differ significantly from each other or from those of either the HP or LP group. Similarly carcasses of HP progeny were significantly heavier than those of LP offspring but did not differ significantly from those of the LP_M or LP_H neonates. Mean liver weights for newborns were 278 mg for HP, 188 mg for LP, 222 mg for LP_M, and 225 mg for LP_H groups. The value for HP differed significantly from LP and LP_M ($P < 0.05$) and approached being different from LP_H ($0.05 < P < 0.10$). The value for LP differed significantly from LP_M and approached being significantly different from LP_H; transitory feeding of 10% casein in gestation, therefore, partially alleviated the effect of protein restriction on liver weight. The LP_H group should have been like the LP group because the treatment during pregnancy was the same. Kidney and spleen weights of new-

Table 7. Mean body and organ weights of newborn female rats in Experiment I

Experi- mental group	No. of rats	Body wt. (g)	Carcass (g)	Liver (mg)	Kidney (mg)	Spleen (mg)
HP	16	5.98 ^{a*}	4.16 ^a	278 ^a	55 ^a	9.8 ^a
LP	14	5.30 ^b	3.62 ^b	188 ^b	49 ^a	9.2 ^a
LP _M	10	5.41 ^{ab}	3.83 ^{ab}	222 ^c	48 ^a	8.5 ^a
LP _H	13	5.60 ^{ab}	3.91 ^{ab}	225 ^{abc}	51 ^a	10.0 ^a

* Means with the same superscripts are not different ($P > 0.05$).

born pups did not differ significantly among adequately nourished and protein-restricted groups in Experiment I.

When neonatal body and organ weights for rats from litters 2 and 3 in Experiment II were combined, none of the group means for HP and LP pups differed significantly (Table 8). When data for litter 2 only were considered, however, body, carcass, liver, kidney, and spleen weights of protein-restricted offspring were significantly smaller than those of adequately fed progeny. The average body weights for HP and LP neonates sacrificed from litter 2 were 6.17 g and 5.52 g, respectively; mean carcass weights for the 2 groups were 4.26 g (HP) and 3.81 g (LP). Livers for litter 2 weighed 274 mg and 235 mg for HP and LP pups, respectively, while the average kidney weights were 64 and 54 mg. Spleens of newborn HP pups averaged 16.2 mg compared with 12.1 mg for LP pups. Only 18 pups (9 HP and 9 LP) from litter 3 were available for sacrifice compared with 72 (23 HP and 49 LP) for litter 2, and the LP neonates from litter 3 which were examined

Table 8. Mean body and organ weights of newborn female rats in Experiments II and III

Experi- ment II		HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
Body wt. ^a	g	6.22 ^a (32) ^b	5.62(58)	NS ^c **	NS _d	NS
Litter 2		6.17 (23)	5.52(49)	--	--	--
Litter 3		6.36 (9)	6.15 (9)	NS	--	--
Carcass ^a	g	4.30	3.89	NS ^c **	NS	NS
Litter 2		4.26	3.81	--	--	--
Litter 3		4.41	4.28	NS	--	--
Liver ^a	mg	286	241	NS ^c **	NS	NS
Litter 2		274	235	--	--	--
Litter 3		317	272	NS	--	--
Kidney ^a	mg	63	56	NS ^c **	NS	NS
Litter 2		64	54	--	--	--
Litter 3		60	62	NS	--	--
Spleen ^a	mg	15.7	12.6	NS ^c **	NS	NS
Litter 2		16.2	12.1	--	--	--
Litter 3		14.4	15.4	NS	--	--

^aArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^bNumber of rats.

^cNS = not significant at least at 0.10 level.

^d-- not applicable to analysis of individual litters.

** $P < 0.01$.

Table 8. (Continued)

Experi- ment III		HP _p	LP _p	<u>Statistical evaluation</u>		
				Diet	Litter	Diet x litter
Body wt. ^a	g	6.19(19)	5.32(14)	--*	NS	NS
Litter 1		6.00(11)	5.59 (4)	NS*	--	--
Litter 2		6.46 (8)	5.21(10)	--*	--	--
Carcass ^a	g	4.30	3.69	--*	NS	NS
Litter 1		4.18	3.86	NS*	--	--
Litter 2		4.48	3.63	--*	--	--
Liver ^a	mg	276	212	--*	NS	NS
Litter 1		272	235	NS**	--	--
Litter 2		282	203	--**	--	--
Kidney ^a	mg	63	52	<0.10 ^e	NS	NS
Litter 1		60	63	NS**	--	--
Litter 2		68	48	--**	--	--
Spleen ^a	mg	14.5	11.5	<0.10	NS	NS
Litter 1		11.6	11.3	NS*	--	--
Litter 2		18.4	11.6	--*	--	--

^e0.05 < P < 0.10.

* P < 0.05.

were appreciably larger than those from litter 2 (6.15 vs 5.52 g). Consequently, none of the body or organ weights for litter 3 neonates differed significantly between HP and LP treatments.

In Experiment III, the body, carcass and liver weights of neonates from litters 1 and 2 or from litter 2 only were significantly reduced by protein restriction (Table 8). Kidney and spleen weights of protein-restricted pups in litter 2 were significantly smaller than those of adequately nourished newborn pups, also. When data for litters 1 and 2 were considered

together, however, kidney and spleen weights only approached being different ($0.05 < P < 0.10$). None of the parameters measured were significantly affected by protein restriction in litter 1 which included only 4 neonates from the LP treatment. The mean body, carcass, liver, and kidney weights of LP pups in the first experimental litter were larger than those for the second while values for pups in the first HP litter tended to be smaller than those in the second. That is, neonates in the LP treatment weighed on the average 5.59 g in litter 1 and 5.21 g in litter 2 while HP neonates weighed 6.00 and 6.46 g in litters 1 and 2, respectively. As a result, body and organ weight differences between experimental groups were smaller in litter 1 than in litter 2, and treatment means did not differ significantly.

Relative weights of carcass, liver, kidney, and spleen for newborn progeny of adequately nourished and protein-restricted dams can be compared in Tables 9 and 10. Only the liver was more seriously affected by protein restriction than body weight in Experiment I (Table 9). Mean liver weights expressed in mg/g body weight were 46.5 for HP, a value significantly higher than 35.7, 40.9, and 39.8 for LP, LP_M, and LP_H offspring. The mean for LP_M group was significantly larger than the value for the LP group indicating that the transitory supplement of 10% casein on days 15, 16, and 17 of gestation may have exerted a protective effect on relative liver weight for neonates. Although LP_H and LP groups had been treated identically prior to parturition, the mean relative liver weight for the LP_H neonates approached being significantly larger than the value for the LP pups ($0.05 < P < 0.10$).

In Experiment II, only the relative spleen weight of newborn pups from litter 2 was significantly reduced by protein restriction (Table 10). Relative spleen weight for protein-restricted pups averaged 2.2 mg/g body weight compared with 2.6 mg/g body weight for adequately nourished neonates.

Table 9. Mean relative carcass and organ weights of newborn female rats in Experiment I

Experimental group	No. of rats	Carcass/ BW ¹ (g/100g)	Liver/ BW (mg/g)	Kidney/ BW (mg/g)	Spleen/ BW (mg/g)
HP	16	69.55 ^{a*}	46.5 ^a	9.1 ^a	1.7 ^a
LP	14	68.30 ^a	35.7 ^b	9.2 ^a	1.7 ^a
LP _M	10	71.04 ^a	40.9 ^c	9.0 ^a	1.6 ^a
LP _H	13	69.87 ^a	39.8 ^c	9.2 ^a	1.8 ^a

¹BW = body weight.

* Means with the same superscripts are not different ($P > 0.05$).

Average relative liver and kidney weights of LP offspring were smaller than those of HP progeny in Experiment II, but none of the values differed significantly.

Relative liver and kidney weight of newborn pups were reduced by protein restriction in Experiment III (Table 10). The effect was significant only in litter 2, however, when the mean relative liver weight for HP_p neonates was 43.8 mg/g body weight compared with 38.8 mg/g body weight for protein-restricted pups. Average relative kidney weights for litter 2 pups were 10.4 and 9.3 mg/g body weight for HP_p and LP_p groups, respectively.

Postnatal survival, growth, and development Survival, growth and development of offspring during the suckling period may indicate effects of experimental manipulation on lactation performance. Survival rate at weaning was poor among both adequately nourished and protein-restricted offspring in Experiments I, II, and III (Tables 5 and 6). On the average,

Table 10. Mean relative carcass and organ weights of newborn female rats in Experiments II and III

Experi- ment II		HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
Carcass/BW ^{ab}	g/100 g	69.16(32) ^c	69.15(58)	NS ^d	NS ^e	NS
Litter 2		69.07(23)	69.09(49)	NS	--	--
Litter 3		69.36 (9)	69.47 (9)	NS	--	--
Liver/BW ^b	mg/g	45.8	42.8	NS	NS	NS
Litter 2		44.2	42.5	NS	--	--
Litter 3		49.9	44.4	NS	--	--
Kidney/BW ^b	mg/g	10.1	9.9	NS	NS	NS
Litter 2		10.4	9.9	NS	--	--
Litter 3		9.4	10.1	NS	--	--
Spleen/BW ^b	mg/g	2.5	2.2	NS*	NS	NS
Litter 2		2.6	2.2	--*	--	--
Litter 3		2.3	2.5	NS	--	--

^aBW = body weight.

^bArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^cNumber of rats.

^dNS = not significant at least 0.10 level.

^e-- not applicable to analysis of individual litters.

*P<0.05.

Table 10. (Continued)

Experi- ment III		HP _p	LP _p	Statistical evaluation		
				Diet	Litter	Diet x litter
Carcass/BW ^b	g/100 g	69.48(19)	69.47(14)	NS	NS	NS
Litter 1		69.56(11)	68.99 (4)	NS	--	--
Litter 2		69.36 (8)	69.67(10)	NS	--	--
Liver/BW ^b	mg/g	44.6	39.8	NS	NS	NS
Litter 1		45.2	42.2	NS*	--	--
Litter 2		43.8	38.8	--	--	--
Kidney/BW ^b	mg/g	10.1	9.8	NS	NS	NS
Litter 1		9.9	11.1	NS*	--	--
Litter 2		10.4	9.3	--	--	--
Spleen/BW ^b	mg/g	2.3	2.2	NS	NS	NS
Litter 1		1.9	2.0	NS	--	--
Litter 2		2.8	2.2	NS	--	--

30, 66, and 56% of the adequately nourished pups nursed were weaned while only 5 to 8, 39, and 14% of the pups from the protein-restricted groups survived to weaning in the 3 experiments. More young survived in the second litter of HP rats in Experiment I, but fewer survived in litter 2 vs litter 3 in Experiment II. Survival of HP_p pups was similar for litters 1 and 2 in Experiment III. Survival for protein-restricted groups was similar in both litters in all experiments. Due to the large variation within groups, none of the treatments affected mean survival rates at weaning differently in Experiment I; in Experiment II, both diet and parity and in Experiment III only diet influenced survival at weaning significantly. The interaction of diet and parity on survival to weaning was not significant in Experiments I or III but approached significance ($0.05 < P < 0.10$) in Exper-

iment II. Greenwood (1940) reported from her observation of Wistar rats that more young were weaned from the second litter than from the first or subsequent litters under normal conditions. Therefore, parity may have exerted an important influence on lactation performance among dams in Experiment II because first litters had been delivered on stock diets. Consequently, litter 2, the first experimental litter, probably represented optimal performance under the conditions of the study, 90 and 44% survival for HP and LP treatments, respectively. Survival rate for HP pups decreased to 39% in litter 3 while that for the LP pups decreased to 33%.

The limited number of litters weaned in Experiment I was too small for valid regression analyses; therefore, only means for preweaning body weights in this experiment are reported in Table 11. In Experiment II, adequately nourished pups were significantly heavier than protein restricted animals at birth, 7, 14, and 21 days of age. In Experiment III, the weights of pups receiving different dietary treatment which survived to weaning were not different at birth and only approached being significantly different at 7 days of age ($0.05 < P < 0.10$). Well nourished pups (HP_p) were significantly heavier than LP_p rats by 14 and 21 days of age, however. In both Experiments II and III, weight differences of adequately nourished and protein-restricted pups increased with age. Weight gain of protein-restricted offspring was most seriously affected during Experiment III; these rats had been most severely restricted in protein, and their offspring weighed 57% as much as the HP_p pups when weaned, 25.71 g vs 44.94 g. Weaning weights for LP pups in Experiments I and II averaged 70 and 66% of those of HP pups in the respective experiments.

Table 11. Mean preweaning body weights of rat pups in Experiments I, II, and III

Experiment	Experimental group	No. of litters	No. of pups	Average body wt. (g)				
				Day 0	Day 7	Day 14	Day 21	
I [#]	HP ¹			6.36	12.46	26.61	43.78	
	Litter 1	1	8	6.30	10.16	20.58	33.60	
	Litter 2	3	22	6.38	13.23	28.62	47.18	
	LP ¹			6.10	8.84	21.29	33.30	
	Litter 1	1	4	6.70	8.02	21.58	37.18	
	Litter 2	1	4	5.51	9.65	21.00	29.43	
	LP _M ¹			6.01	9.39	21.41	32.64	
	Litter 1	1	5	6.01	9.39	21.41	32.64	
	Litter 2	0	0	--	--	--	--	
	LP _H ¹			6.22	9.48	19.85	30.65	
	Litter 1	0	0	--	--	--	--	
	Litter 2	1	4	6.22	9.48	19.85	30.65	
	II	HP ¹			6.70 ^{a*}	14.44 ^a	31.24 ^a	50.42 ^a
		Litter 2	10	71	6.69	15.08	32.02	50.95
Litter 3		5	27	6.71	13.16	29.68	49.37	
LP ¹				6.18 ^b	10.55 ^b	20.94 ^b	33.28 ^b	
Litter 2		13	70	6.09	10.53	21.50	33.59	
Litter 3		6	37	6.37	10.60	19.74	32.62	
III	HP ¹			6.46 ^a	11.79 ^a	26.40 ^a	44.94 ^a	
	Litter 1	5	29	6.43	12.19	25.77	44.06	
	Litter 2	4	23	6.51	11.28	27.17	46.04	
	LP ¹			6.40 ^a	8.62 ^a	14.84 ^b	25.71 ^b	
	Litter 1	3	8	6.36	8.01	14.96	27.71	
	Litter 2	2	15	6.46	9.53	14.65	22.70	

¹Arithmetic mean for litters 1 and 2, Experiments I, III; for litters 2 and 3, Experiment II.

[#]Insufficient number of litters for statistical analyses.

^{*}Means within an experiment with the same superscript are not different ($P > 0.05$).

The average body and organ weights of female weanlings sacrificed on day 21 in Experiments II and III are presented in Table 12. No pups from Experiment I were sacrificed at weaning. Body, carcass, liver, kidney, and spleen weights of female rats born to and nursed by dams in the LP treatment were significantly smaller than those of rats born to and nursed by dams in the HP treatment in Experiment II. Spleen weights were significantly heavier for both HP and LP animals from litter 2 than for those from litter 3; no other body or organ weight was significantly affected by parity.

Body, carcass, liver, kidney, and spleen weights of LP_p weanlings in Experiment III were significantly smaller than those of HP_p pups when data from litters 1 and 2 were considered together or when those from only litter 1 were considered (Table 12). Although mean body, carcass, liver, and spleen weights of LP_p weanlings from litter 2 were reduced to about 50% of those of HP_p pups, variation in this litter was larger than that in litter 1; as a result, mean values for these parameters in litter 2 only approached being significantly different ($0.05 < P < 0.10$) as a function of diet. Kidneys of LP_p pups were significantly lighter ($P < 0.05$) than those of HP_p weanlings in litter 2. Renal weights were decreased among both HP_p and LP_p pups from litter 2 when compared with those from litter 1 ($0.05 < P < 0.10$), and the interaction of diet and parity on weanling kidney weight was significant ($P < 0.05$), a finding which indicated that the effect of the diets differed in the 2 litters.

Relative carcass and spleen weights of weanling females were not significantly influenced by protein restriction in Experiment II (Table 13). Relative liver weight of LP pups from litter 3 which was 3.72 g/100 g on

Table 12. Mean body and organ weights of weanling female rats in Experiments II and III

Experiment	II	HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
Body wt. ^a	g	49.30(33) ^b	32.86(36)	--**	NS ^c	NS
Litter 2		50.04(20)	33.63(22)	--**	-- ^d	--
Litter 3		48.18(13)	31.64(14)	--**	--	--
Carcass ^a	g	36.50	24.03	--**	NS	NS
Litter 2		37.24	24.53	--**	--	--
Litter 3		35.36	23.24	--**	--	--
Liver ^a	g	1.84	1.17	--**	NS	NS
Litter 2		1.80	1.17	--**	--	--
Litter 3		1.90	1.18	--**	--	--
Kidney ^a	g	0.61	0.35	--**	NS	NS
Litter 2		0.62	0.36	--**	--	--
Litter 3		0.60	0.35	--**	--	--
Spleen ^a	g	0.22	0.15	--*	--*	NS
Litter 2		0.22	0.17	--*	--	--
Litter 3		0.22	0.13	--**	--	--

^aArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^bNumber of rats.

^cNS = not significant at least at 0.10 level.

^d-- not applicable to analysis of individual litters.

* $P < 0.05$.

** $P < 0.01$.

Table 12. (Continued)

Experiment III		HP _p	LP _p	Statistical evaluation		
				Diet	Litter	Diet x litter
Body wt. ^a	g	44.43(24)	23.70(13)	--**	NS	NS
Litter 1		45.88(12)	27.60 (6)	--**	--	--
Litter 2		42.97(12)	20.35 (7)	<0.10 ^e	--	--
Carcass ^a	g	32.47	16.81	--**	NS	NS
Litter 1		33.32	19.67	--**	--	--
Litter 2		31.63	14.35	<0.10	--	--
Liver ^a	g	1.64	0.88	--**	NS	NS
Litter 1		1.72	1.00	--**	--	--
Litter 2		1.55	0.77	<0.10	--	--
Kidney ^a	g	0.52	0.26	--**	<0.10	--*
Litter 1		0.55	0.28	--**	--	--
Litter 2		0.49	0.24	--*	--	--
Spleen ^a	g	0.22	0.09	--*	NS	NS
Litter 1		0.22	0.12	--*	--	--
Litter 2		0.23	0.07	<0.10	--	--

^e0.05 < P < 0.10.

the average was significantly smaller than that of HP pups which averaged 3.94 g/100 g. Relative liver weight did not differ significantly between HP and LP pups in litter 2 or when the litters were combined, however. Kidney weight was reduced to a greater extent than body weight among LP weanlings from litter 2. The values were 1.24 g/100 g for HP and 1.06 g/100 g for LP pups. Relative kidney weight did not differ significantly for pups from litter 3 or when data from litters 2 and 3 were combined.

Among female weanlings in Experiment III, neither relative liver nor relative spleen weight was significantly affected by protein restriction

Table 13. Mean relative carcass and organ weights of weanling female rats in Experiments II and III

Experiment	II	HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
Carcass/BW ^{ab}	g/100 g	74.01(33) ^c	73.04(36)	NS ^d	NS ^e	NS
Litter 2		74.38(20)	72.78(22)	NS	--	--
Litter 3		73.43(13)	73.44(14)	NS	--	--
Liver/BW ^b	g/100 g	3.74	3.57	NS	NS	NS
Litter 2		3.61	3.48	NS*	--	--
Litter 3		3.94	3.72	--	--	--
Kidney/BW ^b	g/100 g	1.24	1.08	NS**	NS	NS
Litter 2		1.24	1.06	--	--	--
Litter 3		1.24	1.11	NS	--	--
Spleen/BW ^b	g/100 g	0.45	0.46	NS	NS	NS
Litter 2		0.44	0.49	NS	--	--
Litter 3		0.46	0.41	NS	--	--

^aBW = body weight.

^bArithmetic mean for litters 2 and 3, Experiment II; litters 1 and 2, Experiment III.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e-- not applicable for analysis of individual litters.

*P<0.05.

**P<0.01.

Table 13. (Continued)

Experiment III		HP _p	LP _p	<u>Statistical evaluation</u>		
				Diet	Litter	Diet x litter
Carcass/BW	g/100 g	73.08(24)	70.62(13)	NS	NS	NS
Litter 1		72.61(12)	70.81((6)	NS	--	--
Litter 2		73.55(12)	70.47 (7)	<0.10 ^f	--	--
Liver/BW	g/100 g	3.68	3.69	NS	NS	NS
Litter 1		3.75	3.60	NS	--	--
Litter 2		3.60	3.77	NS	--	--
Kidney/BW	g/100 g	1.18	1.12	NS*	NS	NS
Litter 1		1.20	1.03	--	--	--
Litter 2		1.16	1.20	NS	--	--
Spleen/BW	g/100 g	0.50	0.37	NS	NS	NS
Litter 1		0.48	0.40	NS	--	--
Litter 2		0.53	0.35	NS	--	--

^f0.05 < P < 0.01.

(Table 13). Relative carcass weight tended to be smaller for young in the LP_p treatment for litter 2 but not for litter 1; in litter 1, the values were 70.81 and 72.61% while in litter 2 they were 70.47 and 73.55%. Relative kidney weight of LP_p pups from litter 1 was 1.03 g/100 g on the average and was significantly smaller than that of HP_p weanlings which was 1.20 g/100 g on the average. Relative kidney weights for pups from litter 2 were 1.16 and 1.20 g/100 g for HP_p and LP_p treatments, respectively; neither these values nor those for the combined litters differed significantly.

Postweaning Growth, Development,
and Metabolic Efficiency

Growth

Data on growth will be summarized individually for each experiment. The parameters included are body weight during growth and body, carcass, adipose deposit, liver, kidney, and spleen weights at autopsy. Brain development will be considered in a later section together with behavioral data.

Body weight

Experiment I Growth as indicated by body weight was reduced by protein restriction before and after weaning in Experiment I (Table 14). Males weaned by females fed 6% casein in gestation and 10% casein in lactation weighed 36 g when weaned at 3 weeks of age compared with 40 g for males weaned by dams fed 24% casein in gestation and lactation in Experiment I_a. Protein restriction was extended by feeding 10% casein to weaned rats until autopsy at about 36 weeks of age. By 15 weeks, restricted rats (LP/LP) weighed only 285 g, 75% as much as rats which had received an adequate protein supply throughout life (HP/HP). From 15 to 19 weeks of age, both groups grew more slowly than before, but the effect of decreased growth rate with maturity was more marked in the control group which gained 23 g during this period than in the restricted group which gained 32 g. Water deprivation schedules in anticipation of behavioral measurements were instituted for rats in Experiment I_a between the ages of 19 and 20 weeks. As a result, food intake decreased for both HP/HP and LP/LP groups, and most animals lost weight (5 and 11 g for HP/HP and LP/LP groups, respectively).

Table 14. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment I

Experiment Group	I _a		I _b		I _c	
	HP/HP	LP/LP	St/St	4 _g 15 ₁ /15	HP/HP	LP/HP
Number of rats	7	6	6	8 7	13	5
	(g)	(g)	(g)	(g)	(g)	(g)
3 weeks ^a	40	36	54	49	48	32
6 weeks	124	95	158	169	126	117
9 weeks	256	182	302	300	262	245
12 weeks	313	236	391	380	341	324
15 weeks	379	285	451	395	399	371
18 weeks	398	312	481	444	427	402
20 weeks	397	306	517	455	441	413

^aAll rats were weaned at 3 weeks of age.

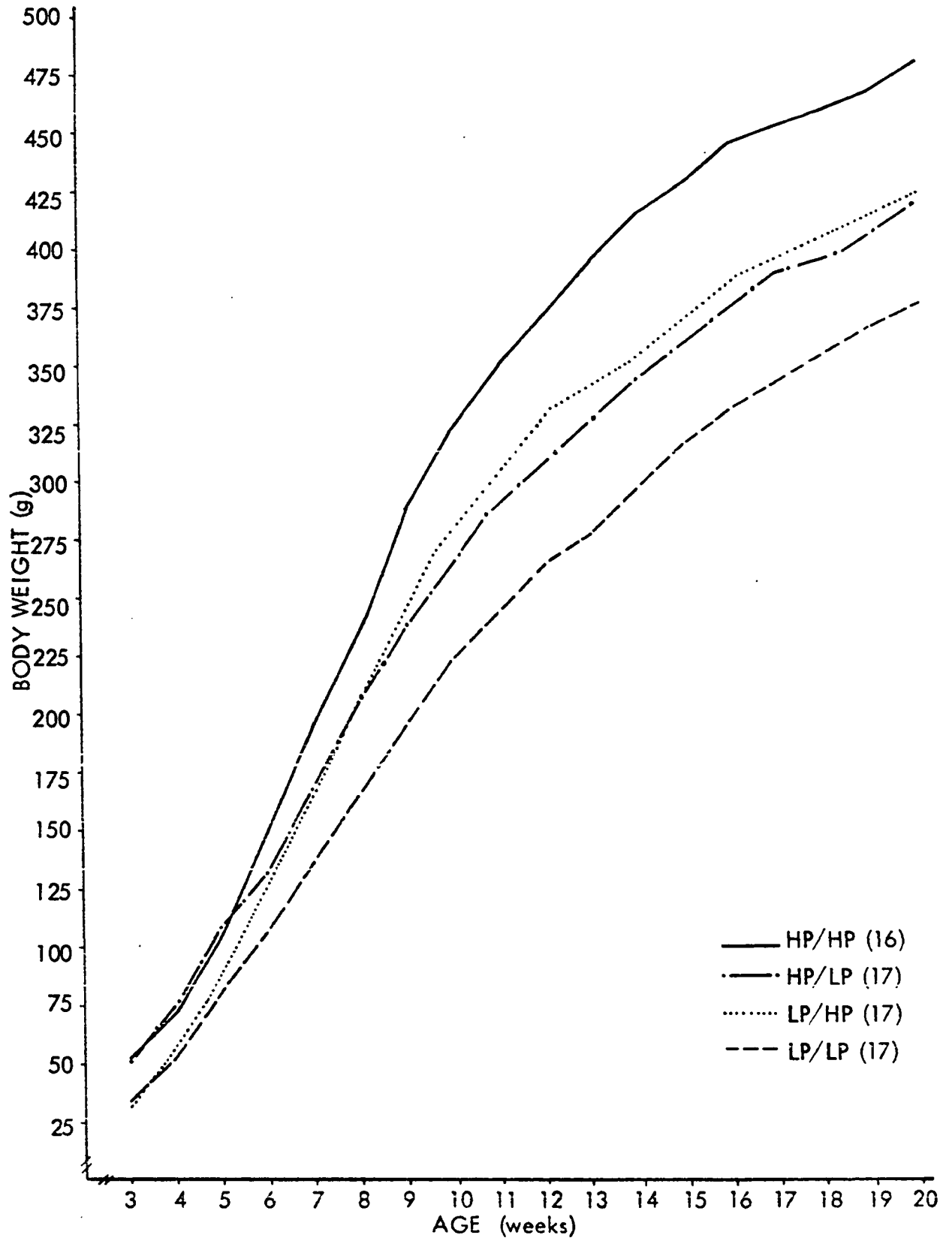
The dietary treatment in Experiment I_b examined the course of severe protein restriction during gestation. Dams were fed diets containing 4 and 15% protein supplied by methionine-supplemented casein in gestation and lactation, respectively (Table A2). After weaning, the offspring were maintained on 15% protein until autopsy at 40 to 43 weeks of age; they have been designated 4_g 15₁/15. The St/St rats were the progeny of females fed Steenbock XV, a ration which supplies about 24% protein from mixed sources (Table A1), during gestation and lactation. The offspring were maintained on Steenbock XVII, a ration which contains about 23% protein from mixed sources (Table A1), from weaning for the duration of the experiment. Both 4_g 15₁/15 and St/St males grew at practically identical rates through 12 weeks of age (Table 14). However, for no apparent reason, all rats in the 4_g 15₁/15 group failed to gain or lost weight during at least 1 week between 12 and 16 weeks of age. As a result, the slope of the growth curve for the

4 $\frac{15_1}{15}$ group changed; rats lost 8 g on the average during the 13th week. The mean rate of growth for the 2 groups became parallel again after 14 weeks; by 20 weeks, body weight of rats restricted in protein in utero was approximately 12% below that of stock animals, 455 vs 517 g.

Rats for Experiment I_c were weaned from the second litters of dams in Experiment I (Figure 4a). Those born of and nursed by restricted females weighed 32 g on the average at weaning while offspring of adequately nourished dams averaged 48 g (Table 14). All rats in Experiment I_c were fed 24% casein after weaning. Rehabilitated rats (LP/HP) grew rapidly on the high protein ration. The growth curve for LP/HP animals was almost parallel to that of the HP/HP rats, and they maintained their initial weight deficit of about 16 g through 12 weeks of age. After 12 weeks of age, LP/HP rats grew more slowly than HP/HP rats; by 20 weeks of age, HP/HP rats outweighed LP/HP animals by an average of 28 g.

Experiment II Statistical analysis of growth curves for male progeny of the first experimental litter (litter 2), Experiment II, revealed significant differences due to protein restriction before and after weaning and the interaction of restriction with time, i.e., whether it occurred before or after weaning or throughout life (Figure 9). Males reared by females restricted to 6 and 10% casein in gestation and lactation, respectively, (LP/LP and LP/HP) exhibited a weight deficit of about 37% at weaning compared with HP/HP and HP/LP rats. For 2 weeks after weaning, weeks 4 and 5, males fed 10% casein maintained growth rates equal to those of their littermates fed 24% casein. All groups gained an average of 18 to 23 g during week 4 and 31 to 34 g during week 5. By 6 weeks of age, the effects of the restricted protein supply after weaning were evident;

Figure 9. Postweaning growth of male offspring from litter 2 in Experiment II



both HP/LP and LP/LP rats were smaller than their littermates fed 24% casein after weaning (HP/HP and LP/HP) (Table 15). At 6 weeks of age, rats restricted in protein before weaning and adequately fed afterward (LP/HP) weighed 126 g on the average compared to 132 g for animals restricted in protein only after weaning (HP/LP). Growth curves for HP/LP and LP/HP animals were similar for the remaining 14 weeks of the experiment. Weight gains of rats fed adequate protein after weaning were 46 and 40 g/week for HP/HP and LP/HP groups, respectively, during weeks 6 to 9; these gains were larger than those of rats restricted after weaning (35 g/week for HP/LP and 30 g/week for LP/LP). From 9 to 15 weeks, gains for the 4 groups were similar; rates of gain ranged from 23 g/week for HP/HP to 20-21 g/week for the remaining 3 groups. During weeks 15 to 20, all groups gained an average of 11-12 g/week.

Average weaning weights among litter 3 males in Experiment II were 49 g for HP/HP, 53 g for HP/LP, 31 g for LP/HP, and 34 g for LP/LP and were similar to values in litter 2 (Table 15). Growth after weaning, however, did not follow a consistent pattern for any group. Several factors may have influenced these results. According to Greenwood (1940), progeny from the third litter of stock animals did not grow as well as offspring from litter 2. She suggested that perhaps the detrimental effects of respiratory infections, which were prevalent among confined laboratory rats, became more evident with parity. In the present experiment, temperature controls in the animal laboratories malfunctioned for a period of about 3 weeks, and the ambient temperature was increased to 74-76°F, about 5° above the usual range. During this period, most animals ate less than usual and failed to gain or actually lost weight. Fourteen of 30 rats from

Table 15. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment II

	HP/HP (g)	HP/LP (g)	LP/HP (g)	LP/LP (g)
3 weeks ^a				
Litter 2	53(16) ^b	51(17)	32(17)	34(17)
Litter 3	49 (7)	53 (6)	31 (9)	34 (8)
6 weeks				
Litter 2	150	132	126	107
Litter 3	146	129	125	103
9 weeks				
Litter 2	288	237	245	197
Litter 3	225	198	226	195
12 weeks				
Litter 2	371	307	327	264
Litter 3	288	286	298	269
15 weeks				
Litter 2	426	361	369	316
Litter 3	330	334	338	329
18 weeks				
Litter 2	458	394	403	354
Litter 3	389	374	356	355
20 weeks				
Litter 2	479	418	423	375
Litter 3	406	387	369	370

^aAll rats were weaned at 3 weeks of age.

^bNumber of rats.

litter 3, Experiment II, i.e., 3 of 7 HP/HP, 2 of 6 HP/LP, 5 of 9 LP/HP, and 4 of 8 LP/LP rats, were 2 to 8 weeks old when the temperature control in the animal laboratory failed; therefore, these animals did not grow as rapidly as expected during this period. The average body weight for ani-

mals in litter 3 compared with that of animals from litter 2 was depressed for all groups from the 9th through the 20th week except those restricted in protein both before and after weaning (LP/LP). HP/HP rats in litter 3 weighed 406 g on the average at 20 weeks, a value even less than the weight of rats in litter 2 of the HP/LP and LP/HP groups, 418 and 423 g, respectively. HP/LP rats in litter 3 weighed 387 g at 20 weeks while LP/HP rats averaged 369 g and LP/LP rats weighed 370 g.

Experiment III Growth was followed for 5 males in litter 1 and for 19 in litter 2 in Experiment III. Because of the small number of rats in litter 1, data from the 2 litters are combined in Table 16. Growth patterns among rats in Experiment III were very similar to those of animals from the first experimental litter (litter 2) in Experiment II (Figure 9). Reduced growth as a consequence of protein restriction either before or after weaning or throughout life was evident when body weights of HP_p/LP , LP_p/HP , and LP_p/LP groups were compared with that of HP_p/HP rats. All groups from Experiment III weighed less on the average of 3 weeks and at 20 weeks of age and all periods in between than their counterparts in Experiment II, litter 2; the weight deficits were more marked among those rats which were restricted before weaning than among those adequately nourished during gestation and lactation. At 20 weeks of age, HP_p/HP rats were 5%, HP_p/LP rats 3%, LP_p/HP 10%, and LP_p/LP 13% lighter than comparable animals in litter 2, Experiment II. The prolonged restriction of the LP_p dams in Experiment III produced larger weight deficits in their offspring, the LP_p/HP and LP_p/LP groups than the shorter LP restriction in Experiment II produced in LP/HP and LP/LP rats when comparisons are made with those rats well nourished before weaning in the respective experiments.

Table 16. Mean body weights of male offspring at intervals from 3 to 20 weeks of age in Experiment III

	HP _p /HP (g)	HP _p /LP (g)	LP _p /HP (g)	LP _p /LP (g)
3 weeks ^a	48 ^b (8) ^c	47(6)	25(5)	24(5)
6 weeks	143	125	117	90
9 weeks	259	226	235	182
12 weeks	341	301	296	246
15 weeks	398	355	342	287
18 weeks	436	383	371	319
20 weeks	454	406	382	325

^aAll rats were weaned at 3 weeks of age.

^bArithmetic means for observations in litters 1 and 2.

^cNumber of rats.

Body, carcass, adipose deposit, and organ weights Body, carcass, and organ weight data for adult males in Experiments I, II, and III were collected when rats were sacrificed after completing behavioral training. Perirenal and epididymal fat deposits also were excised and weighed in Experiments II and III. Rats were 35 to 45 weeks of age in Experiment I and 26 to 28 weeks of age in Experiments II and III when sacrificed. In addition to the effects of diet before and after weaning, body and organ weights at autopsy reflected the effects of water deprivation which was instituted when rats were about 20 weeks of age and maintained throughout behavioral training. Rats in Experiments I_a and I_b were given water ad libitum for at least 1 week before being sacrificed while those in Experiments I_c, II, and III were given free access to water for 2 days prior to autopsy.

Experiment 1 Autopsy data for adult males in Experiments I_{a,b,c} are presented in Table 17. Protein restriction throughout life resulted in significantly smaller body, carcass, and liver weights for LP/LP rats compared with HP/HP animals in Experiment I_a. Kidney weights of LP/LP and HP/HP rats approached being significantly different ($0.05 < P < 0.10$), but spleen weights did not.

Adult males in Experiment I_b were subjected to intrauterine protein restriction then maintained on 15% protein through lactation and after weaning; they were significantly lighter at autopsy than animals fed a stock ration containing 23-24% protein from mixed sources. Rats of the 4 15_g₁/15 group weighed 488 g on the average when autopsied compared with 593 g for St/St animals. Carcass and kidney weights of 4 15_g₁/15 rats were also significantly lighter than those of the St/St rats, but liver and spleen weights did not differ significantly.

Rats reared by females fed 24% casein in gestation and lactation and continued on that ration after weaning were divided into 2 groups when water deprivation was initiated prior to behavioral training in Experiment I_c. Treatments of the groups were identical except that one group, HP/HP_{nt}, was not trained in the visual discrimination maze. When water deprivation began, mean weights were 492 g and 490 g for HP/HP_{nt} and HP/HP, respectively. At autopsy, HP/HP_{nt} rats had gained an average of 12 g while HP/HP animals had lost 4 g on the average; as a result, body and carcass weights of the 2 groups were significantly different. Kidneys of HP/HP rats were also significantly lighter than those of the HP/HP_{nt} group.

Males deprived of adequate protein before weaning and fed 24% casein after weaning (LP/HP) also were compared with HP/HP rats. The HP/LP rats

Table 17. Mean body and organ weights of adult male rats in Experiment I

Experiment Group	Number of rats	I _a			I _b			I _c				
		HP/HP 7	LP/LP 6	P	St/St 5	4 ^g 15 ¹ / 7 ¹ 15	P	P ^a	HP/HP _{nt} 7	HP/HP 6	LP/HP 4	P ^b
Autopsy wt.	g	487	374	--**	593	488	--*	--**	504	486	447	<0.10 ^c
Carcass	g	396	303	--*	476	399	--*	--*	397	383	357	NS ^d
Liver	g	12.30	9.17	--*	15.19	13.05	NS	NS	14.66	14.25	10.36	--*
Kidney	g	2.60	1.72	<0.10	3.26	2.81	--*	--*	3.03	2.89	2.74	NS
Spleen	g	0.71	0.58	NS	0.72	0.72	NS	NS	0.78	0.72	0.73	NS

^aHP/HP_{nt} vs HP/HP.

^bHP/HP vs LP/HP.

^c0.05 < P < 0.10.

^dNS = not significant at least at 0.10 level.

* P < 0.05.

** P < 0.01.

weighed 447 g on the average when sacrificed compared with 486 g for the HP/HP group. These values approached being significantly different ($0.05 < P < 0.10$). Liver weights of the animals deprived of adequate protein early in life (LP/HP) were significantly smaller than those of the HP/HP rats, but carcass, kidney, and spleen weights were not.

Experiment II Rats restricted in protein before weaning (LP/LP + LP/HP) were smaller when sacrificed than those which received adequate protein before weaning (HP/HP + HP/LP). Only body weights for litter 2 (479 g for HP/HP + HP/LP vs 417 g for LP/LP + LP/HP) differed significantly, however (Table 18). Also in litter 2, rats restricted after weaning (LP/LP + HP/LP) weighed 423 g on the average and were significantly smaller than those adequately fed after weaning (HP/HP + LP/HP) which weighed an average of 472 g. In litter 2, the effects of adequate or restricted protein before and after weaning on body weight differed greatly (511 and 396 g for HP/HP and LP/LP rats, respectively); therefore, the influence of these groups was important in the significant preweaning and postweaning effects of protein restriction which were observed.

Both HP/HP and LP/HP groups behaved differently in litter 3 compared with litter 2. The greatest change from expected growth patterns occurred between the ages of 6 and 9 weeks and probably reflected the influence of the failure of laboratory temperature control discussed previously. Growth and therefore final body weight in the LP/LP treatment were not very different from those in litter 2, and HP/LP deviation was less marked than that of the HP/HP and LP/HP groups. Consequent to the unusual growth patterns of the rats in litter 3, neither the effect of preweaning nor post-

Table 18. Mean body, adipose deposit, and organ weights of adult male rats in Experiment II

		HP/HP	HP/LP	LP/HP	LP/LP
Body wt. ^b	g	483 (22) ^c	444 (23)	414 (26)	394 (25)
Litter 2		511 (15)	450 (17)	437 (17)	396 (17)
Litter 3		424 (7)	426 (6)	369 (9)	389 (8)
Carcass ^b	g	383	356	332	317
Litter 2		403	360	350	318
Litter 3		342	344	297	314
Adipose deposit ^b	g	18.88(18)	15.88(19)	13.32(22)	12.65(21)
Litter 2		20.50(15)	16.30(17)	13.62(13)	12.03(13)
Litter 3		10.79 (3)	12.30 (2)	12.87 (9)	13.67 (8)
Liver ^b	g	14.68	12.88	12.14	10.86
Litter 2		16.04	13.11	12.74	10.84
Litter 3		11.77	12.22	11.00	10.91
Kidney ^b	g	3.13	2.44	2.64	2.08
Litter 2		3.32	2.50	2.76	2.04
Litter 3		2.71	2.29	2.43	2.15
Spleen ^b	g	0.82	0.71	0.69	0.65
Litter 2		0.85	0.70	0.71	0.68
Litter 3		0.76	0.74	0.64	0.58

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cNumber of observations.

^dNS = not significant at least at 0.10 level.

^e0.05 < P < 0.10.

^fInsufficient observations in litter 3 for regression analyses.

* P < 0.05.

** P < 0.01.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP/HP +	LP/LP +	P	HP/HP +	LP/LP +	P
	HP/LP	LP/HP		LP/HP	HP/LP	
NS ^d	463 (45)	404 (51)	NS ^{**}	446 (48)	418 (48)	NS ^{**}
NS	479 (32)	417 (34)	-- ^{**}	472 (32)	423 (34)	-- ^{**}
NS	425 (13)	379 (17)	NS	393 (16)	405 (14)	NS
NS	369	325	NS ^{**}	356	336	NS ^{**}
NS	380	334	-- ^{**}	375	339	-- ^{**}
NS	343	305	<0.10 ^e	317	327	NS
-- ^f	17.34(37)	12.99(43)	-- ^{**}	15.82(40)	14.18(40)	--
NS	18.27(32)	12.83(26)	-- ^{**}	17.31(28)	14.45(30)	NS
--	11.39 (5)	13.25(17)	--	12.35(12)	13.39(10)	--
NS	13.76	11.51	NS ^{**}	13.30	11.83	NS ^{**}
NS	14.48	11.79	-- ^{**}	14.29	11.97	-- ^{**}
NS	11.98	10.96	NS	11.34	11.47	NS
NS	2.78	2.37	NS ^{**}	2.87	2.25	-- [*]
NS	2.89	2.39	-- ^{**}	3.02	2.27	-- ^{**}
NS	2.52	2.30	NS	2.55	2.21	<0.10
NS	0.76	0.67	NS	0.75	0.68	NS [‡]
NS	0.77	0.70	<0.10 ^{**}	0.78	0.69	--
NS	0.75	0.61	-- ^{**}	0.69	0.65	NS

weaning restriction on body weight was significant for litter 3 or when litters 2 and 3 were considered together.

Protein restriction before and after weaning affected carcass weights and body weights similarly. Carcasses of HP/HP and HP/LP rats tended to be heavier than those of LP/LP and LP/HP animals; they were significantly different due to preweaning restriction for litter 2 (380 vs 334 g) and approached significance ($0.05 < P < 0.10$) for litter 3 (343 vs 305 g). Carcass weights for rats in litter 2 vs litter 3 (356 and 321 g) approached being significantly different in Experiment II ($0.05 < P < 0.10$); as a result, when values for litters 2 and 3 were combined, the effect of preweaning restriction on carcass weight was not significant.

Carcasses for rats in litter 2 given adequate protein after weaning (HP/HP + LP/HP) weighed an average of 375 g and were significantly heavier than carcasses of rats protein-restricted after weaning (LP/LP + HP/LP) which weighed 339 g on the average. Neither carcass weights for litter 3 nor for litters 2 and 3 combined were significantly affected by postweaning protein restriction.

Perirenal and epididymal adipose deposits were measured for most rats in litter 2 and from a limited number in litter 3. Only data from litter 2 were evaluated statistically. The diet fed before weaning influenced adipose tissue deposits while the diet fed after weaning had no modifying effects. Rats in the LP/LP and LP/HP treatments had deposits which weighed 12.03 and 13.62 g, respectively, while those in the HP/HP and HP/LP treatments had deposits of 20.50 and 16.30 g, respectively.

Livers of rats given adequate protein prior to weaning (HP/HP + HP/LP) were heavier at autopsy than those of animals restricted during this period

(LP/LP + LP/HP). The effect was significant in litter 2 where livers of HP/HP + HP/LP rats weighed 14.48 g on the average compared with 11.79 g for livers of LP/LP + LP/HP animals. Livers of rats restricted in protein after weaning (LP/LP + LP/HP) weighed 11.97 g on the average for litter 2 while those of rats adequately fed after weaning averaged 14.29 g ($P < 0.01$). Liver weights in litter 3 for the HP/HP treatment averaged only 11.77 g compared with 16.04 g for HP/HP rats in litter 2. This deviation from expected hepatic weights among HP/HP rats was probably another manifestation of the deviant growth patterns observed in litter 3; consequently, hepatic weight did not differ significantly due to the influence of protein restriction before or after weaning when data from only litter 3 or litters 2 and 3 combined were considered.

Those rats which received adequate protein before weaning (HP/HP + HP/LP) had larger kidneys than those restricted during this period (LP/LP + LP/HP). The effect was significant among rats in litter 2 where kidneys from the HP/HP and HP/LP treatments averaged 2.89 g compared with 2.39 g for kidneys from the LP/LP and LP/HP treatments. Restriction after weaning reduced kidney size to a greater extent, however, than restriction before weaning. Among litter 2 animals, kidneys of HP/HP + LP/HP rats weighed an average of 3.02 g at autopsy and were significantly heavier than those of LP/LP + HP/LP rats which weighed only 2.27 g on the average. The effect of postweaning restriction on kidney weight approached significance ($0.05 < P < 0.10$) among rats in litter 3 and was significant ($P < 0.05$) when data from litters 2 and 3 were combined.

Splenic weights of rats in litter 2 were 0.77 g for HP/HP + HP/LP treatments and 0.70 for the LP/LP + LP/HP treatments ($0.05 < P < 0.10$).

Spleens of rats adequately nourished before weaning in litter 3 weighed 0.75 g on the average and were significantly larger than those of rats restricted in protein before weaning which averaged 0.61 g. When the data for the 2 litters were combined, average values of 0.77 g for HP/HP + HP/LP and 0.70 for LP/LP + LP/HP did not differ significantly, however. Due to the unequal numbers in the 2 litters and the approximate nature of the regression analyses, such results occurred occasionally. It is believed that the analyses of the data for the individual litters are more reliable than the analyses for the combined litters, however. Therefore, splenic weight reduction as a result of preweaning protein restriction was considered significant among rats in Experiment II.

Protein restriction after weaning resulted in significantly smaller spleens of LP/LP and HP/LP rats in litter 2 (0.69 g) compared with those among HP/HP + LP/HP animals (0.78 g). The effect of postweaning protein restriction on splenic weight was not significant among rats in litter 3 or when data from litters 2 and 3 were combined, however.

Experiment III Autopsy data for Experiment III is presented in Table 19. Since litter 1 was represented by only 5 animals (1 restricted and 1 adequately nourished litter), data from this litter alone were not analyzed statistically. Statistical analyses for litters 1 and 2 combined and for litter 2 only are reported. Adult progeny of dams restricted in protein from weaning through lactation ($LP_p/LP + LP_p/HP$) weighed significantly less at autopsy than offspring of dams adequately fed throughout life ($HP_p/HP + HP_p/LP$); carcasses of the rats restricted in protein before weaning were also lighter than those of animals adequately nourished prior to weaning. The effect was significant when litters 1 and 2 were combined

Table 19. Mean body, adipose deposit, and organ weights of adult male rats in Experiment III

		HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
Body wt. ^b	g	469(8) ^c	428(6)	403(5)	339(5)
Litter 1		478(2)	425(1)	392(1)	331(1)
Litter 2		466(6)	429(5)	405(4)	341(4)
Carcass ^b	g	374	345	325	274
Litter 1		376	343	319	264
Litter 2		374	346	326	276
Adipose deposit ^b	g	18.26	17.05	10.50	9.25
Litter 1		24.08	17.05	10.31	11.67
Litter 2		16.32	17.05	10.55	8.64
Liver ^b	g	13.50	12.22	12.38	9.82
Litter 1		14.12	10.79	12.05	9.33
Litter 2		13.30	12.51	12.46	9.94
Kidney ^b	g	2.81	2.38	2.84	2.07
Litter 1		2.92	2.38	2.73	2.02
Litter 2		2.77	2.39	2.87	2.08
Spleen ^b	g	0.84	0.64	0.66	0.59
Litter 1		0.81	0.57	0.60	0.49
Litter 2		0.85	0.66	0.67	0.62

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient number of observations for regression analyses.

^f0.05 < P < 0.10.

* P < 0.05.

** P < 0.01.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP /HP +	LP /LP +	P	HP /HP +	LP /LP +	P
	HP ^p /LP	LP ^p /HP		LP ^p /HP	HP ^p /LP	
NS ^d	451(14)	371(10)	--**	443(13)	388(11)	NS
-- ^e	460 (3)	362 (2)	--	449 (3)	378 (2)	--
NS	449(11)	373 (8)	--*	441(10)	390 (9)	NS
NS	362	299	--**	355	313	NS
--	365	292	--	357	304	--
NS	361	301	--*	355	315	NS
NS	17.74	9.87	--**	15.28	13.50	NS
--	21.74	10.99	--	19.49	14.36	--
NS	16.65	9.60	--*	14.01	13.31	NS
NS	12.96	11.10	<0.10 ^f	13.07	11.13	NS
--	13.01	10.69	--	13.43	10.06	--
NS	12.94	11.20	NS	12.96	11.37	NS
NS	2.63	2.46	NS	2.82	2.24	<0.10
--	2.74	2.38	--	2.86	2.20	--
NS	2.60	2.48	NS	2.81	2.25	<0.10
NS	0.76	0.62	<0.10	0.77	0.62	NS
--	0.73	0.54	--	0.74	0.53	--
NS	0.76	0.64	NS	0.78	0.64	NS

and when only litter 2 was considered. Average values for the combined litters were 451 and 362 g for live body and carcass weights, respectively of $HP_p/HP + HP_p/LP$ rats and 371 and 299 g for those of $LP_p/LP + LP_p/HP$ animals. Although final live body and carcass weights of rats fed restricted protein after weaning (388 and 313 g for $LP_p/LP + HP_p/LP$) were lighter than those of rats adequately fed following weaning (443 and 355 g for $HP_p/HP + LP_p/HP$), the values did not differ significantly.

Adipose deposits in the perirenal region plus the epididymal fat pad for $HP_p/HP + HP_p/LP$ rats from litters 1 and 2 weighed 17.74 g on the average compared with 9.87 g for those of $LP_p/LP + LP_p/HP$ rats. These and similar values for $HP_p/HP + HP_p/LP$ vs $LP_p/LP + LP_p/HP$ rats in litter 2 differed significantly as a result of preweaning protein restriction. The effect of restriction after weaning on adipose deposits was not significant for either the combined litters or only litter 2.

Livers of $HP_p/HP + HP_p/LP$ rats from litters 1 and 2 which were adequately fed before weaning averaged 12.96 g at autopsy, a value which approached being significantly heavier ($0.05 < P < 0.10$) than the average of 11.10 g for $LP_p/LP + LP_p/HP$ animals which were born of mothers restricted in protein through gestation and lactation. The effect of preweaning restriction did not approach significance for litter 2 only; although weights of livers from animals restricted after weaning ($LP_p/LP + HP_p/LP$) were smaller than those of rats adequately fed in the postweaning period ($HP_p/HP + LP_p/HP$), the values, 11.13 g and 13.07 g, did not differ significantly.

Protein restriction before weaning ($LP_p/LP + LP_p/HP$) did not affect renal weights differently than adequate protein before weaning. However,

kidneys of rats from litters 1 and 2 fed restricted protein after weaning ($LP_p/LP + HP_p/LP$) weighed 2.24 g on the average compared with 2.82 g for kidneys of $HP_p/HP + LP_p/HP$ rats. These values approached significance ($0.05 < P < 0.10$) as did those for litter 2 only.

Splenic weights for $HP_p/HP + HP_p/LP$ rats from litters 1 and 2 in Experiment III averaged 0.76 g at autopsy while spleens of $LP_p/LP + LP_p/HP$ animals weighed 0.62 g on the average ($0.05 < P < 0.10$). When only litter 2 was considered, the spleen values due to preweaning restriction did not differ significantly. Spleen weights for rats restricted or adequately fed after weaning were not significantly different.

In summary, prolonged protein restriction prior to conception and through gestation and lactation in Experiment III affected body and organ weights, except kidneys, more severely than did postweaning restriction. Reductions of body and organ weights due to preweaning restriction were significant or approached significance when data from litters 1 and 2 were combined or when those from only litter 2 were considered for each parameter measured except renal weight. Body and organ weights also were generally smaller among rats restricted after weaning than among those adequately fed after weaning in Experiment III, but only the reduction in kidney weight approached significance.

Relative carcass weights, adipose deposits, and organ weights

Experiment I Reductions in body and carcass and organ weights which occurred with protein restriction in LP/LP rats were proportional so that relative carcass and organ weights remained similar to those of adequately fed rats (HP/HP) in Experiment I_a (Table 20). Kidneys of LP/LP rats in Experiment I_a which weighed 0.46 g/100 g body weight compared with

Table 20. Mean relative carcass and organ weights of adult male rats in Experiment I

Experiment Group		I _a			I _b				I _c			
		HP/HP 7	LP/HP 6	P	St/St 5	4 g	15 7 ¹	/15 P	P ^a	HP/HP _{nt} 7	HP/HP 6	LP/HP 4
Carcass	g/100g	81.43	81.18	NS ^c	80.33	81.91	NS	NS	78.81	78.90	79.84	NS
Liver	g/100 g	2.52	2.46	NS	2.56	2.66	NS	NS	2.91	2.93	2.28	--*
Kidney	g/100 g	0.54	0.46	NS	0.55	0.58	NS	NS	0.61	0.60	0.62	NS
Spleen	g/100 g	0.15	0.16	NS	0.12	0.15	<0.10 ^d	NS	0.16	0.15	0.16	NS

^aHP/HP_{nt} vs HP/HP.

^bHP/HP vs LP/HP.

^cNS = not significant at least at 0.10 level.

^d0.05 < P < 0.10.

*P < 0.05.

0.54 g/100 g for HP/HP animals were reduced to a greater extent than carcass, liver, or spleen in relation to body weight, but the values did not differ significantly.

In Experiment I_b, average relative carcass, liver, and kidney weights of 4 $\frac{15}{g_1}$ /15 rats which had been subjected to protein restriction in utero were slightly but not significantly larger than those of St/St rats which had been reared on a stock ration containing 23 to 24% protein from mixed sources. Relative spleen weights of 4 $\frac{15}{g_1}$ /15 animals which averaged 0.15 g/100 g body weight compared with 0.12 g/100 g for St/St rats approached being significantly different, however ($0.05 < P < 0.10$).

Among rats from Experiment I_c, only liver weight was affected to a greater extent than body weight by preweaning protein restriction. Livers of HP/HP rats weighed 2.93 g/100 g body weight on the average and were significantly heavier than those of LP/HP rats which averaged 2.28 g/100 g. Relative carcass, kidney, and spleen weights for HP/HP and LP/HP rats were not significantly different. Weights of carcass, liver, kidney, and spleen in relation to body weight were similar for HP/HP and HP/HP_{nt} rats.

Experiment II Relative carcass weights, adipose deposits, and organ weights for adult males from Experiment II are presented in Table 21. Carcass, adipose deposits, and spleen weights in relation to body weight did not differ significantly as a result of preweaning or postweaning protein restriction. Average carcass weight accounted for about 80 g/100 g body weight for all experimental groups, adipose deposits for 2.46 to 4.01 g/100 g, and spleen 0.15 to 0.18 g/100 g.

In litter 2, the interaction of protein restriction before and after weaning on carcass weight approached significance ($0.05 < P < 0.10$). Relative

Table 21. Mean relative carcass, adipose deposit, and organ weights of adult male rats in Experiment II

		HP/HP	HP/LP	LP/HP	LP/LP
Carcass ^b	g/100 g	79.47(22) ^c	80.16(23)	80.44(26)	80.58(25)
Litter 2		78.84(15)	79.89(17)	80.39(17)	80.55(17)
Litter 3		80.80 (7)	80.91 (6)	80.55 (9)	80.64 (8)
Adipose deposit ^b	g/100 g	3.75(18)	3.55(19)	3.20(22)	3.22(21)
Litter 2		4.01(15)	3.59(17)	3.13(13)	3.04(13)
Litter 3		2.46 (3)	3.22 (2)	3.30 (9)	3.51 (8)
Liver ^b	g/100 g	3.02	2.89	2.91	2.76
Litter 2		3.14	2.90	2.88	2.74
Litter 3		2.77	2.86	2.96	2.80
Kidney ^b	g/100 g	0.65	0.55	0.64	0.53
Litter 2		0.65	0.55	0.63	0.52
Litter 3		0.64	0.54	0.66	0.55
Spleen ^b	g/100 g	0.17	0.16	0.17	0.17
Litter 2		0.17	0.16	0.16	0.17
Litter 3		0.18	0.18	0.17	0.15

^aDiet before weaning x diet after weaning.

^bArithmetic means for litters 2 and 3.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e0.05 < P < 0.10.

^fInsufficient number of observations for regression analyses.

* P < 0.05.

** P < 0.01.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^d	79.82(45)	80.51(51)	NS	79.99(48)	80.38(48)	NS
<0.10 ^e	79.40(32)	80.47(34)	NS	79.66(32)	80.22(34)	NS
NS	80.85(13)	80.59(17)	NS	80.66(16)	80.76(14)	NS
-- ^f	3.65(37)	3.21(43)	--	3.45(40)	3.38(40)	--
NS	3.79(32)	3.08(26)	NS	3.60(28)	3.35(30)	NS
--	2.76 (5)	3.40(17)	--	3.09(12)	3.45(10)	--
NS	2.96	2.84	<0.10*	2.96	2.82	NS
NS	3.02	2.81	--	3.01	2.82	<0.10
NS	2.81	2.89	NS	2.88	2.83	NS
NS	0.60	0.58	NS*	0.64	0.54	--**
NS	0.60	0.57	--*	0.64	0.54	--**
NS	0.59	0.61	NS	0.65	0.54	--**
NS	0.17	0.17	NS	0.17	0.16	NS
NS	0.16	0.17	NS	0.16	0.16	NS
NS	0.18	0.16	NS	0.18	0.16	NS

carcass weights in g/100 g body weight for litter 2 rats were 78.84 for HP/HP, 79.89 for HP/LP, 80.39 for LP/HP, and 80.55 for LP/LP. Therefore, carcass weight was reduced slightly less than body weight by protein restriction, but effects of restriction before and after weaning differed and the effect of restriction before and after weaning was not cumulative.

Liver weight relative to body weight for rats adequately nourished before weaning (HP/HP + HP/LP) was larger than that of rats restricted in protein before weaning (LP/LP + LP/HP) in litter 2 and when data from litters 2 and 3 were combined. Average values for litter 2 expressed in percent body weight were 3.02 for HP/HP + HP/LP and 2.81 for LP/LP + LP/HP groups. These values differed significantly while similar values for litters 2 plus 3, 2.96 and 2.84, approached significance ($0.05 < P < 0.10$). Relative liver weight for rats from litter 2 restricted after weaning (LP/LP + HP/LP) averaged 2.82 g/100 g body weight compared with 3.01 g/100 g for that of HP/HP + LP/HP ($0.05 < P < 0.10$).

Average relative renal weights expressed as percent body weight of rats in litters 2 and 3 for the 4 experimental groups were 0.65 for HP/HP, 0.55 for HP/LP, 0.64 for LP/HP, and 0.53 for LP/LP treatments. Kidney weight, therefore, was more seriously affected by postweaning protein restriction than was body weight among rats from litter 2, from litter 3, and from the combined litters ($P < 0.01$). Kidneys of rats restricted after weaning in litters 2 and 3 (LP/LP + HP/LP) averaged 0.54 g/100 g compared with 0.64 g/100 g for rats adequately nourished after weaning (HP/HP + LP/HP). Since relative renal weight values for the LP/HP and LP/LP treatments were slightly smaller than those for the HP/HP and HP/LP groups, respectively, the mean values for HP/HP + HP/LP treatments were larger than

those of the LP/LP + LP/HP groups indicating a slight effect of preweaning protein restriction which was significant in litter 2 (0.60 vs 0.57 g/100 g body weight; $P < 0.05$). The effect of preweaning protein restriction on relative renal weight was much smaller than the effect of restriction after weaning, however.

Experiment III Neither relative carcass weight nor relative liver weight nor relative spleen weight was significantly altered by protein restriction before or after weaning in Experiment III (Table 22). As in Experiments I and II, carcass represented about 80% of the adult male body weight, liver about 3%, and spleen about 0.16%.

Relative adipose deposits were significantly reduced by protein restriction prior to weaning for combined litters 1 and 2 or for litter 2. Perirenal and epididymal fat deposits accounted for 3.92% of body weight in $HP_p/HP + HP_p/LP$ rats from litters 1 and 2 and 2.68% for $LP_p/LP + LP_p/HP$ rats. The effect of postweaning protein restriction on relative weight of the adipose deposits was not significant.

The effect of protein restriction on kidney weight was much less marked than the effect on body weight among LP_p/HP rats. Thus, relative kidney weight appeared to be significantly increased by preweaning protein restriction and significantly decreased by postweaning restriction in Experiment III. Average values in g/100 g body weight for rats from litters 1 and 2 were 0.58 for HP_p/HP , 0.56 for HP_p/LP , 0.70 for LP_p/HP , and 0.61 for LP_p/LP . Of these 4 values, 3 were similar; only LP_p/HP differed. Therefore, when data were analyzed statistically, the combination of LP_p/HP with the data from either HP_p/HP or LP_p/LP rats produced a significant effect, in one case related to preweaning diet and in the other to the

Table 22. Mean relative carcass, adipose deposit, and organ weights of adult male rats in Experiment III

		HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
Carcass ^b	g/100 g	79.86(8) ^c	80.68(6)	80.74(5)	80.74
Litter 1		78.77(2)	80.71(1)	81.17(1)	79.76
Litter 2		80.23(6)	80.68(5)	80.63(4)	80.99
Adipose deposit ^b	g/100 g	3.88	3.97	2.61	2.75
Litter 1		5.01	4.01	2.62	3.53
Litter 2		3.50	3.97	2.61	2.56
Liver ^b	g/100 g	2.88	2.85	3.07	2.90
Litter 1		2.96	2.54	3.07	2.82
Litter 2		2.85	2.91	3.07	2.92
Kidney ^b	g/100 g	0.58	0.56	0.70	0.61
Litter 1		0.62	0.56	0.69	0.61
Litter 2		0.58	0.56	0.71	0.61
Spleen ^b	g/100 g	0.18	0.15	0.16	0.18
Litter 1		0.17	0.13	0.15	0.15
Litter 2		0.18	0.16	0.16	0.18

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats

^dNS = not significant at least at 0.10 level.

^eInsufficient number of observations for regression analyses.

*P<0.05.

**P<0.01.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP /HP + HP ^p /LP _p	LP /LP + LP ^p /HP _p	P	HP /HP + LP ^p /HP _p	LP /LP + HP ^p /LP _p	P
NS ^d	80.22(14)	80.74(10)	NS	80.20(13)	80.71(11)	NS
-- ^e	79.42 (3)	80.46 (2)	--	79.57 (3)	80.24 (2)	--
NS	80.43(11)	80.81 (8)	NS	80.39(10)	80.82 (9)	NS
NS	3.92	2.68	--**	3.39	3.42	NS
--	4.68	3.08	--*	4.21	3.77	--
NS	3.71	2.58	--*	3.14	3.34	NS
NS	2.86	2.98	NS	2.95	2.87	NS
--	2.82	2.94	--	2.99	2.68	--
NS	2.88	3.00	NS	2.94	2.91	NS
NS	0.57	0.66	--*	0.63	0.58	--*
--	0.60	0.65	--*	0.64	0.58	--*
NS	0.57	0.66	--*	0.63	0.58	--*
NS	0.17	0.17	NS	0.17	0.16	NS
--	0.16	0.15	--	0.16	0.14	--
NS	0.17	0.17	NS	0.17	0.17	NS

postweaning diet. No interaction of restriction before and after weaning was observed. The explanation for the large relative kidney weights observed in LP_p/HP rats in Experiment III is not known.

Food consumption

Experiment I Postweaning food intake, expressed per g metabolic weight per day, is reported in Table 23 in Experiments I_{a,c}. Food intake was not measured in Experiment I_b. Male rats deprived of an adequate protein supply both before and after weaning (LP/LP) in Experiment I_a tended to consume greater quantities of food per g body wt.^{0.75} than those supplied adequate protein before and after weaning (HP/HP). Average intakes differed significantly during the 4th (0.37 vs 0.32 g food/g metabolic weight/day for LP/LP and HP/HP rats, respectively; $0.05 < P < 0.10$) and 6th (0.38 vs 0.33 g food/g metabolic weight/day; $P < 0.01$) weeks of life only, however.

Food intake measurements evaluated in this section were made before rats were 20 weeks of age; consequently, HP/HP rats in Experiment I_c had not been divided into training and nontraining groups, HP/HP and HP/HP_{nt}. Therefore, food consumption and utilization data will be compared for the 13 HP/HP and 5 LP/HP rats in Experiment I_c. Though relative food intakes for the LP/HP treatment were larger than those for the HP/HP treatment during weeks 4 through 6 on the average, the values did not differ significantly.

Experiment II Male offspring in Experiment II restricted in protein after weaning, LP/LP + HP/LP, consumed more food per g metabolic mass per day than those fed adequate protein after weaning, HP/HP + LP/HP (Table 24). Values differed significantly or approached significance for

Table 23. Mean postweaning food intake of male offspring in Experiment I

Experiment Group	I _a			I _c		
	HP/HP	LP/LP	P	HP/HP	LP/HP	P
Number of rats	7	6		13	5	
	Food intake ((g/g body wt. ^{0.75})/day) x 100					
Week 4	32	37	<0.10 ^a	28	33	NS ^b
Week 5	38	39	NS	32	35	NS
Week 6	33	38	--**	31	33	NS
Week 7	33	33	NS	33	32	NS
Week 8	30	33	NS	31	31	NS
Week 12	22	25	NS	24	22	NS
Week 16	18	20	NS	20	19	NS
Week 20	18	18	NS	18	17	NS

^a0.05 < P < 0.10.

^bNS = not significant at least at 0.10 level.

**P < 0.01.

litter 2 or litter 3 or for data from the combined litters from the 4th week (0.36 vs 0.30 g food/g metabolic weight/day) through the 20th week (0.18 vs 0.16 g food/g metabolic weight/day) of life with the exception of the 6th week. During this week, average food intake/g metabolic weight/day was 0.33 for rats fed either adequate (HP/HP + LP/HP) or restricted protein (LP/LP + HP/LP) after weaning.

Food intake among animals restricted in protein before weaning (LP/LP + LP/HP) did not differ from that of HP/HP + HP/LP rats except for rats from litter 2 during the 5th week when LP/LP + LP/HP rats ate 0.40 g food/g metabolic weight/day on the average compared with 0.37 g for HP/HP + HP/LP rats.

Rats restricted in protein before weaning only, LP/HP, tended to eat more food per unit metabolic mass per day than rats adequately fed before

Table 24. Mean postweaning food intake of male offspring in Experiment II

	HP/HP	HP/LP	LP/HP	LP/LP
	Food intake ((g/g body wt. ^{0.75})/day) x 100			
Week 4 ^b	29(23) ^c	36(23)	31(26)	36(25)
Litter 2	28(16)	37(17)	31(17)	36(17)
Litter 3	31 (7)	36 (6)	31 (9)	37 (8)
Week 5 ^b	33	41	34	44
Litter 2	32	42	35	44
Litter 3	35	37	33	42
Week 6 ^b	34	32	33	35
Litter 2	35	33	34	36
Litter 3	31	30	32	33
Week 7 ^b	29	31	30	33
Litter 2	30	33	32	33
Litter 3	25	27	27	31
Week 8 ^b	26	32	28	32
Litter 2	27	32	30	31
Litter 3	24	31	24	32
Week 12 ^b	21	24	23	24
Litter 2	22	24	23	24
Litter 3	20	26	22	26
Week 16 ^b	19	19	18	20
Litter 2	18	19	19	20
Litter 3	20	20	17	19
Week 20 ^b	17	17	16	18
Litter 2	16	17	17	18
Litter 3	18	19	15	17

^a Diet before weaning x diet after weaning.

^b Arithmetic mean for litters 2 and 3.

^c Number of rats.

^d NS = not significant at least at 0.10 level.

^e 0.05 < P < 0.10.

* P < 0.05.

** P < 0.01.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP +	LP/LP +	P	HP/HP +	LP/LP +	P
	HP/LP	LP/HP		LP/HP	HP/LP	
	Food intake ((g/g body wt. ^{0.75})/day) x 100					
NS ^d	33(46)	34(51)	NS	30(49)	36(48)	-- **
NS	33(33)	34(34)	NS	30(33)	36(34)	-- *
NS	33(13)	34(17)	NS	31(16)	36(14)	-- **
NS	37	39	NS*	34	42	-- **
NS	37	40	-- *	33	43	-- **
NS	36	37	NS	34	40	-- **
NS	33	34	NS	33	33	NS
NS	34	35	NS	34	34	NS
NS	30	33	NS	32	32	NS
NS	30	31	NS	30	32	NS*
NS	32	33	NS	31	33	-- *
NS	26	29	NS	26	30	NS
NS	29	30	NS	27	32	-- **
NS	30	30	NS	29	32	-- **
NS	27	28	NS	24	32	-- **
NS**	23	24	NS	22	24	-- **
-- **	23	24	NS	22	24	-- *
NS	23	24	NS	22	26	-- **
NS	19	19	NS	18	20	<0.10 ^e
NS	19	19	NS	18	20	-- *
NS	20	18	NS	18	20	<0.10
NS	17	17	NS	16	18	NS**
NS	16	17	NS	16	18	-- **
NS	18	16	NS	16	18	NS

and after weaning (HP/HP) but less than those groups restricted after weaning, HP/LP and LP/LP. During week 12, intakes expressed in g/g metabolic weight/day for rats from litter 2 were 0.22 for HP/HP, 0.24 for HP/LP, 0.23 for LP/HP, and 0.24 for LP/LP rats. Interaction of the effect of protein restriction before and after weaning was significant during week 12. This finding indicated that the effect of protein restriction before and after weaning on food intake differed, i.e., intakes for LP/HP rats were larger than those of HP/HP rats while those for LP/LP and HP/LP rats were the same during this week.

Experiment III Relative food consumption data for male offspring from Experiment III are presented in Table 25. Rats fed 10% casein after weaning ($LP_p/LP + HP_p/LP$) consumed significantly more food per g metabolic weight per day than those fed 24% casein ($HP_p/HP + LP_p/HP$) from the 4th through the 8th weeks of life excepting week 6. Expressed in g food/g metabolic weight/day, intake for $LP_p/LP + HP_p/LP$ and $HP_p/HP + LP_p/HP$ groups, respectively, from litters 1 and 2 were 0.38 vs 0.32 for week 4, 0.42 vs 0.33 for week 5, 0.37 vs 0.29 for week 7, and 0.34 vs 0.29 for week 8; during week 6, the values were similar, 0.32 vs 0.33 g food/g metabolic weight/day.

Rats restricted in the amount of protein supplied prior to weaning ($LP_p/LP + LP_p/HP$) did not eat more food per g metabolic weight per day on the average than rats adequately nourished before weaning ($HP_p/HP + HP_p/LP$). Generally, relative food consumption for rats restricted in protein prior to weaning only, LP_p/HP , was between that of the HP_p/HP group and those of the groups restricted after weaning, HP_p/LP and LP_p/LP , however. For rats in litter 2, during week 5, intakes in g food/g metabolic weight/day were

Table 25. Mean postweaning food intake of male offspring in Experiment III

	HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP	Inter- action ^a P
	Food intake ((g/g body wt. ^{0.75})/day) x 100				
Week 4 ^b	31(8) ^c	38(6)	34(5)	39(5)	NS ^d
Litter 1	31(2)	40(1)	36(1)	42(1)	-- ^e
Litter 2	30(6)	37(5)	33(4)	38(4)	NS
Week 5 ^b	31	41	37	43	NS
Litter 1	33	41	37	49	--*
Litter 2	30	41	37	41	--*
Week 6 ^b	31	32	36	32	NS
Litter 1	32	32	37	33	--
Litter 2	30	31	36	32	NS
Week 7 ^b	29	38	30	37	NS
Litter 1	28	38	33	39	--
Litter 2	30	38	29	36	NS
Week 8 ^b	28	33	30	34	NS
Litter 1	26	32	30	35	--
Litter 2	29	33	30	34	NS
Week 12 ^b	24	25	20	24	NS
Litter 1	26	24	22	24	--
Litter 2	23	25	20	24	NS
Week 16 ^b	17	17	18	19	NS
Litter 1	19	19	19	21	--
Litter 2	17	17	18	18	NS
Week 20 ^b	16	16	16	16	NS
Litter 1	18	16	19	15	--
Litter 2	15	17	15	16	NS

^a Diet before weaning x diet after weaning.

^b Arithmetic mean for litters 1 and 2.

^c Number of rats.

^d NS = not significant at least at 0.10 level.

^e Insufficient number of observations for regression analyses.

* P<0.05.

** P<0.01.

Diet before weaning			Diet after weaning		
HP ^P /HP +	LP ^P /LP +	P	HP ^P /HP +	LP ^P /LP +	P
HP ^P /LP	LP ^P /HP		LP ^P /HP	HP ^P /LP	
Food intake ((g/g body wt. ^{0.75})/day) x 100					
34(14)	36(10)	NS	32(13)	38(11)	--*
34 (3)	39 (2)	--	33 (3)	41 (2)	--*
34(11)	36 (8)	NS	32(10)	38 (9)	--*
35	40	NS	33	42	--**
35	43	--	34	45	--**
35	39	NS	33	41	--**
31	34	NS	33	32	NS
32	35	--	34	32	--
31	34	NS	32	32	NS
33	33	NS	29	37	--*
31	36	--	30	39	--*
33	33	--	29	37	--*
30	32	NS	29	34	--*
28	32	--	27	33	--*
30	32	NS	29	34	--*
24	22	NS	23	24	NS
25	23	--	24	24	--
24	22	NS	22	24	NS
17	18	NS	18	18	NS
19	20	--	19	20	--
17	18	NS	17	17	NS
16	16	NS	16	16	NS
17	17	--	18	16	--
16	16	NS	15	16	NS

0.30 for HP_p/HP, 0.41 for HP_p/LP, 0.37 for LP_p/HP, and 0.41 for LP_p/LP rats, and the interaction of the effect of protein supply before and after weaning on food intake was significant.

Food utilization

Food efficiency ratios (FER), g weight gain/g food eaten, for experimental animals were calculated over linear portions of the growth curve from 3 to 6 and 6 to 9 weeks and for the period between 9 and 20 weeks when growth progressively decreased. An overall ratio for 3 to 20 weeks was also determined.

Experiment I Data from Experiments I_{a,c} are presented in Table 26. In Experiment I_a, rats were placed on a water deprivation schedule between the ages of 19 and 20 weeks in preparation for behavioral testing. Since food intakes decreased for most rats as a result of the water deprivation during the 20th week, FER values were determined for the periods of 9 to 19 and 3 to 19 weeks in Experiment I_a. Protein restriction before and after weaning significantly decreased food efficiency ratios during rapid growth from 3 to 6 and 6 to 9 weeks in LP/LP rats. Protein restriction did not affect food efficiency when growth rate was relatively slow, i.e., from 9 to 19 weeks. FER of the LP/LP rats (0.19) was significantly lower than that of the HP/HP animals (0.23) for the total period measured, 3 to 19 weeks.

For the first 6 weeks after weaning when growth was rapid, LP/HP animals had higher rates of utilization than HP/HP rats, but FER values were not significantly different.

Table 26. Mean postweaning food efficiency ratios of male offspring in Experiment I

Experiment Group Number of rats	I _a			I _c		
	HP/HP 7	LP/LP 6	P	HP/HP 13	LP/HP 5	P
FER ^a wk. 3-6	47 ^b	34	--*	45	50	NS ^c
FER wk. 6-9	42	30	--*	39	41	NS
FER wk. 9-20 ^d	13	13	NS	13	13	NS
FER wk. 3-20 ^d	23	19	--**	20	22	NS

^aFER = g weight gain/g food eaten.

^bMean x 100.

^cNS = not significant at least at 0.10 level.

^dFER wk. 9-19 and wk. 3-19 in Experiment I_a.

*P<0.05.

**P<0.01.

Experiment II Efficiency of food utilization was reduced significantly among rats restricted in protein after weaning (LP/LP + HP/LP) compared with the efficiency among animals adequately fed in the postweaning period (HP/HP + LP/HP) in Experiment II (Table 27). Average FER values were 0.51 vs 0.37 for weeks 3 to 6, 0.39 vs 0.31 for weeks 6 to 9, and 0.23 vs 0.21 for weeks 3 to 20 for HP/HP + LP/HP vs LP/LP + HP/LP groups, respectively.

Animals restricted in protein before weaning tended to utilize food more efficiently than unrestricted rats when they were growing rapidly, i.e., from 3 to 6 weeks and from 6 to 9 weeks. However, the FER values

Table 27. Mean postweaning food efficiency ratios of male offspring in Experiment II

	HP/HP	HP/LP	LP/HP	LP/LP
FER ^b wk. 3-6 ^c	48 ^d (23) ^e	36(23)	54(26)	38(25)
Litter 2	48 (16)	36(17)	54(17)	38(17)
Litter 3	49 (7)	36 (6)	55 (9)	38 (8)
FER wk. 6-9 ^c	39	30	39	32
Litter 2	42	31	39	32
Litter 3	31	27	37	32
FER wk. 9-20 ^c	15	15	14	15
Litter 2	14	15	15	15
Litter 3	16	15	13	15
FER wk. 3-20 ^c	23	20	22	21
Litter 2	23	20	23	21
Litter 3	23	20	22	20

^aDiet before weaning x diet after weaning.

^bFER = g weight gain/g food eaten.

^cArithmetic mean for litters 2 and 3.

^dMean x 100.

^eNumber of rats.

^fNS = not significant at least at 0.10 level.

**P<0.01.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP +	LP/LP +	P	HP/HP +	LP/LP +	P
	HP/LP	LP/HP		LP/HP	HP/LP	
NS ^f	42(46)	46(51)	NS	51(49)	37(48)	-- **
NS	41(33)	46(34)	-- **	51(33)	37(34)	-- **
NS	43(13)	47(17)	NS	52(16)	37(14)	-- **
NS	34	35	NS	39	31	-- **
NS	37	36	NS	41	32	-- **
NS	29	35	NS	34	30	-- **
NS	15	15	NS	15	15	NS
NS	15	15	NS	15	15	NS
NS	16	14	NS	15	15	NS
NS	22	22	NS	23	20	-- **
NS	22	22	NS	23	21	-- **
NS	21	22	NS	22	20	-- **

were significantly different among rats in litter 2 from 3 to 6 weeks of age only; at that time, LP/LP + LP/HP rats had an average FER of 0.46 compared with 0.41 for HP/HP + HP/LP animals. Beyond 3 to 6 weeks of age, no significant difference due to preweaning restriction was observed; therefore, catch-up growth during weeks 3 to 6 by rats restricted prior to weaning may be responsible for the unanticipated impression that preweaning protein restriction improved efficiency of food utilization after weaning.

Experiment III Food efficiency ratios for males in Experiment III are presented in Table 28. As in Experiment II, postweaning protein restriction significantly decreased food utilization for all periods measured except weeks 9 to 20 when the growth rate was declining. Average FER values for LP_p/LP + HP_p/LP vs HP_p/HP + LP_p/HP in litter 2 were 0.38 vs 0.52 during weeks 3 to 6, 0.30 vs 0.40 during weeks 6 to 9, and 0.20 vs 0.23 from 3 to 20 weeks of age.

Average FER among animals in litters 1 and 2 from 3 to 6 weeks of age for LP_p/HP rats (0.54) was larger than that of HP_p/HP animals (0.50) and that for LP_p/LP rats (0.41) was larger than that of HP_p/LP animals (0.36). Thus when data for LP_p/LP and LP_p/HP groups were combined, the average value for rats protein-restricted before weaning (0.48) was significantly larger than that achieved by HP_p/HP + HP_p/LP rats (0.44). During weeks 9 to 20, however, mean FER for HP_p/HP + HP_p/LP rats was 0.15 compared with 0.13 for LP_p/LP + LP_p/HP animals ($0.05 < P < 0.10$). Therefore, catch-up growth which occurred during weeks 3 to 6 in the rats restricted before weaning was probably primarily responsible for their improved efficiency of food utilization for a short period. Growth rates in g per week for week 3 to 6 were 31 for HP_p/HP, 26 for HP_p/LP, 31 for LP_p/HP, and 22 for LP_p/LP rats.

Table 28. Mean postweaning food efficiency ratios of male offspring in Experiment III

	HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP	Inter-action ^a P
FER ^b wk. 3-6 ^c	50 ^d (8) ^e	36(6)	54(5)	41(5)	NS ^f
Litter 1	52 (2)	39(1)	54(1)	45(1)	-- ^g
Litter 2	50 (6)	35(5)	54(4)	40(4)	NS
FER wk. 6-9 ^c	38	29	40	32	NS
Litter 1	36	32	33	30	--
Litter 2	38	29	42	32	NS
FER wk. 9-20 ^c	16	14	13	13	NS
Litter 1	16	15	15	10	--
Litter 2	16	14	13	14	NS
FER wk. 3-20 ^c	23	20	23	20	NS
Litter 1	22	21	22	18	--
Litter 2	23	20	23	20	NS

^aDiet before weaning x diet after weaning.

^bFER = g weight gain/g food eaten.

^cArithmetic mean for litters 1 and 2.

^dMean x 100.

^eNumber of rats.

^fNS = not significant at least at 0.10 level.

^gInsufficient number of observations for regression analyses.

^h0.05 < P < 0.10.

* P < 0.05.

** P < 0.01.

Diet before weaning			Diet after weaning		
HP /HP + HP _p ^p /LP	LP /LP + LP _p ^p /HP	P	HP /HP + LP _p ^p /HP	LP /LP + HP _p ^p /LP	P
44(14)	48(10)	--**	52(13)	38(11)	--*
48 (3)	50 (2)	--*	53 (3)	42 (2)	--*
43(11)	47 (8)	--	52(10)	38 (9)	--*
34	36	NS	39	30	NS
34	32	--	35	31	--*
34	37	NS	40	30	--*
15	13	<0.10 ^h	15	14	NS
15	12	--	15	12	--
15	14	NS	14	14	NS
22	21	NS	23	20	<0.10
22	20	--	22	20	--
22	22	NS	23	20	--*

Average food intakes were 62, 73, 55, and 50 g per week for the 4 groups, respectively. LP_p/LP and LP_p/HP rats were, therefore, maintaining growth rates similar to those of HP_p/HP and HP_p/LP rats on smaller amounts of food during this period of rapid growth.

Brain Development and Behavior

Brain weight

Newborn females Absolute and relative neonatal brain weights for females sacrificed shortly after birth are presented in Tables 29 and 30.

Experiment I Absolute neonatal brain weights for Experiment I were 243, 229, 227, and 239 mg for offspring of HP, LP, LP_M , and LP_H dams, respectively (Table 29). None of the values were significantly different from any of the others. Relative to body weight, brains of LP progeny were significantly larger at 43.2 mg/g body weight than those of HP offspring which averaged 40.7 mg/g of body weight. Average relative brain weight values for LP_M and LP_H progeny were intermediate to these groups and were not significantly different from those of either the HP or the LP offspring.

Experiment II Absolute brain weights for HP and LP newborn female progeny in Experiment II were not significantly different when animals from litters 2 and 3 were considered together (Table 30). A significant interaction between diet and litter, however, indicated that in the experimental sample, the effect of protein restriction differed from litter 2 to litter 3. Among litter 2 progeny, brains of pups from adequately fed dams (HP) weighed significantly more on the average (251 mg) than those of LP progeny (237 mg). In litter 3 brains from protein-restricted pups

Table 29. Mean absolute and relative brain weights of newborn female rats in Experiment I

Experimental Group		HP	LP	LP _M	LP _H
Brain	mg	243 ^{a*} (16) ¹	229 ^a (14)	227 ^a (10)	239 ^a (13)
Brain/BW ²	mg/g	40.7 ^a	43.2 ^b	42.4 ^{ab}	43.0 ^{ab}

¹Number of rats.

²BW = body weight.

*Means with the same superscripts within a line are not different ($P > 0.05$).

were larger and weighed 249 mg on the average compared with 240 mg for adequately fed neonates; these values did not differ significantly. Only 18 pups from litter 3 (9 HP and 9 LP) were available for neonatal measurements compared with 72 (23 HP and 49 LP) from litter 2. Mean birth weight for LP neonates examined in litter 3 was large, 6.15 g compared with 5.52 g for the LP newborns autopsied from litter 2. Therefore, the small number of neonates examined in litter 3 may not have been representative of the total population of neonates subjected to in utero protein restriction; consequently, the significant interaction is likely to be a spurious observation.

Relative brain weights of neonates were significantly smaller for HP (40.8 mg/g) than for LP (43.2 mg/g) offspring in litter 2 (Table 30). Relative brain weights of litter 3 newborns were 37.8 mg/g for the HP and 40.5 mg/g for the LP treatment; these values approached being significantly different ($0.05 < P < 0.10$). When the data for the 2 litters were combined,

Table 30. Mean absolute and relative brain weights of newborn and weanling female rats in Experiments II and III

Newborn pups Experiment II		HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
Brain ^a	mg	248(32) ^b	239(58)	NS ^c *	NS ^d	--*
Litter 2		251(23)	237(49)	--*	--	--
Litter 3		240 (9)	249 (9)	NS	--	--
Brain/BW ^{ae}	mg/g	39.9	42.8	NS*	NS	NS
Litter 2		40.8	43.2	--*	--	--
Litter 3		37.8	40.5	<0.10 ^f	--	--
Experiment III		HP _p	LP _p			
Brain ^a	mg	251(19)	232(14)	<0.10	NS	NS
Litter 1		253(11)	225 (4)	NS*	--	--
Litter 2		248 (8)	235(10)	--*	--	--
Brain/BW ^a	mg/g	40.8	43.9	--*	NS	NS
Litter 1		42.2	40.5	NS*	--	--
Litter 2		38.7	45.2	--*	--	--

^aArithmetic mean for litters 2 and 3, Experiment II; for litters 1 and 2, Experiment III.

^bNumber of rats.

^cNS = not significant at least at 0.10 level.

^d-- not applicable to analysis of individual litters.

^eBW = body weight.

^f0.05 < P < 0.10.

* P < 0.05.

Table 30. (Continued)

Weanling pups Experiment II		HP	LP	<u>Statistical evaluation</u>		
				Diet	Litter	Diet x litter
Brain ^a	g	1.44(33)	1.36(36)	--*	NS	NS
Litter 2		1.46(20)	1.38(22)	--*	--	--
Litter 3		1.41(13)	1.33(14)	--*	--	--
Brain/BW ^a	g/100 g	2.97	4.22	--**	NS	NS
Litter 2		2.94	4.22	--**	--	--
Litter 3		3.02	4.23	--**	--	--
Experiment III		HP _p	LP _p			
Brain ^a	g	1.40(24)	1.21(13)	--**	NS	NS
Litter 1		1.41(12)	1.21 (6)	--**	--	--
Litter 2		1.38(12)	1.21 (7)	<0.10	--	--
Brain/BW ^a	g/100 g	3.18	5.30	--**	NS	NS
Litter 1		3.10	4.49	--*	--	--
Litter 2		3.27	6.00	--*	--	--

**
P<0.01.

however, values of 39.9 and 42.8 mg/g for HP and LP progeny, respectively, did not differ significantly. This unexpected finding was probably due to the uneven numbers in the litters and experimental groups within litters and to the approximate nature of the regression analyses which resulted in spurious observations on occasion. The analyses of data from the individual litters are believed to be more accurate than the analyses of data from the combined litters, however; therefore, protein restriction in utero is believed to have affected brain weight less than body weight in neonates in Experiment II.

Experiment III The trend noted in Experiment II for reduced absolute and increased relative brain weights among newborn females as a

result of gestational protein restriction was observed also in Experiment III (Table 30). When data from litters 1 and 2 were combined, brains from HP_p pups weighed 251 mg on the average while those of LP_p pups averaged 232 mg ($0.05 < P < 0.10$). Due to large variation within both groups and the small number (4) of LP_p neonates autopsied from litter 1, brains of adequately nourished pups did not differ significantly from those of protein-restricted animals; the brains weighed 253 and 225 mg, respectively. Brains of HP_p progeny were significantly heavier than those of LP_p offspring in litter 2, however (248 mg for HP_p vs 235 mg for LP_p).

Relative brain weights of HP_p pups were significantly smaller than those of LP_p pups when litters 1 and 2 were considered together (HP 40.8 vs LP_p 43.9 mg/g) or when only litter 2 was considered (HP_p 38.7 vs LP_p 45.2 mg/g). Values for pups from litter 1, 42.2 mg/g for HP_p rats and 40.5 mg/g for LP_p pups, did not differ significantly.

Weanling females Absolute and relative brain weights of females sacrificed at weaning (3 weeks of age) for both Experiments II and III are presented in Table 30.

Experiment II Brains of adequately nourished female pups (HP) were significantly heavier at 3 weeks of age than those of pups born of and suckled by dams fed restricted quantities of protein during gestation and lactation (LP) (Table 30). Brains of HP rats from litters 2 and 3 weighed 1.44 g on the average compared with 1.36 g for brains of LP rats. Brain weight accounted for significantly smaller portions of body weight among adequately nourished weanlings (about 3%) than among protein-restricted pups (about 4.2%).

Experiment III Brains of HP_p weanlings from litters 1 and 2 weighed 1.40 g on the average while those for LP_p pups averaged 1.21 g (Table 30). These values and those for litter 1 (1.41 and 1.21 g, respectively, for HP_p and LP_p pups) differed significantly, and the tendency for heavier brain weight among HP_p than LP_p rats approached significance ($0.05 < P < 0.10$) in litter 2. Brain weight per unit of body weight was significantly different for HP_p and LP_p treatments when data for litters 1 and 2 were combined or examined separately. The average relative brain weight for HP_p weanlings from the combined litters was 3.18% while that for LP_p weanlings was 5.30%.

Adult males Brain weight data for males sacrificed at approximately 6 to 10 months of age following behavioral training are presented in Tables 31, 32, and 33. Absolute and relative weights of the total brain were compared. In addition, the brain was divided into cortical and subcortical sections so that weight and cholinesterase (ChE) activity of these sections could be examined. Ratios of cortical:subcortical weight and ChE activity were also determined.

Experiment I Body weight was reduced to a greater extent than brain weight among adult males restricted in protein before and after weaning (LP/LP) in Experiment I_a (Table 31). Relative brain weights for protein-restricted animals averaged 0.54 g/100 g body weight compared with 0.44 for HP/HP rats ($P < 0.05$). Total brain, cortical and subcortical weights, and cortical/subcortical ratios (C/SC) did not differ significantly between experimental groups in Experiment I_a.

Brain weight accounted for an average of 0.47% of total body weight in 4_g 15₁/15 rats in Experiment I_b (Table 31). This value was significantly

Table 31. Mean absolute and relative brain weights of adult male rats in Experiment I

Experiment Group	Number of rats	I _a			I _b			I _c				
		HP/HP 7	LP/LP 6	P	St/St 5	4 ^g 15 ¹ / ₁₅	P	P ^a	HP/HP _{nt} 7	HP/HP 6	LP/HP 4	P ^b
Brain	g	2.099	2.026	NS ^c	2.270	2.287	NS	NS	2.207	2.197	2.084	NS
Brain/BW ^d	g/100 g	0.44	0.54	--*	0.39	0.47	--**	NS	0.44	0.45	0.47	NS
Cortex	g	0.830	0.825	NS	0.924	0.923	NS	NS	0.926	0.935	0.874	NS
Subcortex	g	1.148	1.122	NS	1.264	1.255	NS	NS	1.211	1.182	1.135	NS
C/SC ^e x 10 ³		729	744	NS	733	744	NS	NS	767	797	774	NS

^aHP/HP_{nt} vs HP/HP.

^bHP/HP vs LP/HP.

^cNS = not significant at least at 0.10 level.

^dBW = body weight.

^eC/SC = g cortex/g subcortex.

*P<0.05.

**P<0.01.

larger than 0.39%, the relative brain weight of St/St rats. Values for total brain, cortical and subcortical weights, and C/SC ratios were not significantly different between groups in Experiment I_b.

Brain weight and cholinesterase activity of HP/HP rats in Experiment I_c were compared with those of animals which were treated identically by diet, in handling, and water deprivation; the latter animals were not subjected to behavioral training and are therefore designated as HP/HP_{nt}. Animals restricted in protein prior to weaning only (LP/HP) were also compared with HP/HP rats. Total brain, cortical and subcortical weights, relative brain weights, and C/SC ratios did not differ significantly for HP/HP and HP/HP_{nt} treatments nor for LP/HP and HP/HP treatments (Table 31).

Experiment II Absolute and relative brain weight data for adult males in Experiment II are presented in Table 32. In general, the preweaning diet affected brain weight to a greater extent than the postweaning diet. Such a finding might be expected since cell division in rat brains is largely completed before weaning (Winick and Noble, 1966). Postweaning brain growth has been shown to involve increased cell size rather than cell number by these same investigators.

Among animals in litter 2, brains of rats given adequate protein before weaning (HP/HP + HP/LP) weighed 2.251 g, a value significantly larger than 2.113 which was the average brain weight for rats restricted in protein before weaning (LP/LP + LP/HP). Total brain weights due to preweaning diet approached being significantly different when litters 2 and 3 were combined ($0.05 < P < 0.10$) but not when litter 3 was analyzed separately.

Postweaning treatment did not influence total brain weight when rats from litters 2 and 3 were combined or when only litter 2 was examined.

Table 32. Mean absolute and relative brain weights of adult male rats in Experiment II

		HP/HP	HP/LP	LP/HP	LP/LP
Brain ^b	g	2.192(22) ^c	2.237(23)	2.115(26)	2.098(25)
Litter 2		2.247(15)	2.255(17)	2.130(17)	2.095(17)
Litter 3		2.075 (7)	2.183 (6)	2.087 (9)	2.102 (8)
Brain/BW ^{bf}	g/100 g	0.46	0.51	0.52	0.54
Litter 2		0.44	0.51	0.50	0.53
Litter 3		0.50	0.52	0.58	0.54
Cortex ^b	g	0.887	0.907	0.881	0.853
Litter 2		0.898	0.894	0.901	0.865
Litter 3		0.865	0.941	0.843	0.827
Subcortex ^b	g	1.225	1.243	1.146	1.151
Litter 2		1.266	1.266	1.132	1.129
Litter 3		1.137	1.177	1.172	1.197
C/SC ^{bg} x 10 ³		730	735	775	748
Litter 2		711	710	802	774
Litter 3		770	806	722	692

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e0.05 < P < 0.10.

^fBW = body weight.

^gC/SC = g cortex/g subcortex.

* P < 0.05.

** P < 0.01.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP +	LP/LP +	P	HP/HP +	LP/LP +	P
	HP/LP	LP/HP		LP/HP	HP/LP	
NS ^d	2.215(45)	2.106(51)	<0.10 ^e	2.150(48)	2.164(48)	NS
NS	2.251(32)	2.113(34)	--**	2.184(32)	2.176(34)	NS
NS	2.125(13)	2.094(17)	NS	2.082(16)	2.137(14)	<0.10
NS	0.49	0.53	NS**	0.50	0.52	NS**
NS	0.48	0.51	--	0.47	0.52	--**
NS	0.51	0.56	NS	0.54	0.53	NS
NS	0.897	0.867	NS	0.884	0.879	NS
NS	0.896	0.883	NS	0.900	0.880	NS
NS	0.900	0.836	NS	0.853	0.876	<0.10
NS	1.234	1.148	--*	1.182	1.195	NS
NS	1.266	1.131	--**	1.195	1.198	NS
NS	1.155	1.184	NS	1.157	1.188	NS
NS	733	761	NS	754	742	NS
NS	711	788	<0.10	760	742	NS
NS	787	708	NS	743	741	NS

However, rats in litter 3 fed 10% casein after weaning, LP/LP + HP/LP, appeared to have heavier brains (2.137 g) than those fed 24% casein after weaning, HP/HP + LP/HP (2.082 g). This unexpected finding approaches statistical significance ($0.05 < P < 0.10$). Therefore, the data for the 4 treatment groups were examined. Mean brain weights were 2.075 g for HP/HP, 2.183 g for HP/LP, 2.087 g for LP/HP, and 2.102 g for LP/LP rats. Brains from the HP/LP treatment were from 81 to 108 mg larger on the average than those of any other group. The LP/LP rats also had brains with a mean weight larger than HP/HP and LP/HP. As a result, when data from these 2 groups were combined as an estimate of the postweaning effect of protein restriction, HP/LP influence was accentuated. With the exception of HP/HP and HP/LP treatments from litter 3, little variation in brain weight occurred between littermates receiving the same diet before weaning in either litter 2 or 3. It seems unlikely, therefore, that protein restriction after weaning among HP/LP rats in litter 3 increased brain weight; biological variation between rats assigned to HP/HP and HP/LP groups at weaning is perhaps a more logical explanation.

Mean relative brain weight for litter 2 was 0.51% when rats were restricted in protein before weaning, LP/LP + LP/HP, compared with 0.48% for animals fed adequate protein before weaning, HP/HP + HP/LP. Postweaning protein restriction (LP/LP + HP/LP) among litter 2 animals resulted in an average relative brain weight of 0.52% compared with 0.47% for animals given 24% casein after weaning (HP/HP + HP/LP). Average relative brain weights expressed as g/100 g body weight among litter 2 animals were 0.44 for HP/HP, 0.51 for HP/LP, 0.50 for LP/HP, and 0.53 for LP/LP treatments. The effects of an adequate or restricted protein supply were accentuated by

the HP/HP and LP/LP treatments. However, restriction before or after weaning produced significant effects on relative brain weight in litter 2 but not in litter 3 nor in the combined litters.

Protein restriction before weaning did not influence cortical weight of brains among adult males in Experiment II. However, rats given adequate protein after weaning tended to have smaller cortices in litter 3 than rats restricted in protein after weaning, LP/LP + HP/LP; cortices from the former treatment (HP/HP + LP/HP) weighed 0.853 g while those from the latter weighed 0.876 g ($0.05 < P < 0.10$). Cortices for the HP/LP group weighed an average of 76 to 114 mg more than those of the other groups and therefore followed the same trend as did the total brain weights among groups. Cortical weights of the littermates from the HP/HP and HP/LP groups in litter 3 differed by 76 mg while those of the other 2 groups of littermates, LP/LP and LP/HP, differed by only 16 mg. Among rats from litter 2, cortical weight of littermates differed by 4 (HP/HP and HP/LP) and 36 (LP/LP and LP/HP) mg. Therefore, it is not likely that the postweaning protein restriction increased cortical weight. Rather this unexpected finding like the previously discussed results about the effect of postweaning restriction on brain weight is probably due to biological variation among littermates assigned at random to HP/HP and HP/LP groups when weaned.

The effect of diet, particularly diet before weaning, was more apparent among subcortices than cortices in Experiment II. Subcortical weights for rats supplied adequate protein before weaning were significantly larger than those for rats restricted in protein before weaning when litter 2 or litters 2 and 3 together were considered. Average subcortical weights among rats from litter 2 were 1.266 g for HP/HP + HP/LP groups and 1.131 g

for LP/LP + LP/HP groups. Subcortical weights were not significantly different as a result of preweaning restriction among litter 3 rats or as a result of postweaning restriction among rats from litters 2 and 3 or either litter alone.

Protein restriction before or after weaning did not significantly affect the C/SC ratio among rats in Experiment II although rats in litter 2 restricted before weaning tended to have larger C/SC ratios (788) than those adequately fed before weaning (711) ($0.05 < P < 0.10$).

Experiment III Neither preweaning nor postweaning treatments affected total nor subcortical brain weights of rats in Experiment III (Table 33). Total and subcortical brain weights, respectively, averaged 2.162 and 1.184 g for $HP_p/HP + HP_p/LP$ and 2.038 and 1.104 g for $LP_p/LP + LP_p/HP$ treatments; mean total and subcortical brain weights for $HP_p/HP + LP_p/HP$ and $LP_p/LP + HP_p/LP$ groups were 2.136 and 1.180 g and 2.080 and 1.116 g, respectively.

Protein restriction before or after weaning reduced body weight to a greater extent than brain weight for rats from litters 1 and 2 in Experiment III (Table 33). Only the postweaning restriction was significant for litter 2. Mean relative brain weight for rats from litters 1 and 2 given inadequate quantities of protein before weaning, $LP_p/LP + LP_p/HP$, was 0.55 g/100 g body weight compared with 0.48 g/100g for $HP_p/HP + HP_p/LP$ rats. Rats restricted in protein after weaning ($LP_p/LP + HP_p/LP$) had an average relative brain weight of 0.54 g/100 g which was significantly larger than 0.48 g/100 g for rats adequately nourished after weaning ($HP_p/HP + LP_p/HP$).

Table 33. Mean absolute and relative brain weights of adult male rats in Experiment III

		HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
Brain ^b	g	2.203(8) ^c	2.108(6)	2.028(5)	2.047(5)
Litter 1		2.148(2)	2.166(1)	2.018(1)	2.067(1)
Litter 2		2.221(6)	2.097(5)	2.031(4)	2.042(4)
Brain/BW ^{bf}	g/100 g	0.47	0.50	0.50	0.60
Litter 1		0.45	0.51	0.51	0.62
Litter 2		0.48	0.49	0.50	0.60
Cortex ^b	g	0.877	0.898	0.823	0.860
Litter 1		0.839	0.872	0.801	0.810
Litter 2		0.889	0.904	0.829	0.873
Subcortex ^b	g	1.225	1.130	1.110	1.099
Litter 1		1.181	1.186	1.120	1.155
Litter 2		1.239	1.119	1.108	1.084
C/SC ^{bh} x 10 ³		720	797	746	785
Litter 1		714	736	716	701
Litter 2		721	809	753	806

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient observations for regression analyses.

^fBW = body weight.

^g0.05 < P < 0.10.

^hC/SC = g cortex/g subcortex.

* P < 0.05.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP /HP +	LP /LP +	P	HP /HP +	LP /LP +	P
	HP _p ^p /LP	LP _p ^p /HP		LP _p ^p /HP	HP _p ^p /LP	
NS ^d	2.162(14)	2.038(10)	NS	2.136(13)	2.080(11)	NS
-- ^e	2.154 (3)	2.043 (2)	--	2.104 (3)	2.117 (2)	--
NS	2.164(11)	2.036 (8)	NS	2.145(10)	2.072 (9)	NS
--*	0.48	0.55	--*	0.48	0.54	--*
--	0.47	0.56	--	0.47	0.56	--
<0.10 ^g	0.48	0.55	NS	0.49	0.54	--*
NS	0.886	0.842	--*	0.856	0.891	NS
--	0.850	0.806	--	0.826	0.841	--
NS	0.896	0.851	<0.10	0.865	0.890	<0.10
NS	1.184	1.104	NS	1.180	1.116	NS
--	1.183	1.138	--	1.161	1.171	--
NS	1.184	1.096	NS	1.186	1.104	NS
NS	753	765	NS	730	792	<0.10
--	722	708	--	715	718	--*
NS	761	779	NS	734	808	--*

A significant interaction of the effects of diet before and after weaning on relative brain weight was present when data from litters 1 and 2 were combined; the interaction approached significance ($0.05 < P < 0.10$) for litter 2 alone. Average relative brain weights for litters 1 and 2 were 0.47, 0.50, 0.50, and 0.60 g/100 g body weight for HP_p/HP , HP_p/LP , LP_p/HP , and LP_p/LP groups, respectively. Protein restriction either before (LP_p/HP) or after (HP_p/LP) weaning only resulted in slightly altered relative brain weights while restriction during both periods (LP_p/LP) accentuated the distortion between brain and body weight.

Adult rats restricted in protein prior to weaning had significantly smaller cortices than those given adequate protein during gestation and suckling (0.842 g for $LP_p/LP + LP_p/HP$ vs 0.886 g for $HP_p/HP + HP_p/LP$) when rats from litters 1 and 2 were considered. The effect of restriction before weaning on cortical weight approached significance for litter 2 ($0.05 < P < 0.10$).

The effect of postweaning restriction on cortical weight was not significant for the combined litters but approached significance ($0.05 < P < 0.10$) among litter 2 rats when average cortical weights were 0.865 and 0.890 g for $HP_p/HP + LP_p/HP$ and $LP_p/LP + HP_p/LP$, respectively. Cortical weight for individual experimental treatments were 0.889 g for HP_p/HP , 0.904 g for HP_p/LP , 0.829 g for LP_p/HP , and 0.873 g for LP_p/LP . Cortices of LP_p/HP rats were 44 mg lighter on the average than those of their littermates (LP_p/LP) and 60 and 75 mg lighter than those of HP_p/HP and HP_p/LP animals, respectively. Therefore, when data for the LP_p/HP group were combined with those for the LP_p/LP group, the next smallest value, as an estimate of the effect of protein restriction before weaning or with those of the HP_p/HP group,

the 3rd smallest value, as an estimate of the effect of adequate protein after weaning, the resulting means were smaller than those obtained when data for HP_p/HP and HP_p/LP or LP_p/LP and HP_p/LP groups were combined. It is not likely that adequate protein after weaning inhibited development of the LP_p/HP cortices more than restricted protein inhibited those of the LP_p/LP treatment. Biological variation or experimental error in separation of cortical and subcortical brain sections, therefore probably provide a more reasonable explanation than dietary treatment for the trend toward larger cortical weights observed with postweaning protein restriction among rats in litter 2.

Protein restriction before weaning did not significantly influence C/SC ratios among rats from Experiment III. For litter 2, C/SC ratios were significantly larger than for rats which underwent protein restriction after weaning compared with those of rats adequately nourished postweaning (808 for $LP_p/LP + HP_p/LP$ vs 734 for $HP_p/HP + LP_p/HP$). The tendency for larger C/SC ratios with postweaning protein restriction approached significance ($0.05 < P < 0.10$) when data from the 2 litters were combined. Although cortical and subcortical weights observed among LP_p/LP and HP_p/LP rats were not significantly different from those of HP_p/HP and LP_p/HP animals, the actual values varied in opposite directions; therefore C/SC ratios accentuated the variation. Since a similar effect was not present in Experiment II, the biological significance of this finding is doubtful. It may instead be a result of experimental errors in separation of brain sections and biological variations among experimental animals.

Brain cholinesterase activity

Newborn females Specific cholinesterase (ChE/g brain) and total (ChE/brain) activities for newborn females sacrificed in Experiments I, II, and III are found in Tables 34 and 35.

Experiment I Both specific and total brain ChE activities were somewhat lower among newborns deprived of adequate protein before birth than among those born of adequately nourished dams (Table 34). None of the values differed significantly from any of the others, however.

Experiment II Compared with values for protein-restricted pups (LP), specific and total ChE activities for adequately nourished neonates (HP) were increased in litter 2, Experiment II (Table 35). Specific ChE activity for HP pups was 1.76 moles acetylthiocholine (ASCh) hydrolyzed per minute $\times 10^6$ on the average compared with 1.61 for LP neonates; total brain ChE values were 0.44 vs 0.38 moles ASCh hydrolyzed per minutes $\times 10^6$ for HP and LP neonates, respectively. Although adequately nourished newborn pups from litter 3 had higher specific and total ChE activities on the average than protein-restricted pups, the differences were smaller than those between groups in litter 2. As a result, the effect of in utero protein restriction on brain ChE was not significant among pups from litter 3 or when pups from litters 2 and 3 were considered together.

Experiment III Data for specific and total ChE activities for newborn females from Experiment III are presented in Table 35. The differences between means for HP and LP rats in Experiment III were smaller than those observed among neonates in Experiment II, and fewer newborn pups were measured in Experiment III than in Experiment II. As a result, neither

Table 34. Mean specific and total brain ChE activities of newborn female rats in Experiment I

		HP	LP	LP _M	LP _H
ChE/g brain	R ¹ x 10 ⁶	1.75 ^{a*} (16) ²	1.68 ^a (14)	1.68 ^a (10)	1.66 ^a (13)
ChE/brain	R x 10 ⁶	0.43 ^a	0.39 ^a	0.38 ^a	0.39 ^a

¹R = rate in moles ASCh hydrolyzed per minute.

²Number of rats.

*Means with the same superscripts within a line are not different ($P > 0.05$).

specific nor total brain ChE values differed significantly for litter 1 or 2 or for the combined litters.

Weanling females Specific ChE activity in the brains of rats has been shown to increase rapidly in early life (Im et al., 1971). In the present experiment, average activity of this enzyme increased approximately 4 fold between birth and 21 days of age for both adequately nourished and protein-restricted female pups (Table 35). Average specific brain ChE activities for all newborn pups in Experiments II and III, respectively, were 1.64 and 1.60 moles ASCh hydrolyzed per minute x 10⁶. For all weanlings, mean specific brain ChE activities were 6.65 and 6.43 moles ASCh hydrolyzed per minute x 10⁶ in Experiments II and III, respectively.

Experiment II Specific brain ChE activity among females weaned in Experiment II was not different between adequately nourished and protein-restricted groups for litter 2 or 3 or the combined litters (Table 35). Because brains of adequately nourished pups (HP) were larger than

Table 35. Mean specific and total brain ChE activities of newborn and weanling female rats in Experiments II and III

Newborn pups Experiment II		HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
ChE/g brain ^a	R ^b x 10 ⁶	1.74(32) ^c	1.61(58)	NS ^d *	NS ^e	NS
Litter 2		1.76(23)	1.61(49)	--*	--	--
Litter 3		1.70 (9)	1.62 (9)	NS	--	--
ChE/brain ^a	R x 10 ⁶	0.43	0.39	NS*	NS	NS
Litter 2		0.44	0.38	--*	--	--
Litter 3		0.41	0.40	NS	--	--
Experiment III		HP _p	LP _p			
ChE/g brain ^a	R x 10 ⁶	1.63(19)	1.55(14)	NS	NS	NS
Litter 1		1.69(11)	1.58 (4)	NS	--	--
Litter 2		1.55 (8)	1.53(10)	NS	--	--
ChE/brain ^a	R x 10 ⁶	0.41	0.36	NS	NS	NS
Litter 1		0.43	0.36	NS	--	--
Litter 2		0.38	0.36	NS	--	--

^aMean for litters 2 and 3, Experiment II; for litters 1 and 2, Experiment III.

^bR = rate in moles ASCh hydrolyzed per minute.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e-- not applicable to analysis of individual litters.

*P < 0.05.

Table 35. (Continued)

Weanling pups Experiment II		HP	LP	Statistical evaluation		
				Diet	Litter	Diet x litter
ChE/g brain ^a	R x 10 ⁶	6.63(33)	6.66(36)	NS	NS	NS
Litter 2		6.81(20)	6.57(22)	NS	--	--
Litter 3		6.34(13)	6.81(14)	NS	--	--
ChE/brain ^a	R x 10 ⁶	9.55	9.04	<0.10 ^f	NS	NS
Litter 2		9.93	9.03	--**	--	--
Litter 3		8.97	9.06	NS	--	--
Experiment III		HP _p	LP _p			
ChE/g brain ^a	R x 10 ⁶	6.58(24)	6.28(13)	NS	<0.10	NS
Litter 1		6.27(12)	5.76 (6)	NS	--	--
Litter 2		6.89(12)	6.72 (7)	NS	--	--
ChE/brain ^a	R x 10 ⁶	9.19	7.58	NS*	--**	--*
Litter 1		8.85	6.96	--*	--	--
Litter 2		9.54	8.11	NS	--	--

^f 0.05 < P < 0.10.

** P < 0.01.

those of restricted animals (LP), average total ChE activity per brain was significantly higher for the HP (9.93 moles ASCh hydrolyzed per minute x 10⁶) than for the LP (9.03 moles) treatment in litter 2; the values from the combined litters approached being significantly different (0.05 < P < 0.10). Total ChE activity in brains of HP and LP groups from litter 3 did not differ significantly.

Experiment III The actual values for specific activities for ChE in brains of female weanlings tended to be higher among adequately nourished than among protein-restricted animals in Experiment III (Table 35). Since brains of adequately nourished weanlings were generally heavier than those of protein-restricted weanlings, total ChE activities were gen-

erally higher also among adequately nourished pups. However, the specific ChE activities due to diet were not significantly different among rats from litter 1 or litter 2 or when data from the litters were combined. Total brain ChE activities were significantly higher for the HP_p than for the LP_p treatment in litter 1 (8.85 vs 6.96 moles ASCh hydrolyzed per minute $\times 10^6$). Specific ChE activities in brains of rats in litter 2 were generally higher than those in brains of litter 1 rats, and the tendency approached significance ($0.05 < P < 0.10$). Total ChE activity per brain was significantly higher in brains of rats in litter 2 than in brains of litter 1 animals. A significant interaction of diet and litter on total ChE activity per brain was also observed, indicating that the effect of diet differed from litter 1 to litter 2.

Adult males Mean specific and total ChE activities for whole brain, cortex, subcortex, and the ratios of cortical to subcortical activities (C:SC ChE ratio) for adult males from Experiments I, II, and III are presented in Tables 36, 37, and 38. The mean specific brain ChE activity for adult males in these experiments ranged from 6.90 to 8.70 moles ASCh hydrolyzed per minute $\times 10^6$, values about 1.33 times the activities observed in weanling females.

Though information regarding variation of brain ChE activity with sex is not available, Dobbing and McCance (1964) reported no significant sexual differences in brain weight until 21 days and no differences in specific brain cholesterol through 56 days among rats suckled in large and small litters then fed adequately after weaning. Presumably as a result of sexual differences in brain weight, total cholesterol activity did become significantly different in male and female rats by 35 days of age. In the

present studies, average specific ChE activities of weanling females (5.76 to 6.89 moles ASCh hydrolyzed per minute $\times 10^6$) were similar to those reported by Im et al. (1971) for male weanlings adequately nourished or restricted during suckling; these values ranged from 6.61 to 6.92 moles ASCh hydrolyzed per minute $\times 10^6$. Therefore, values for neonate and weanling females observed in the present studies should have been comparable to those of their male littermates at the same ages and may be compared in a developmental sequence with those of males measured as adults at 35 to 45 weeks of age in Experiment I and 26 to 28 weeks of age in Experiments II and III.

Experiment I Specific and total ChE activities for cortex, subcortex, and whole brain and C:SC ChE ratios did not differ between rats restricted in protein (LP/LP) and those adequately nourished (HP/HP) both before and after weaning in Experiment I_a (Table 36). Neither did values for ChE activity in brains of St/St and $4 \frac{15}{15}$ rats differ in Experiment I_b (Table 36). Because Krech et al. (1962) hypothesized that brain ChE activity increased with maze training, values for brain ChE activities of adequately nourished animals trained in the visual discrimination maze (HP/HP) in Experiment I_c were compared with those of adequately nourished animals not trained (HP/HP_{nt}). No differences were observed due to maze training (Table 36). Protein restriction before weaning (LP/HP) also did not affect ChE activities significantly in Experiment I_c (Table 36).

Experiment II Mean ChE activities for rats in Experiment II are presented in Table 37. Protein restriction before weaning (LP/LP + LP/HP vs HP/HP + HP/LP) resulted in a significant effect on specific cortical and total subcortical ChE activities and the C:SC ChE ratio of rats in

Table 36. Mean brain ChE activities of adult male rats in Experiment I

Experiment Group	Number of rats	I _a			I _b				I _c			
		HP/HP 7	LP/LP 6	P	St/St 5	4 8	15 ₁ 7 ¹	15 P	P ^a	HP/HP _{nt} 7	HP/HP 6	LP/HP 4
ChE/g cortex	R ^c × 10 ⁶	8.18	7.34	NS ^d	8.26	7.23	NS	NS	7.79	7.84	7.78	NS
ChE/cortex	R × 10 ⁶	6.83	6.10	NS	7.69	6.72	NS	NS	7.24	7.37	6.79	NS
ChE/g subcortex	R × 10 ⁶	8.36	9.31	NS	8.33	8.35	NS	NS	7.56	7.90	7.84	NS
ChE/subcortex	R × 10 ⁶	9.55	10.49	NS	10.56	10.47	NS	NS	9.16	9.37	8.89	NS
C:SC ChE ratio ^e × 10 ³		993	808	NS	1012	893	NS	NS	1051	1002	998	NS
ChE/g brain	R × 10 ⁶	8.29	8.53	NS	8.33	7.90	NS	NS	7.68	7.90	7.80	NS
ChE/brain	R × 10 ⁶	17.38	17.27	NS	18.93	18.03	NS	NS	16.94	17.36	16.26	NS

^aHP/HP_{nt} vs HP/HP.

^bHP/HP vs LP/HP.

^cR = rate in moles ASCh hydrolyzed per minute.

^dNS = not significant at least at 0.10 level.

^eC:SC ratio = (ChE/g cortex)/(ChE/g subcortex).

Table 37. Mean brain ChE activities of adult male rats in Experiment II

		HP/HP	HP/LP	LP/HP	LP/LP
ChE/g cortex ^b	R ^c x 10 ⁶	6.85(22) ^d	6.83(23)	7.23(26)	7.33(25)
Litter 2		6.52(15)	6.87(17)	7.50(17)	7.64(17)
Litter 3		7.56 (7)	6.72 (6)	6.72 (9)	6.68 (8)
ChE/cortex ^b	R x 10 ⁶	6.07	6.20	6.43	6.26
Litter 2		5.85	6.15	6.83	6.61
Litter 3		6.55	6.35	5.67	5.53
ChE/g subcortex ^b	R x 10 ⁶	8.12	7.69	7.96	7.90
Litter 2		8.22	7.94	8.12	7.91
Litter 3		7.92	6.98	7.65	7.88
ChE/subcortex ^b	R x 10 ⁶	9.94	9.61	9.12	9.14
Litter 2		10.36	10.09	9.19	8.99
Litter 3		9.03	8.25	8.97	9.45
C:SC ChE ratio ^{bf} x 10 ³		860	908	925	940
Litter 2		813	882	941	981
Litter 3		960	982	896	853
ChE/g brain ^b	R x 10 ⁶	7.64	7.36	7.67	7.65
Litter 2		7.57	7.52	7.88	7.77
Litter 3		7.79	6.90	7.26	7.40
ChE/brain ^b	R x 10 ⁶	16.73	16.45	16.22	16.03
Litter 2		17.00	16.94	16.79	16.25
Litter 3		16.15	15.05	15.15	15.56

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cR = rate in moles ASCh hydrolyzed per minute.

^dNumber of rats.

^eNS = not significant at least at 0.10 level.

^fC:SC ChE ratio = (ChE/g cortex)/(ChE/g subcortex).

*P<0.05.

**P<0.01.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^e	6.84(45)	7.28(51)	NS*	7.06(48)	7.09(48)	NS
NS	6.71(32)	7.57(34)	--*	7.04(32)	7.26(34)	NS
NS	7.17(13)	6.70(17)	NS	7.09(16)	6.70(14)	NS
NS	6.14	6.35	NS	6.27	6.23	NS
NS	6.01	6.72	NS	6.37	6.38	NS
NS	6.46	5.60	NS	6.05	5.88	NS
NS	7.90	7.93	NS	8.03	7.80	NS
NS	8.07	8.01	NS	8.16	7.93	NS
NS	7.49	7.76	NS	7.77	7.50	NS
NS	9.77	9.13	NS*	9.49	9.36	NS
NS	10.22	9.09	--*	9.74	9.54	NS
NS	8.67	9.20	NS	8.99	8.94	NS
NS	884	933	NS*	895	925	NS
NS	850	961	--*	881	931	NS
NS	970	876	NS	924	908	NS
NS	7.50	7.66	NS	7.66	7.51	NS
NS	7.55	7.82	NS	7.74	7.64	NS*
--**	7.38	7.32	NS	7.49	7.19	--*
NS	16.59	16.13	NS	16.45	16.23	NS
NS**	16.97	16.52	NS	16.89	16.60	NS**
--**	15.64	15.34	NS	15.59	15.34	--

litter 2. Specific cortical ChE activities (7.57 vs 6.71 moles ASCh hydrolyzed per minute $\times 10^6$) and CSC:ChE ratios (961 vs 850) were increased, and total subcortical ChE activity (9.09 vs 10.22 moles ASCh hydrolyzed per minute $\times 10^6$) was decreased by preweaning protein restriction. No other values for specific or total ChE activity among brains of animals in litter 2, litter 3, or litters 2 and 3 combined differed significantly as a result of preweaning restriction.

Specific and total ChE activities for the whole brain among litter 3 rats were significantly reduced by postweaning protein restriction (LP/LP + HP/LP vs HP/HP + LP/HP). An interaction between diet before and after weaning was significant also in litter 3 for both specific and total whole brain ChE activity. Average specific ChE activities for brains of animals in litter 3 expressed as moles ASCh hydrolyzed per minute $\times 10^6$ were 7.79 for HP/HP, 6.90 for HP/LP, 7.26 for LP/HP, and 7.40 for LP/LP treatments, respectively. The time of initiation and duration of protein restriction appeared to influence the final value; protein restriction introduced after weaning (HP/LP) reduced ChE activity more severely than protein restriction both before and after weaning (LP/LP). No additional parameters of ChE activity measured varied significantly due to postweaning protein restriction among brains of rats from either litter 2 or litter 3 or the combined litters.

Experiment III Protein restriction before weaning ($LP_p/LP + LP_p/HP$ vs $HP_p/HP + HP_p/LP$) tended to decrease specific and total cortical ChE activity and C:SC ChE ratios among adult males in Experiment III (Table 38). Average specific cortical ChE activity expressed in moles ASCh hydrolyzed per minute $\times 10^6$ for rats from litters 1 and 2 was 8.40 for rats

Table 38. Mean brain ChE activities of adult male rats in Experiment III

		HP _p /HP	HP _p /LP	LP _p /LP	LP _p /LP
ChE/g cortex ^b	R ^c x 10 ⁶	8.15(8) ^d	8.73(6)	7.59(5)	7.97(5)
Litter 1		7.00(2)	8.65(1)	6.98(1)	7.15(1)
Litter 2		8.54(6)	8.75(5)	7.74(4)	8.17(4)
ChE/cortex ^b	R x 10 ⁶	7.18	7.87	6.29	6.84
Litter 1		5.98	7.55	5.59	5.79
Litter 2		7.58	7.93	6.46	7.11
ChE/g subcortex ^b	R x 10 ⁶	8.52	8.22	8.65	8.49
Litter 1		9.30	8.62	9.92	9.31
Litter 2		8.25	8.14	8.34	8.29
ChE/subcortex ^b	R x 10 ⁶	10.41	9.30	9.64	9.32
Litter 1		11.00	10.22	11.11	10.75
Litter 2		10.21	9.11	9.28	8.96
C:SC ChE ratio ^{bh}	x 10 ³	972	1062	898	956
Litter 1		757	1004	704	769
Litter 2		1043	1073	947	1003
ChE/g brain ^b	R x 10 ⁶	8.37	8.44	8.24	8.26
Litter 1		8.40	8.63	8.70	8.42
Litter 2		8.36	8.41	8.13	8.22
ChE/brain ^b	R x 10 ⁶	18.44	17.84	16.72	16.89
Litter 1		18.04	18.70	17.54	17.41
Litter 2		18.57	17.67	16.51	16.76

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cR = rate in moles substrate hydrolyzed per minute.

^dNumber of rats.

^eNS = not significant at least at 0.10 level.

^f0.05 < P < 0.10.

^gInsufficient observations for regression analyses.

^hC:SC ChE ratio (ChE/g cortex)/(ChE/g subcortex).

* P < 0.05.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP /HP +	LP /LP +	P	HP /HP +	LP /LP +	P
	HP ^p /LP	LP ^p /HP		LP ^p /HP	HP ^p /LP	
NS ^e	8.40(14)	7.78(10)	<0.10 ^f	7.94(13)	8.38(11)	NS
-- ^g	7.55 (3)	7.07 (2)	--	6.99 (3)	7.90 (2)	--
NS	8.63(11)	7.96 (8)	NS	8.22(10)	8.49 (9)	NS
NS	7.48	6.57	--*	6.84	7.40	NS
--	6.50	5.69	--	5.85	6.67	--
NS	7.74	6.79	<0.10	7.14	7.56	NS
NS	8.39	8.57	NS	8.57	8.34	<0.10
--	9.08	9.61	--	9.51	8.96	--
NS	8.20	8.31	NS	8.29	8.20	NS
NS	9.93	9.48	NS	10.11	9.31	<0.10
--	10.74	10.93	--	11.04	10.49	--
NS	9.71	9.12	NS	9.84	9.05	<0.10
NS	1010	927	--*	944	1014	NS
--	839	736	--	739	886	--
NS	1057	975	NS	1005	1042	NS
NS	8.40	8.25	NS	8.32	8.36	NS
--	8.48	8.56	--	8.50	8.53	--
NS	8.38	8.18	NS	8.27	8.32	NS
NS	18.18	16.80	NS	17.77	17.41	NS
--	18.26	17.48	--	17.88	18.05	--
NS	18.16	16.64	NS	17.74	17.27	NS

given adequate protein before weaning ($HP_p/HP + HP_p/LP$) and 7.78 for those restricted in protein before weaning ($LP_p/LP + LP_p/HP$) ($0.05 < P < 0.10$). Total cortical ChE activity for $LP_p/LP + LP_p/HP$ rats from litters 1 and 2 expressed in moles ASCh hydrolyzed per minute $\times 10^6$ was 6.57 on the average, a value significantly smaller than that of 7.48 for $HP_p/HP + HP_p/LP$ rats. Mean total cortical ChE activities for these groups in litter 2 were 6.79 for $LP_p/LP + LP_p/HP$ and 7.74 for $HP_p/HP + HP_p/LP$, but since fewer animals were being compared than for the combined litters, the values differed only at the 0.10 level. When data from the combined litters were considered, C:SC ChE ratios were significantly larger among rats adequately nourished before weaning (1010 for $HP_p/HP + HP_p/LP$) than among those restricted in protein prior to weaning (927 for $LP_p/LP + LP_p/HP$). No difference was obtained when data from only litter 2 were considered, however. Neither specific nor total subcortical nor whole brain ChE activity was significantly affected by preweaning protein restriction.

Protein restriction after weaning ($LP_p/LP + HP_p/LP$ vs $HP_p/HP + LP_p/HP$) tended to influence only ChE activity of the subcortex in Experiment III (Table 38). Specific subcortical ChE activity was decreased among rats fed restricted quantities of protein after weaning, 8.34 vs 8.57 moles ASCh hydrolyzed per minute $\times 10^6$ for $LP_p/LP + HP_p/LP$ and $HP_p/HP + LP_p/HP$ rats, respectively, when data from litters 1 and 2 were combined ($0.05 < P < 0.10$). A reduction in total ChE activity with postweaning protein restriction, 9.31 vs 10.11 moles ASCh hydrolyzed per minute $\times 10^6$ for $LP_p/LP + HP_p/LP$ and $HP_p/HP + LP_p/HP$ groups, respectively, also approached significance ($0.05 < P < 0.10$) when data from the 2 litters were combined or when only litter 2 was considered (9.05 vs 9.84 moles ASCh hydrolyzed per minute $\times 10^6$).

Behavior

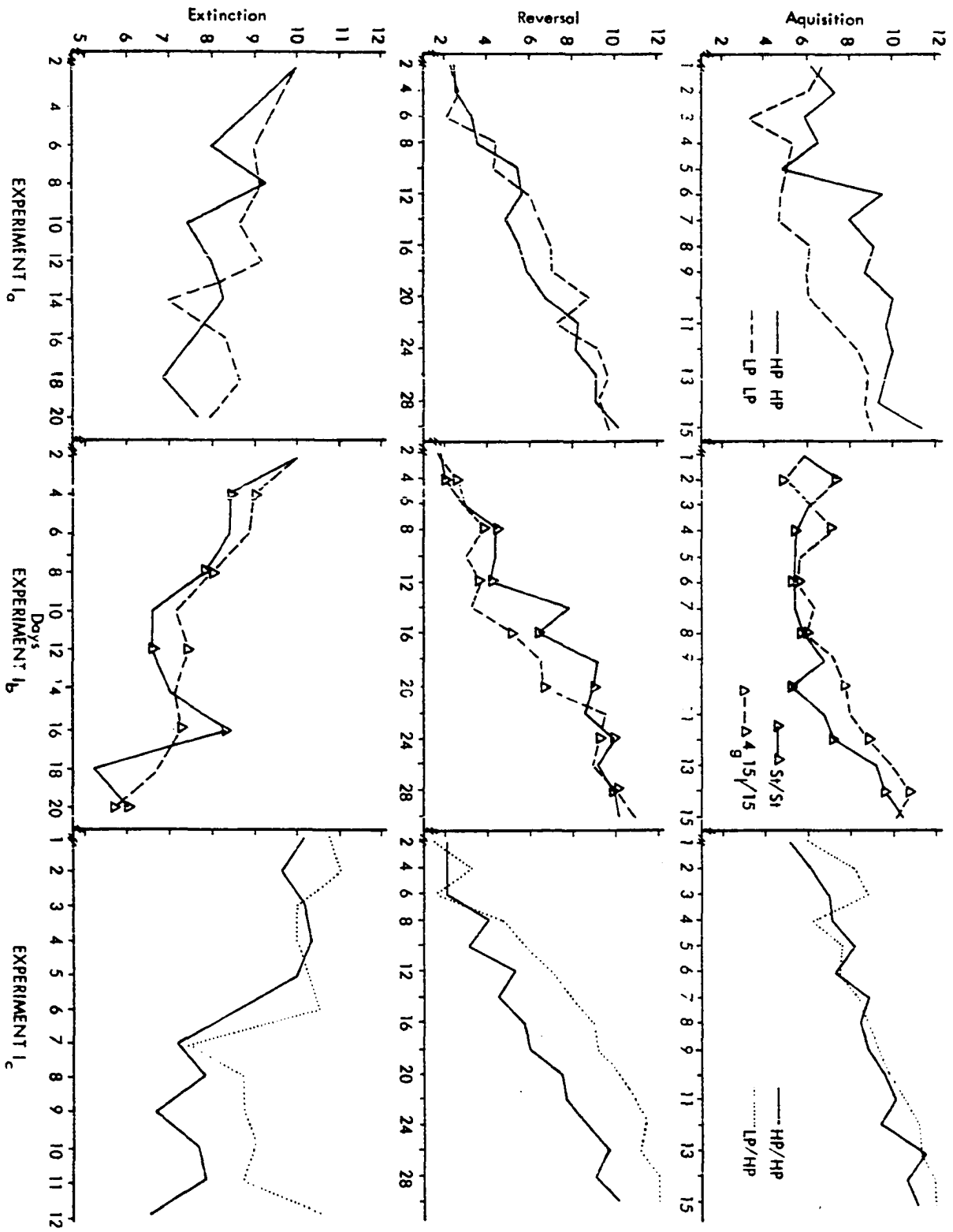
The effects of protein restriction on behavior in these experiments were assessed through observation of the performance of adult males in a single-choice visual discrimination maze. All rats were adjusted to a water deprivation regimen in which they were without water $23\frac{1}{2}$ hours per 24-hour period for several days preceding and during training. The number of correct choices during daily training periods, the number of trials required to reach an arbitrary criterion of success, and the latencies, i.e., the time in seconds required from entry into the maze to completion of the choice, for selected days were observed in each experiment.

Experiment I Training was divided into 3 phases: 1) acquisition, 2) reversal, and 3) extinction. During the initial or acquisition phase, rats received a small amount of water as a reward for each correct choice, i.e., by choosing the presence or absence of a light as a signal to enter the arm of the maze where the water was located. An average of 10 correct choices in 12 daily trials for 2 successive days was established as the criterion of success. Time required to attain this criterion varied from 3 to 29 days in Experiment I, but 28 out of 36 animals approached criterion within the first 15 days of acquisition training. Scores (number of correct choices per day) for this period are presented graphically in Figure 10.

The day after criterion was reached in acquisition, reversal training began; in this phase, the reward was located in the opposite arm of the maze. Choices which had been wrong during acquisition became correct for reversal training. When rats chose correctly on an average of 10 out of 12 trials on 2 successive days, extinction training began; for extinction, no

Figure 10. Learning curves of adult male rats during acquisition, reversal, and extinction training in Experiment I

NUMBER OF CORRECT CHOICES



rewards were given. The range in time required to solve the reversal problem was 12 to 49 days. Twenty-three out of 35 animals approached criterion within 30 days, and average performances on even numbered days 2 through 30 are presented in Figure 10. Extinction training was continued for 20 days in Experiments I_a and I_b. Results for even numbered days 2 through 20 are shown in Figure 10. Since 19 of 25 animals approached a chance level of performance (6 correct choices of 12) within 10 to 12 days of extinction training, this phase of training was limited to 12 days in Experiment I_c (Figure 10). Latencies were measured for each trial on the first or second day and on 1 of the final 2 days of acquisition training; latencies were also measured on 1 of the final 2 days of reversal training in Experiment I.

Rats in the HP/HP treatment in Experiment I_a made more correct choices on the average than LP/LP rats after the 5th day of training during acquisition (Figure 10). Performance differed significantly only on day 6, however. Average trials required to achieve criterion in acquisition for HP/HP rats were 159 compared with 236 for members of the LP/LP group; however, due to large variance within groups and the small number of animals in each group (HP/HP, 7; LP/LP, 6), these values were not significantly different (Table 39). Initial and final latencies and latency reciprocals during acquisition were similar for the 2 experimental groups in Experiment I_a (Table 40).

All animals in Experiments I_{a,b,c} required more training to master the reversal problem than had been needed for acquisition. The learning curves during reversal training of the two groups in Experiment I_a were nearly identical (Figure 10). Trials to criterion during reversal (Table 39) and final latencies and reciprocals during reversal training were similar for

Table 39. Mean trials to criterion during acquisition and reversal training of adult male rats in Experiment I

Experiment Group	I _a			I _b				I _c		
	HP/HP	LP/LP	P	St/St	4 _g 15 ₁ /15			HP/HP	LP/HP	P
Number of rats	7	6		5	8	7 ¹		6	5	
TCA ^a	159	236	NS ^b	211	197		NS	172	134	NS
TCR ^c	360	364	NS	367	365		NS	328	255(4) ^d	NS

^aTCA = trials to criterion in acquisition.

^bNS = not significant at least at 0.10 level.

^cTCR = trials to criterion in reversal.

^dNumber of rats when different from line 3.

the 2 groups also (Table 40). The extinction curves for HP/HP and LP/LP rats in Experiment I_a were erratic but also did not differ significantly throughout the 20 days of extinction training (Figure 10).

Behavioral results for Experiment I_b are presented in Figure 10 and Tables 39 and 40. Severe gestational protein restriction (4% protein) apparently did not affect learning performance under conditions of this experiment. Learning curves were very similar for St/St and 4_g 15₁/15 rats during acquisition, reversal, and extinction (Figure 10). On the average, St/St animals required 211 trials to attain criterion in acquisition compared with 197 trials for 4_g 15₁/15 rats (Table 39). In reversal, St/St rats reached criterion after an average of 367 trials while the 4_g 15₁/15 group required almost exactly the same number of trials, 365. Latencies and latency reciprocals during acquisition or reversal were also not significantly different between groups in Experiment I_b (Table 40).

Learning curves of adequately fed rats (HP/HP) during acquisition and those of rats restricted in protein during gestation and suckling (LP/HP)

Table 40. Mean latencies and latency reciprocals of adult male rats during acquisition and reversal training in Experiment I

Experiment Group	I _a			I _b			I _c		
	HP/HP	LP/LP	P	St/St	4 15 ₈ / 15 ₇ ¹	P	HP/HP	LP/HP	P
Number of rats	7	6		5			6	5	
	(seconds)								
Initial latency acquisition	14.1	16.0	NS ^a	50.8	24.4	NS	24.9	13.0	NS
Final latency acquisition	8.2	7.2	NS	9.4	8.7	NS	6.2	4.3	NS*
Final latency reversal	5.4	6.6	NS	5.7	6.5	NS	4.1	3.2(4) ^b	--*
	((1/seconds) x 1000)								
Reciprocal initial latency acq.	86	90	NS	29	50	NS	72	118	NS
Reciprocal final latency acq.	154	158	NS	137	146	NS	174	257	<0.10 ^c
Reciprocal final latency rev.	188	157	NS	190	162	NS	250	315(4)	--*

^aNS = not significant at least at 0.10 level.

^bNumber of rats when different from line 3.

^c0.05 < P < 0.10.

*P < 0.05.

were similar in Experiment I_c (Figure 10). Mean trials to criterion in acquisition were 172 for HP/HP rats and 134 for LP/HP animals, but as a result of within group variation and the small number of animals measured (6 HP/HP and 5 LP/HP), these values were not significantly different (Table 39). Average initial latency time for HP/HP animals in acquisition was 24.9 sec. while that for LP/HP rats was 13.0 sec. These values were not significantly different, and their reciprocal values of 0.072 and 0.118 also did not differ (Table 40). Final latencies in acquisition were not different for HP/HP (6.2 sec.) and LP/HP (4.3 sec.) treatments, but their reciprocal values of 0.174 for HP/HP and 0.257 for LP/HP approached being significantly different ($0.05 < P < 0.10$).

Rehabilitated rats (LP/HP) seemingly were able to reverse the learned task more easily than HP/HP animals; the LP/HP group averaged more correct choices daily after day 8 of reversal training (Figure 10). Group means differed significantly only on days 14, 20, and 22, however. Trials to criterion in reversal training were 328 and 255 for HP/HP and LP/HP groups, respectively, but again within group variation was large ($R = 144-444$ for HP/HP and $144-348$ for LP/HP), and the values were not statistically different (Table 39). Final latencies in reversal were 4.1 sec. for HP/HP and 3.2 sec. for LP/HP animals (Table 40), and these values as well as their reciprocal values differed significantly. Extinction curves for the 2 groups did not differ significantly in Experiment I_c (Figure 10).

Experiment II Considering the findings from Experiment I and the time required to train large numbers of animals scheduled for Experiments II and III, acquisition training was limited to 15 days, reversal training was omitted, and extinction was limited to 10 days in Experiments II and

III. Each rat was given 10 rather than 12 trials daily, and criterion during acquisition was a minimum of 9 correct choices out of 10 trials for 2 consecutive days. Since performance during acquisition and extinction was not significantly different for litters 2 and 3 in Experiment II, results for the combined litters only are presented in Figure 11. From days 5 through 15 of acquisition training, rats fed 24% casein after weaning (HP/HP + LP/HP) consistently averaged more correct choices than those fed 10% casein during this period (LP/LP + HP/LP). The values were significantly different only on day 14, however.

Means trials to criterion during acquisition for rats in litters 2 and 3, Experiment II, were 102 for HP/HP, 134 for HP/LP, 95 for LP/HP, and 119 for LP/LP rats (Table 41). Protein restriction before weaning (LP/LP + LP/HP vs HP/HP + HP/LP) or after weaning (LP/LP + HP/LP vs HP/HP + LP/HP) did not result in significantly different values for this measurement. Rats in the HP/HP and HP/LP treatments required 118 trials to reach criterion on the average while those from LP/LP and LP/HP groups required 107. Criterion was reached in 98 trials by rats in the HP/HP + LP/HP treatments compared with 126, the average for LP/LP + HP/LP animals. The tendency for animals restricted after weaning to require more trials to attain criterion than animals adequately nourished after weaning was significant among rats in litter 3; the rats which were fed 24% casein after weaning (HP/HP + LP/HP) reached criterion in an average of 88 trials compared with 133 trials for those fed 10% casein after weaning, LP/LP + HP/LP.

Mean latencies and latency reciprocals for acquisition trials on days 1, 5, 10, and 15 in Experiment II are presented in Tables 42 and 43, respectively. As training progressed, latency or time required to complete

Figure 11. Learning curves of adult male rats during acquisition and extinction training in Experiment II

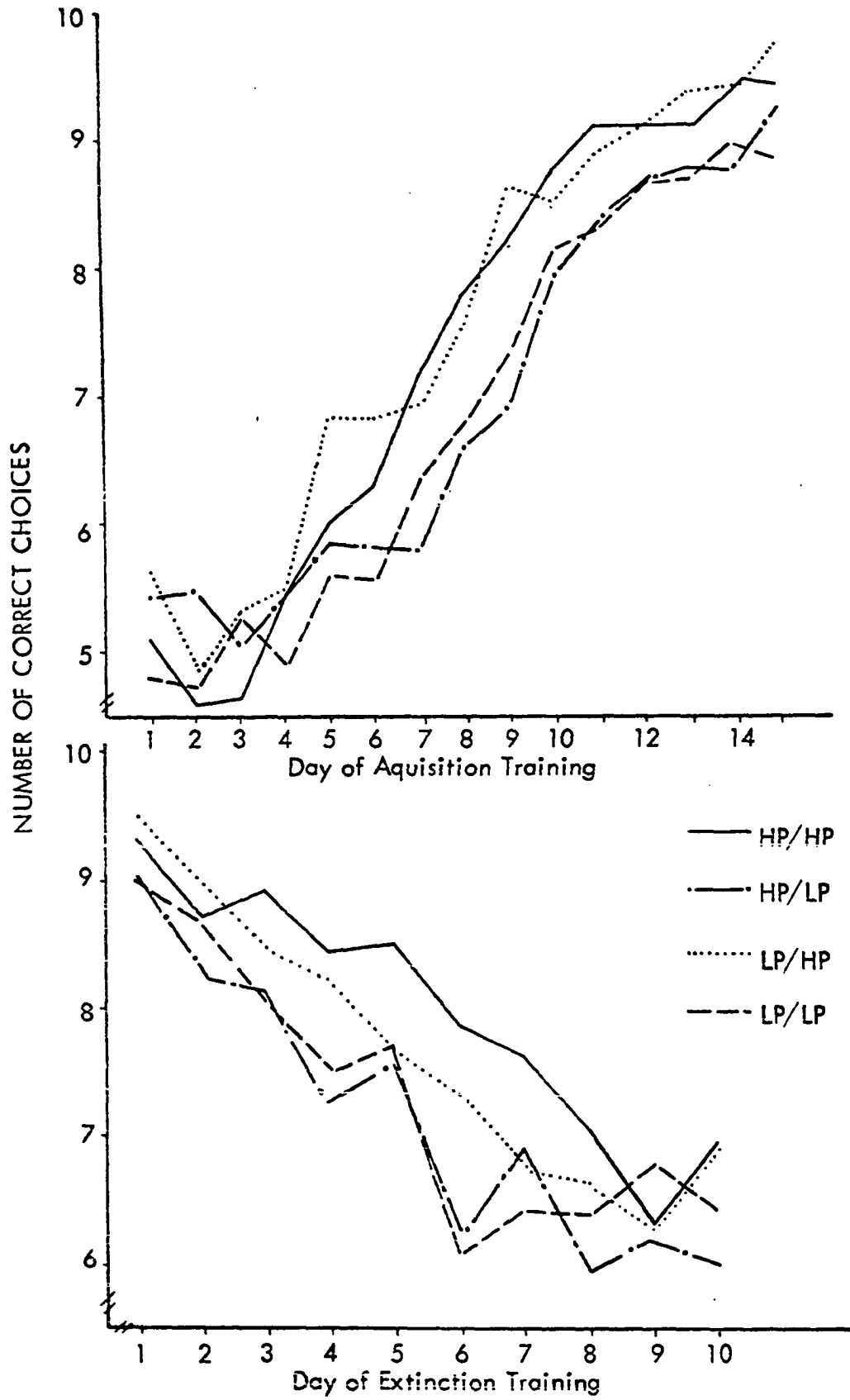


Table 41. Mean trials to criterion during acquisition training of adult male rats in Experiments II and III

Experiment II	HP/HP	HP/LP	LP/HP	LP/LP
TCA ^{bc}	102(22) ^d	134(23)	95(26)	119(25)
Litter 2	108(15)	127(17)	99(17)	119(17)
Litter 3	89 (7)	152 (6)	88 (9)	118 (8)
Experiment III	HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
TCA ^c	108 (8)	115 (6)	88 (5)	132 (5)
Litter 1	110 (2)	100 (1)	80 (1)	90 (1)
Litter 2	108 (6)	118 (5)	90 (4)	142 (4)

^aDiet before weaning x diet after weaning.

^bTCA = trials to criterion in acquisition.

^cArithmetic mean for litters 2 and 3, Experiment II; for litters 1 and 2, Experiment III.

^dNumber of rats.

^eNS = not significant at least at 0.10 level.

^fInsufficient observations for regression analyses.

*P<0.05.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^e	118(45)	107(51)	NS	98(48)	126(48)	NS
NS	118(32)	109(34)	NS	103(32)	123(34)	NS*
NS	118(13)	102(17)	NS	88(16)	133(14)	--
	HP ^p /HP + HP ^p /LP	LP ^p /LP + LP ^p /HP		HP ^p /HP + LP ^p /HP	LP ^p /LP + HP ^p /LP	
NS _f	111(14)	110(11)	NS	100(13)	123(11)	NS
-- _f	107 (3)	85 (2)	--	100 (3)	95 (2)	--
NS	113(11)	116 (9)	NS	101(10)	129 (9)	NS

Table 42. Mean latencies of adult male rats during acquisition training in Experiment II

	HP/HP	HP/LP	LP/HP (seconds)	LP/LP
Latency day 1 ^b	16.4(22) ^c	16.9(23)	13.6(26)	16.1(25)
Litter 2	18.8(15)	16.4(17)	15.8(17)	16.1(17)
Litter 3	11.1 (7)	18.3 (6)	9.6 (9)	16.2 (8)
Latency day 5 ^b	9.9	14.6	8.9	9.9
Litter 2	11.5	16.5	10.5	9.8
Litter 3	6.3	9.3	6.0	10.2
Latency day 10 ^b	7.8	9.8	6.7	7.8
Litter 2	8.4	7.2	7.4	7.8
Litter 3	6.4	17.0	5.5	7.9
Latency day 15 ^b	5.2	6.4	5.4	7.2
Litter 2	5.3	6.4	5.6	7.2
Litter 3	5.0	6.5	5.1	7.2

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

* $P < 0.05$.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P (seconds)	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^d	16.6(45)	14.9(51)	NS	14.9(48)	16.5(48)	NS
NS	17.5(32)	15.9(34)	NS	17.2(32)	16.2(34)	NS
NS	14.4(13)	12.7(17)	NS	10.3(16)	17.1(14)	NS
NS	12.3	9.4	NS	9.4	12.2	NS
NS	14.2	10.1	NS	11.0	13.2	NS*
NS	7.7	8.0	NS	6.1	9.8	--*
NS	8.8	7.3	NS	7.2	8.8	NS
NS	7.8	7.6	NS	7.9	7.5	NS
NS	11.3	6.6	NS	5.9	11.8	NS
NS	5.8	6.3	NS	5.3	6.9	NS*
NS	5.9	6.4	NS	5.5	6.8	--*
NS	5.7	6.1	NS	5.0	6.9	--*

Table 43. Mean latency reciprocals of adult male rats during acquisition training in Experiment II

	HP/HP	HP/LP ((1/seconds) x 1000)	LP/HP	LP/LP
Latency reciprocal day 1 ^b	87(22) ^c	87(23)	95(26)	77(25)
Litter 2	76(15)	83(17)	85(17)	77(17)
Litter 3	110 (7)	97 (6)	112 (9)	78 (8)
Latency reciprocal day 5 ^b	143	118	155	123
Litter 2	118	115	138	129
Litter 3	195	126	186	110
Latency reciprocal day 10 ^b	170	139	180	147
Litter 2	172	149	167	146
Litter 3	164	111	205	150
Latency reciprocal day 15 ^b	214	171	217	157
Litter 2	202	167	219	161
Litter 3	241	183	213	147

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e0.50 < P < 0.10.

* P < 0.05.

a choice consistently decreased for all experimental groups from an average of about 16 seconds on day 1 to an average of about 6 seconds on day 15. Rats restricted in protein after weaning, LP/LP + HP/LP, generally demonstrated longer latencies than those which were adequately fed during this period, HP/HP + LP/HP (Table 42). Values differed significantly for rats in litter 3 on day 5 (6.1 sec. for HP/HP + LP/HP vs 9.8 sec. for LP/LP + HP/LP) and for litter 2 on day 15 (5.5 sec. for HP/LP + LP/HP vs 6.8 sec. for LP/LP + HP/LP) and litter 3 on day 15 (5.0 vs 6.9 sec for HP/HP + LP/HP and LP/LP + HP/LP rats, respectively). When data for litters 2 and 3 on day 15 were combined, however, the overall group means (5.3 sec. for HP/HP + LP/HP vs 6.8 sec. for LP/LP + HP/LP rats) did not differ significantly. As occurred with adult spleen weight data and newborn relative brain weight data in Experiment II which have been discussed previously, the uneven numbers in litters 2 and 3 and the approximate nature of the analyses tended to cause illogical results when day 15 latency and latency reciprocal data from the combined litters were evaluated. The analyses of data from the combined litters were considered less accurate than the separate analyses of data from litters 2 and 3, however, and both latencies and their reciprocal values on day 15 of acquisition training were believed to differ due to postweaning treatment.

When latency reciprocals were considered, variation within groups was reduced; consequently, the mean latency reciprocals for rats treated differently after weaning differed when data from litters 2 and 3 were combined on day 10 of acquisition, 0.175 vs 0.143 for HP/HP + LP/HP vs LP/LP + HP/LP (Table 43). Latency reciprocals were also significantly different for rats restricted after weaning in litter 3 on day 5 and for those in

litter 2 on day 15 of acquisition. Values for litter 3 on day 15 were 0.225 vs 0.162 for HP/HP + LP/HP vs LP/LP + HP/LP treatments and approached being significantly different ($0.05 < P < 0.10$).

As illustrated in Figure 11, rats which received adequate protein after weaning (HP/HP + LP/HP) made more correct choices through day 8 of extinction training than those restricted in protein after weaning (LP/LP + HP/LP). The mean values differed significantly only on day 6, however.

Latencies increased during extinction training for all experimental groups (Table 44). Protein restriction before weaning tended to cause shorter latencies among rats in litter 3 on day 5 of extinction training. Latency values were 23.3 sec. for HP/HP + HP/LP rats vs 16.8 sec. for LP/LP + LP/HP animals ($0.05 < P < 0.10$).

Increased latencies had been observed during acquisition among rats which underwent postweaning protein restriction, LP/LP + HP/LP; the same tendency was present in extinction also. Average values approached being significantly different ($0.05 < P < 0.10$) when data from litters 2 and 3 were combined on day 10 and were significantly different for rats in litter 2 on that day (25.0 sec. for HP/HP + LP/HP vs 31.5 sec. for LP/LP + HP/LP).

When latency reciprocals for extinction were considered, the interaction of diet before and after weaning approached significance on the 5th day of extinction for all rats from litters 2 and 3 or for rats from litter 3 only. Average reciprocal values for litters 2 and 3 on day 5 of extinction were 0.056 for HP/HP, 0.057 for HP/LP, 0.065 for LP/HP, and 0.057 for LP/LP. Reciprocals for LP/HP rats differed from those of the 3 other groups indicating that the effect of protein restriction before weaning only (LP/HP) was different than that of the combined restriction

Table 44. Mean latencies and latency reciprocals of adult male rats during extinction training in Experiment II

	HP/HP	HP/LP	LP/HP	LP/LP
	(seconds)			
Latency day 5 ^b	21.4(22) ^c	24.2(23)	21.7(26)	23.2(25)
Litter 2	19.3(15)	25.5(17)	25.5(17)	25.1(17)
Litter 3	25.8 (7)	20.5 (6)	14.5 (9)	19.3 (8)
Latency day 10 ^b	26.4	38.0	24.7	33.8
Litter 2	26.3	32.1	23.9	30.8
Litter 3	26.7	54.8	26.2	40.1
	((1/seconds) x 1000)			
Latency reciprocal day 5 ^b	56	57	65	57
Litter 2	61	58	52	57
Litter 3	45	53	89	55
Latency reciprocal day 10 ^b	56	44	54	38
Litter 2	50	50	54	41
Litter 3	67	27	54	32

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^e0.05 < P < 0.10.

* P < 0.05.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP +	LP/LP +	P	HP/HP +	LP/LP +	P
	HP/LP	LP/HP		LP/HP	HP/LP	
(seconds)						
NS ^d	22.8(45)	22.4(51)	NS	21.5(48)	23.7(48)	NS
NS	22.6(32)	25.3(34)	NS	22.6(32)	25.3(34)	NS
NS	23.3(13)	16.8(17)	<0.10 ^e	19.5(16)	19.8(14)	NS
NS	32.3	29.2	NS	25.5	35.8	<0.10
NS	29.4	27.4	NS	25.0	31.5	--*
NS	39.7	32.7	NS	26.4	46.4	NS
((1/seconds) x 1000)						
<0.10	56	61	NS	61	57	NS
NS	59	54	NS	56	58	NS
<0.10	48	73	<0.10	69	54	NS
NS	50	46	NS	55	41	--*
NS	50	48	NS	52	45	<0.10
NS	49	44	NS	60	30	NS

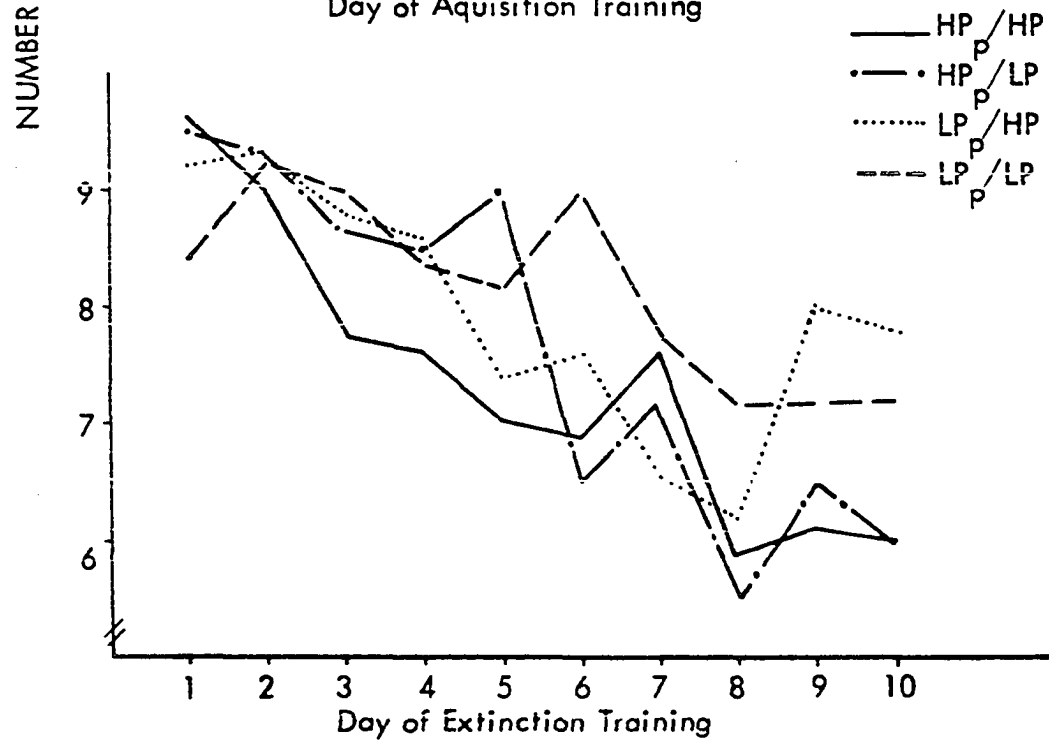
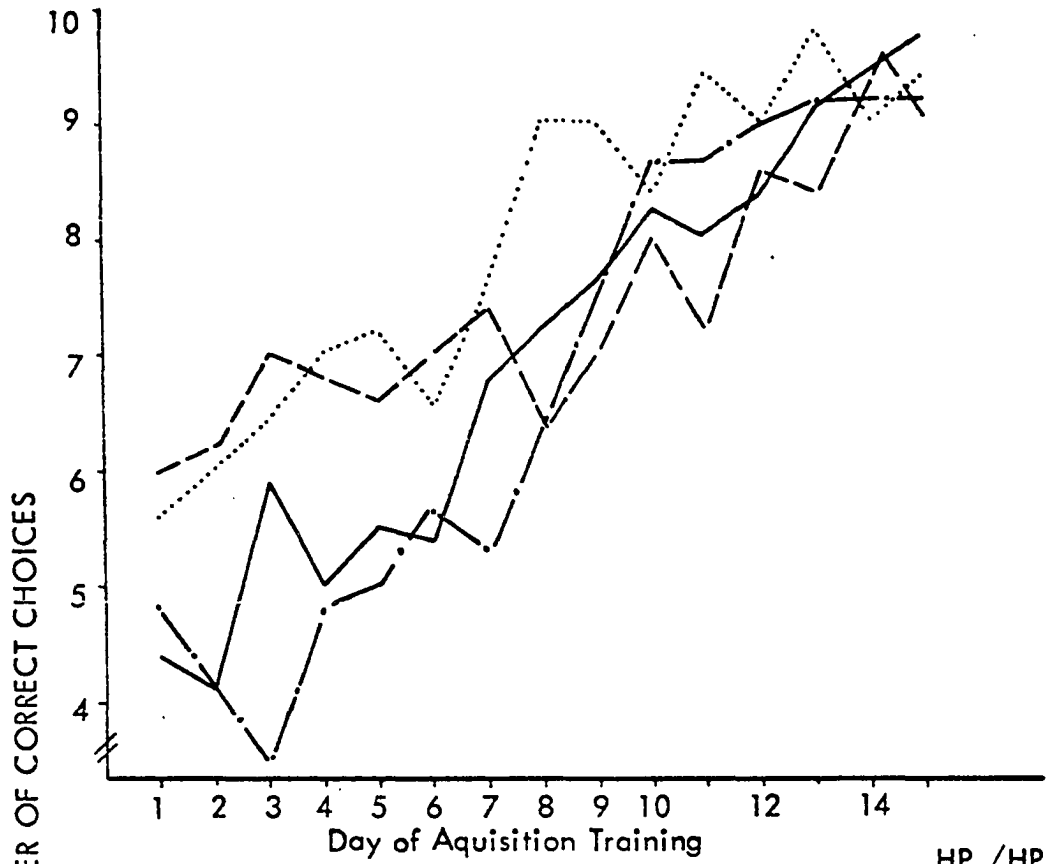
(LP/LP) or restriction after weaning only (HP/LP). When litter 3 only was considered, the deviant effect of restriction only before weaning was again apparent with reciprocal values of 0.089 for LP/HP rats compared with 0.045 for HP/HP, 0.053 for HP/LP, and 0.055 for LP/LP animals.

Experiment III Acquisition and extinction performance of rats in litter 1 were not significantly different from those in litter 2 in Experiment III; therefore, data from the 2 litters were combined in the learning curves presented in Figure 12. Neither the acquisition or the extinction learning curves nor trials to criterion during acquisition were significantly affected by protein restriction before or after weaning in Experiment III (Figure 12 and Table 41).

Similar to findings in Experiment II, latency times tended to decrease with acquisition training and increase with extinction training in all groups (Tables 45 and 47). However, the effect of protein restriction on latency in acquisition was significant only when values for rats from litters 1 and 2 were combined on day 10 of training (Table 45). Groups restricted before weaning tended to require less time to make visual discrimination choices on day 10 ($HP_p/HP + HP_p/LP$ 5.8 sec. vs. 4.2 sec. for $LP_p/LP + LP_p/HP$) ($0.05 < P < 0.10$) while postweaning restriction resulted in significantly increased time for decision making ($HP_p/HP + LP_p/HP$ 4.8 sec. vs 5.5 sec. for $LP_p/LP + HP_p/LP$).

When latency reciprocals for day 10 of acquisition were examined, the difference due to postweaning restriction was significant for both the combined litters and litter 2 alone (Table 46). The interaction of the diets fed before and after weaning also approached significance ($0.05 < P < 0.10$) for reciprocals of the combined litters on day 10. Reciprocal values for the 4

Figure 12. Learning curves of adult male rats during acquisition and extinction training in Experiment III



- HP_p/HP
- HP_p/LP
- LP_p/HP
- - - LP_p/LP

Table 45. Mean latencies of adult male rats during acquisition training in Experiment III

	HP _p /HP	HP _p /LP	LP _p /HP (seconds)	LP _p /LP	Inter- action ^a P
Latency day 1 ^b	8.5(8) ^c	17.0(6)	7.8(5)	6.9(5)	NS ^d
Litter 1	9.2(2)	33.6(1)	9.1(1)	6.8(1)	-- ^e
Litter 2	8.3(6)	13.6(5)	7.4(4)	6.9(4)	NS
Latency day 5 ^b	7.4	6.4	5.0	4.4	NS
Litter 1	14.7	9.2	5.8	4.2	--
Litter 2	4.9	5.9	4.8	4.5	NS
Latency day 10 ^b	5.6	6.0	3.6	4.8	NS
Litter 1	6.2	7.8	3.2	6.0	--
Litter 2	5.4	5.6	3.7	4.6	NS
Latency day 15 ^b	5.5	5.0	4.5	14.2	NS
Litter 1	9.6	7.2	4.4	58.2	--
Litter 2	4.2	4.6	4.5	3.2	NS

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient observations for regression analyses.

^f0.05 < P < 0.10.

*P < 0.05.

Diet before weaning			Diet after weaning		
HP _p /HP +	LP _p /LP +	P (seconds)	HP _p /HP +	LP _p /LP +	P
HP _p ^p /LP	LP _p ^p /HP		LP _p ^p /HP	HP _p ^p /LP	
12.1(14)	7.3(10)	NS	8.2(13)	12.4(11)	NS
17.3 (3)	8.0 (2)	--	9.1 (3)	20.2 (2)	--
10.7(11)	7.2 (8)	NS	8.0(10)	10.6 (9)	NS
7.0	4.7	NS	6.5	5.5	NS
12.9	5.0	--	11.7	6.7	--
5.4	4.6	NS	4.9	5.2	NS
5.8	4.2	<0.10 ^f	4.8	5.5	--*
6.8	4.6	--	5.2	6.9	--
5.5	4.1	NS	4.7	5.2	NS
5.3	9.3	NS	5.1	9.2	NS
8.8	31.3	--	7.8	32.7	--
4.4	3.8	NS	4.3	4.0	NS

Table 46. Mean latency reciprocals of adult male rats during acquisition training in Experiment III

	HP _p /HP	HP _p /LP ((1/seconds) x 1000)	LP _p /HP	LP _p /LP
Latency reciprocal day 1 ^b	134(8) ^c	84(6)	132(5)	148(5)
Litter 1	126(2)	30(1)	110(1)	147(1)
Litter 2	136(6)	95(5)	138(4)	148(4)
Latency reciprocal day 5 ^b	197	172	211	228
Litter 1	107	109	172	238
Litter 2	226	184	221	226
Latency reciprocal day 10 ^b	209	184	285	218
Litter 1	164	128	312	167
Litter 2	224	195	278	230
Latency reciprocal day 15 ^b	226	230	281	256
Litter 1	153	139	227	17
Litter 2	250	248	294	316

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient observations for regression analyses.

^f0.05 < P < 0.10.

* P < 0.05.

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP ^p /HP + HP ^p /LP	LP ^p /LP + LP ^p /HP	P	HP ^p /HP + LP ^p /HP	LP ^p /LP + HP ^p /LP	P
	(1/seconds) x 1000					
NS ^d	112(14)	140(10)	NS	133(13)	113(11)	NS
-- ^e	94 (3)	128 (2)	--	121 (3)	88 (2)	--
NS	117(11)	143 (8)	NS	137(10)	118 (9)	NS
NS	186	220	NS	202	198	NS
--	108	205	--	129	173	--
NS	207	224	NS	224	203	NS
<0.10 ^f	198	252	NS	238	199	--*
--	152	240	--	213	147	--*
NS	211	254	NS	246	210	--
NS	227	269	NS	247	242	NS
--	148	122	--	178	78	--
NS	249	305	NS	268	278	NS

experimental groups were 0.209 for HP_p/HP , 0.184 for HP_p/LP , 0.285 for LP_p/HP , and 0.218 for LP_p/LP . Latencies were shorter and thus reciprocals larger for the LP_p/HP group than for the HP_p/HP treatment showing that protein restriction before weaning tended to shorten latencies. The longest latencies and smallest reciprocals were observed for HP_p/LP rats; thus postweaning restriction tended to increase latency. Intermediate values were observed for both LP_p/LP and HP_p/HP groups.

On the 5th day of extinction training in Experiment III, all rats which were adequately fed after weaning ($HP_p/HP + LP_p/HP$) required an average of 24.0 sec. to make their decisions in the visual discrimination maze compared with 13.9 sec. for those rats fed restricted quantities of protein after weaning ($LP_p/LP + HP_p/LP$) ($P < 0.05$, Table 47). The interaction of dietary treatment before and after weaning on latencies was significant on day 5 when data from litters 1 and 2 were combined and approached significance ($0.05 < P < 0.10$) when only data from litter 2 were considered. Interaction of the preweaning and postweaning diets also approached being significant for latency reciprocal values on day 5 both for the combined litters and for litter 2 alone. These findings indicate that the effect of protein restriction before and after weaning may have been different. Protein restriction begun after weaning (HP_p/LP) resulted in shorter latencies (10.2 sec.) than restriction before weaning only (LP_p/HP 16.0 sec.) or than restriction throughout life (LP_p/LP 18.2 sec.). Rats fed adequately before and after weaning (HP_p/HP) had the longest average latency (29.0 sec.). Significant interaction was not present by day 10 of extinction training; therefore, that observed on day 5 may have been due to unusual behavior of several animals on day 5 rather than to experimental treatment, however.

Table 47. Mean latencies and latency reciprocals of adult male rats during extinction training in Experiment III

	HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
	(seconds)			
Latency day 5 ^b	29.0(8) ^c	10.2(6)	16.0(5)	18.2(5)
Litter 1	62.2(2)	11.5(1)	39.3(1)	22.1(1)
Litter 2	17.9(6)	10.0(5)	10.2(4)	17.2(4)
Latency day 10 ^b	32.0	35.1	16.3	16.9
Litter 1	69.8	86.9	34.7	32.2
Litter 2	19.4	24.8	11.6	13.0
	((1/seconds) x 1000)			
Latency reciprocal day 5 ^b	59	108	96	70
Litter 1	32	87	25	45
Litter 2	68	113	113	72
Latency reciprocal day 10 ^b	54	48	87	77
Litter 1	30	12	29	31
Litter 2	62	55	101	88

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 1 and 2.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient observations for regression analyses.

^f0.05 < P < 0.10.

* P < 0.05.

Inter- action ^a P	Diet before weaning			Diet after weaning		
	HP ^p /HP +	LP ^p /LP +	P	HP ^p /HP +	LP ^p /LP +	P
	HP ^p /LP	LP ^p /HP		LP ^p /HP	HP ^p /LP	
	(seconds)					
--*	21.0(14)	17.1(11)	NS ^d	24.0(13)	13.9(11)	--*
-- ^e	45.3 (3)	30.7 (3)	--	54.6 (3)	16.8 (2)	--
<0.10 ^f	14.3(11)	13.7 (8)	NS	14.8(10)	13.2 (9)	NS
NS	33.3	16.6	NS	25.9	26.8	NS
--	75.5	33.4	--	58.1	59.6	--
NS	21.8	12.3	NS	16.3	19.5	NS
	((1/seconds) x 1000)					
<0.10	80	81	NS	73	90	NS
--	50	35	--	30	66	--
<0.10	88	93	NS	86	95	NS
NS	51	82	NS	66	61	NS
--	24	30	--	29	21	--
NS	59	94	NS	77	70	NS

Correlations

Correlation coefficients of trials to criterion in acquisition (TCA) and selected measurements of brain weight and cholinesterase activity for adult male rats were determined in experimental groups from Experiments I, II, and III. The correlations are presented in Tables 48-52.

Experiment I In Experiment I_a (Table 48), significant negative correlations between TCA and ChE/g subcortex ($r = -0.84$) and TCA and ChE/g whole brain ($r = -0.86$) occurred in the 7 HP/HP rats. A negative correlation between TCA and ChE/subcortex ($r = -0.68$) approached significance ($0.05 < P < 0.10$) in the HP/HP group. None of the correlations between TCA and brain weight or ChE activity were significant for the 6 rats restricted in protein both before and after weaning (LP/LP) in Experiment I_a.

The 5 stock rats (St/St) in Experiment I_b (Table 48) exhibited a significant negative correlation ($r = -0.89$) between TCA and specific ChE activity of the whole brain. Correlation of total brain ChE activity and TCA ($r = -0.87$) approached significance ($0.05 < P < 0.10$) for the St/St group. Neither brain weight nor ChE activity was correlated with TCA for the seven $4 \frac{15_1}{15}$ rats.

Correlations of TCA and total brain weight for 4 LP/HP rats ($r = 0.93$) in Experiment I_c (Table 48) approached significance ($0.05 < P < 0.10$). No measurements of brain weight or cholinesterase activity were significantly correlated with TCA for the 6 HP/HP rats in this experiment; thus the significant correlations in Experiment I_a were not confirmed.

Experiment II None of the correlation coefficients of TCA and the various parameters of brain weight approached significance in Experiment II (Table 49).

Table 48. Correlations of TCA^a with brain weights and ChE activities of adult male rats in Experiment I

Experiment Group	I _a		I _b		I _c	
	HP/HP 7 (r) ^b	LP/LP 6 (r)	St/St 5 (r)	4 15 / 15 8 7 ¹ (r)	HP/HP 6 (r)	LP/HP 4 (r)
Brain wt.	0.49	0.39	-0.67	-0.45	0.40	0.93 ^c
Cortex wt.	0.24	0.65	-0.54	-0.32	0.03	0.04
Subcortex wt.	0.58	-0.73	-0.47	-0.01	0.31	0.64
C/SC ^d	-0.04	0.70	-0.19	-0.19	-0.23	-0.31
ChE/g cortex	-0.37	-0.02	-0.66	0.11	0.28	0.83
ChE/cortex	-0.01*	0.37	-0.64	-0.06	0.22	0.88
ChE/g subcortex	-0.84*	0.10	0.01	-0.03	0.04	-0.77
ChE/subcortex	-0.68 ^c	-0.23	-0.16	-0.03	0.20	-0.15
C:SC ChE ratio ^e	0.47*	0.02	-0.46*	0.04	0.22	0.88
ChE/g brain	-0.86*	0.00	-0.89*	0.07	0.38	0.14
ChE/brain	-0.63	0.28	-0.87 ^c	-0.26	0.46	0.65

^aTCA = trials to criterion in acquisition training.

^bCorrelation coefficient.

^c0.05 < P < 0.10.

^dC/SC = g cortex/g subcortex.

^eCSC ChE ratio = (ChE/g cortex)/(ChE/g subcortex).

*P < 0.05.

Correlations of TCA and brain ChE activity for Experiment II are presented in Table 50. Among 25 rats restricted in protein before and after weaning (LP/LP), a negative correlation between TCA and ChE/g subcortex was significant when litters 2 and 3 were combined ($r = -0.44$) and when only the 17 rats in litter 2 were considered ($r = -0.49$). Correlation between TCA and total subcortical ChE activity for LP/LP rats approached significance ($0.05 < P < 0.10$) for combined observations from litters 2 and 3 ($r =$

Table 49. Correlations of TCA^a with brain weights of adult male rats in Experiment II

	HP/HP (r) ^b	HP/LP (r)	LP/HP (r)	LP/LP (r)
Brain ^c	0.20(22) ^d	-0.22(23)	0.03(26)	-0.09(25)
Litter 2	0.01(15)	-0.19(17)	-0.08(17)	-0.10(17)
Litter 3	0.04 (7)	0.04 (6)	0.33 (9)	-0.01 (8)
Cortex ^c	-0.19	-0.12	0.02	-0.24
Litter 2	-0.03	-0.16	-0.06	-0.28
Litter 3	-0.45	-0.37	0.07	0.12
Subcortex ^c	0.14	-0.03	-0.04	-0.27
Litter 2	-0.30	-0.04	-0.09	-0.32
Litter 3	0.49	0.64	0.25	0.10
C/SC ^{ce}	-0.26	-0.05	0.02	0.03
Litter 2	0.33	-0.06	-0.02	0.03
Litter 3	-0.48	-0.50	-0.13	0.02

^aTCA = trials to criterion in acquisition.

^bCorrelation coefficient.

^cCorrelation coefficients for all observations in litters 2 and 3.

^dNumber of rats.

^eC/SC = g cortex/g subcortex.

Diet before weaning		Diet after weaning	
HP/HP + HP/LP (r)	LP/LP + LP/HP (r)	HP/HP + LP/HP (r)	LP/LP + HP/LP (r)
0.06(45)	-0.05(51)	0.14(48)	-0.02(48)
-0.11(32)	-0.11(34)	0.03(32)	-0.05(34)
0.47(13)	0.24(17)	0.20(16)	0.28(14)
-0.08	-0.16	-0.06	-0.12
-0.13	-0.21	-0.05	-0.21
-0.04	-0.03	-0.28	0.18
0.08	-0.16	0.10	-0.07
-0.13	-0.23	-0.06	-0.12
0.56	0.27	0.38	0.29
-0.11	-0.01	-0.12	-0.02
0.03	-0.02	-0.02	-0.03
-0.28	-0.19	-0.35	0.03

Table 50. Correlations of TCA^a with brain ChE activities of adult male rats in Experiment II

	HP/HP (r) ^b	HP/LP (r)	LP/HP (r)	LP/LP (r)
ChE/g cortex ^c	-0.24(22) ^d	0.09(26)	-0.09(26)	0.07(25)
Litter 2	0.34(15)	0.35(17)	-0.09(17)	0.11(17)
Litter 3	-0.50 (7)	-0.27 (9)	-0.34 (9)	-0.08 (8)
ChE/cortex ^c	-0.32	0.01	-0.03	-0.04
Litter 2	0.31	0.16	-0.07	-0.05
Litter 3	-0.66	-0.33	-0.27	-0.05
ChE/g subcortex ^c	0.02	0.15	0.32	-0.44 [*]
Litter 2	0.15	0.23	0.28	-0.49 [*]
Litter 3	-0.43	0.48	0.31	0.02
ChE/subcortex ^c	0.10	0.12	0.23	-0.38 ^e
Litter 2	0.01	0.18	0.14	-0.44 ^e
Litter 3	-0.04	0.61	0.38	0.07
C:SC ChE ratio ^{cf}	-0.18	-0.04	-0.27	0.36 ^e
Litter 2	-0.04	-0.01	-0.25	0.47 ^e
Litter 3	-0.16	-0.36	-0.40	-0.05
ChE/g brain ^c	-0.19	0.20	0.22	-0.36 ^e
Litter 2	0.14	0.33	0.18	-0.41 ^e
Litter 3	-0.57	0.36	0.09	-0.06
ChE/brain ^c	-0.06	0.10	0.18	-0.41 [*]
Litter 2	0.15	0.26	0.07	-0.47 ^e
Litter 3	-0.62	0.39	0.24	-0.05

^aTCA = trials to criterion in acquisition.

^bCorrelation coefficient.

^cCorrelation coefficient for all observations in litters 2 and 3.

^dNumber of rats.

^e0.05 < P < 0.10.

^fC:SC ChE ratio = (ChE/g cortex)/(ChE/g subcortex).

^{*}P < 0.05.

Diet before weaning		Diet after weaning	
HP/HP + HP/LP (r)	LP/LP + LP/HP (r)	HP/HP + LP/HP (r)	LP/LP + HP/LP (r)
-0.06(45)	0.04(51)	-0.18(48)	0.04(48)
0.38 [*] (32)	0.08(34)	0.03(32)	0.12
-0.53 ^e (13)	-0.18(17)	-0.39(16)	-0.14
-0.10	-0.05	-0.15	-0.03
0.26	-0.07	-0.04	-0.01
-0.45	-0.16	-0.47 ^e	0.00
0.01	-0.11	0.16	-0.14
0.15	-0.23	0.21	-0.13
-0.35	0.27	-0.05	-0.06
0.07	-0.15	0.18	-0.10
0.10	-0.27	0.13	-0.12
0.01	0.34	0.15	0.09
-0.04	0.14	-0.24 ^e	0.18
0.03	0.26	-0.20	0.25
-0.16	-0.27	-0.28	-0.03
-0.05	-0.16	0.00	-0.14
0.24	-0.27	0.11	-0.12
-0.55 ^e	0.10	-0.29	-0.12
0.00	-0.17	0.08	-0.15
0.21	-0.29 ^e	0.11	-0.15
-0.45	0.19	-0.24	0.02

-0.38) and for litter 2 ($r = -0.44$). Correlations of TCA and C:SC ChE ratios for litter 2 ($r = 0.47$) and for litters 2 and 3 ($r = 0.36$) also approached significance ($0.05 < P < 0.10$) for the LP/LP group. Specific and total whole brain ChE activity and TCA for LP/LP rats in litter 2 approached significance ($r = -0.41$ and -0.47 ; $0.05 < P < 0.10$). When data from litters 2 and 3 were combined, TCA and total whole brain ChE activity were significantly correlated ($r = -0.41$; $P < 0.05$) while the correlation coefficient for TCA and specific whole brain ChE activity approached being significant ($r = -0.36$; $0.05 < P < 0.10$).

Among the 45 rats given adequate protein before weaning (HP/HP + HP/LP), specific cortical ChE activity was correlated with TCA. Specific cortical ChE activity and TCA were directly correlated among 32 rats in litter 2 ($r = 0.38$; $P < 0.05$) but were negatively correlated ($r = -0.53$; $0.05 < P < 0.10$) for the 13 rats from litter 3. ChE/g brain and TCA were negatively correlated for HP/HP and HP/LP rats from litter 3 ($r = -0.55$; $0.05 < P < 0.10$). Among rats restricted in protein before weaning (LP/LP + LP/HP), only the negative correlation of total brain ChE activity ($r = -0.29$) for 34 rats in litter 2 approached being significant ($0.05 < P < 0.10$).

Among the rats fed adequate protein after weaning (HP/HP + LP/HP), correlations between TCA and total cortical ChE activity for the 16 rats from litter 3 ($r = -0.47$) and TCA and C:SC ChE ratios for 48 rats from litters 2 and 3 ($r = -0.24$) approached significance ($0.05 < P < 0.10$). Additional correlations of TCA and brain ChE activity among rats from Experiment II did not reach the 10% level of significance.

Experiment III Correlations of TCA with brain weight for Experiment III are presented in Table 51. Positive correlation between TCA and subcortical weight ($r = 0.93$) was significant for the 5 rats from litters 1 and 2 which were restricted in protein before and after weaning (LP_p/LP).

Table 51. Correlations of TCA^a with brain weights of adult male rats in Experiment III

	HP _p /HP (r) ^b	HP _p /LP (r)	LP _p /HP (r)	LP _p /LP (r)	Diet before weaning		Diet after weaning	
					HP _p /HP + HP _p /LP (r)	LP _p /LP + LP _p /HP (r)	HP _p /HP + LP _p /HP (r)	LP _p /LP + HP _p /LP (r)
Brain ^c	0.11(8) ^d	-0.02(6)	-0.59(5)	-0.49(5)	-0.02(14)	-0.34(10)	0.12(13)	-0.24(11)
Litter 1	-- ^e (2)	-- (1)	-- (1)	-- (1)	-- (3)	-- (2)	-- (3)	-- (2)
Litter 2	0.11(6)	0.06(5)	-0.61(4)	-0.71(4)	-0.03(11)	-0.36 (8)	0.07(10)	-0.21 (9)
Cortex ^c	0.50	0.14	-0.32	0.31	0.43	0.12	0.24	0.06
Litter 1	--	--	--	--	--	--	--	--
Litter 2	0.47	0.04	-0.35	-0.34	0.34	0.02	0.14	-0.27
Subcortex ^c	-0.19	-0.11	-0.20	-0.93*	-0.20	-0.37	0.03	-0.40
Litter 1	--	--	--	--	--	--	--	--
Litter 2	-0.07	0.00	-0.20	-0.90	-0.15	-0.32	0.04	-0.26
C/SC ^{cf}	0.44	0.30	-0.05	0.65	0.42	0.24	0.13	0.44
Litter 1	--	--	--	--	--	--	--	--
Litter 2	0.30	0.03	-0.07	0.10	0.31	0.16	0.04	0.01

^aTCA = trials to criterion in acquisition.

^bCorrelation coefficient.

^cCorrelation coefficient for all observations in litters 1 and 2.

^dNumber of rats.

^eInsufficient observations for correlation analyses.

^fC/SC = g cortex/g subcortex.

*P<0.05.

No other correlations between TCA and brain weight among Experiment III animals approached significance ($0.05 < P < 0.10$).

The correlation of TCA and ChE/g brain for the 4 LP_p/LP rats from litter 2 in Experiment III (Table 52) was highly significant ($r = 0.99$; $P < 0.01$). Other correlation coefficients between TCA and brain ChE activity did not approach significance ($0.05 < P < 0.10$).

Table 52. Correlations of TCA^a with brain ChE activities of adult male rats in Experiment III

	HP _p /HP (r) ^b	HP _p /LP (r)	LP _p /HP (r)	LP _p /LP (r)
ChE/g cortex ^c	0.26(8) ^d	0.19(6)	0.13(5)	0.62(5)
Litter 1	-- ^e (2)	-- (1)	-- (1)	-- (1)
Litter 2	0.19(6)	0.19(5)	0.11(4)	0.12(4)
ChE/cortex ^c	0.41	0.18	0.02	0.78
Litter 1	--	--	--	--
Litter 2	0.60	0.14	0.00	-0.46
ChE/g subcortex ^c	0.23	-0.21	-0.18	-0.05
Litter 1	--	--	--	--
Litter 2	0.38	0.02	-0.18	0.81
ChE/subcortex ^c	0.09	-0.17	-0.21	-0.37
Litter 1	--	--	--	--
Litter 2	0.30	0.01	-0.20	0.72
C:SC ChE ratio ^{cf}	0.08	0.33	0.14	0.27
Litter 1	--	--	--	--
Litter 2	-0.09	0.23	0.11	-0.55
ChE/g brain ^c	0.63	0.07	-0.08	0.28
Litter 1	--	--	--	--
Litter 2	0.57	0.15	-0.03	0.99 ^{**}
ChE/brain ^c	0.58	0.02	-0.48	0.01
Litter 1	--	--	--	--
Litter 2	0.54	0.11	-0.63	0.76

^aTCA = trials to criterion in acquisition.

^bCorrelation coefficient.

^cCorrelation coefficient for all observations in litters 1 and 2.

^dNumber of rats.

^eInsufficient observations for correlation analyses.

^fC:SC ChE ratio = (ChE/g cortex)/(ChE/g subcortex).

^{**}P<0.01.

Diet before weaning		Diet after weaning	
HP /HP + HP ^p /LP (r)	LP /LP + LP ^p /HP (r)	HP /HP + LP ^p /HP (r)	LP /LP + HP ^p /LP (r)
0.27(14)	0.30(10)	0.24(13)	0.14(11)
-- (3)	-- (2)	-- (3)	-- (2)
0.19(11)	-0.21 (8)	0.20(10)	-0.10 (9)
0.36	0.26	0.26	0.10
--	--	--	--
0.29	0.16	0.20	-0.22
0.12	-0.15	-0.02	0.00
--	--	--	--
0.29	0.02	0.06	0.40
-0.06	-0.28	0.00	-0.26
--	--	--	--
0.05	-0.14	0.07	0.15
0.15	0.21	0.15	0.10
--	--	--	--
0.01	0.10	0.10	-0.29
0.37	0.06	0.30	0.08
--	--	--	--
0.34	0.22	0.33	0.22
0.20	-0.16	0.27	-0.10
--	--	--	--
0.17	-0.01	0.22	-0.01

DISCUSSION

The effects of protein restriction on reproduction, growth, and behavior depend on 1) the type of restriction, 2) the age at which the restriction is implemented, and 3) the severity and duration of the restriction (Barnes et al., 1973; Hsueh et al., 1974). Thirteen percent casein supplemented with 0.2% cystine or DL-methionine is recommended for gestation, lactation, and growth in rats by the Committee on Animal Nutrition, National Academy of Science-National Research Council (1962). In practice, however, diets with 18-30% casein have been considered optimal by various investigators (Nelson and Evans, 1948, 1958a, 1958b; Barnes et al., 1973).

In the present series of studies, a diet containing 6% casein supplemented with methionine was fed to achieve protein restriction during gestation. During lactation and for growth from 3 weeks until autopsy at about 35 to 45 weeks of age in Experiment I and 26 to 28 weeks of age in Experiments II and III, casein for restricted animals was increased to 10%. Adequately nourished animals were fed 24% casein during each of these periods. Part of the offspring from dams fed 6% casein in gestation and 10% casein in lactation were fed diets containing 24% casein from weaning. Part of the progeny from dams fed 24% casein in gestation and lactation was switched to 10% casein after weaning. These groups then were examined for the effects of protein restriction occurring only before or after weaning.

Reproductive Performance

Maternal adjustments

Food intake Total food intake during gestation was similar for females fed 6 or 24% protein in Experiments I and II. Less total food was

consumed during pregnancy by females fed 6% casein from weaning (LP_p) than by those reared on 24% casein (HP_p) in Experiment III; but relative to body weight at conception, intakes during gestation were similar. These findings were in agreement with those of Guilbert and Goss (1932), Macomber (1933), Wang et al. (1966), Berg (1967), Zeman (1967), Chou (1970), Zamenhof et al. (1972), Barton (1973), and Turner (1973).

Daily distribution of food intake was distinctly different for adequately fed and protein-restricted dams during pregnancy in each of the 3 experiments (Figures 6 and 7). Intakes of protein-restricted dams were higher than those of adequately fed females during the first 15 days of gestation in Experiments I and II. Absolute food intake of protein-restricted dams in Experiment III was 3 or 4 g less per 2 days than that of adequately nourished animals during the first 2 weeks of gestation. Relative to 100 g body weight, intakes of the LP_p dams were 1 or 2 g larger than those of the HP_p rats for each 2-day period during days 0 through 15, however. After day 15 of gestation, food intakes for the protein-restricted rats in all experiments decreased so sharply that on days 20 and 21, they ate about 12 to 18 g, approximately half as much food as they had eaten during the first 2 days of pregnancy. Adequately nourished dams in the 3 experiments demonstrated a slight decrease in food eaten during the last 3 days of gestation but generally consumed as much food, or more, on days 20 and 21 of gestation as they had eaten on days 0 and 1.

Zeman (1967), Chou (1970), Barton (1973), and Lee (1973) also observed patterns of food intake in protein-restricted pregnant rats that were similar to those observed in the present studies. Zeman (1967) pair-fed adequately nourished and protein-restricted animals and found no significant

difference in any parameter between offspring of adequately nourished pair-fed and control rats at birth, weaning, or at 40 days of age. Therefore, the divergent eating pattern seen with protein restriction may not be significant in successful reproductive performance or subsequent development of the progeny.

Total food intake during lactation was markedly reduced among dams fed low protein rations in Experiments I, II, and III. When total maternal intakes during lactation were compared on the basis of number of pups weaned, food eaten per pup weaned did not differ significantly between adequately fed and protein-restricted dams, however. Protein-restricted rats in Experiments I, II, and III increased their food intakes per 2 days by only about 50% between postpartum days 3 and 19 while adequately fed rats approximately doubled their 2-day intake during the same period.

Fewer rats in surviving litters reared by protein-restricted rats than in those nursed by rats fed adequate amounts of protein may have been partially responsible for their smaller food intakes. However, when Menaker and Navia (1973) maintained a litter size of 8 during lactation (no deaths 0-19 days), they found that dams fed 8% protein nevertheless ate only about one-half as much as controls fed 25% protein. Therefore, it seems probable that the food intake of rats fed a low protein diet in lactation would be less than that of rats fed adequate protein regardless of litter size. The failure of protein-restricted dams in the current studies to increase their food intake during lactation may have contributed to reduced milk synthesis by these animals which in turn adversely affected survival rates among their offspring.

Weight change Net weight change (postpartum weight minus weight at mating) during gestation for rats fed diets low in protein was 67 to 80% smaller than that of rats fed adequate amounts of protein in Experiments I, II, and III (Table 3 and 4). These findings confirm those of numerous earlier investigators (Goettsch, 1949; Curtiss, 1953; Nelson and Evans, 1953, 1958b; Wang et al., 1966; Zeman, 1967; Tagle and Donoso, 1969; Chou, 1970; Barton, 1973; Turner, 1973). The weight loss or reduced gain of the protein-restricted animals cannot be attributed to reduced energy intake since total amounts of the isocaloric rations eaten by protein-restricted and adequately fed animals in gestation were similar in Experiments I and II in which weights of females were similar when bred and intake relative to body weight for the 2 groups was comparable in Experiment III where LP_p females were 68 g smaller than HP_p rats at mating. It is likely then that reduced protein rather than reduced energy intake adversely affected gestational weight gain in the restricted animals.

Both adequately nourished and protein-restricted dams lost weight during lactation in each of the 3 experiments. Because adequately nourished females usually weaned 6 to 8 pups per litter compared with 4 to 5 for restricted dams, it is difficult to compare weight loss between the 2 groups. In Experiment II where adequately nourished dams weaned an average of 6.3 pups from each of 2 litters compared with an average of 5.1 pups for protein-restricted dams, weight loss during lactation averaged 37 and 45 g for HP and LP females, respectively.

The weight loss during lactation of 35 g on the average for animals fed 24% methionine-supplemented casein in the 3 experiments reported here was not expected. Several other investigators (Nelson and Evans, 1958a,

1958b; Widdowson and Cowen, 1972; Turner, 1973) have observed weight maintenance or a net gain during lactation among rats fed 18 to 30% protein during lactation. Supplementation of casein with methionine in the current experiments was identical with that of Nelson and Evans (1958b) who observed an average gain of 17 g during lactation among rats fed 24% casein.

However, Nelson and Evans' ration contained 8% fat while the ration used in the current experiments contained 5%. Their ration, consequently, supplied approximately 15 kcal. more energy per 100 g ration than the one used in the experiments reported here. Adequately nourished rats in the current studies consumed an average of 645 g food during lactation. For an animal otherwise in energy balance, the difference in available energy from the rations fed in the 2 laboratories could account for a weight change of about 12 g from the consumption of about 600 g diet. Consequently, the energy content of the ration could account for some but not all of the 52 g (-35 vs +17 g) discrepancy in weight change with lactation seen in the 2 different laboratories.

In summary, protein restriction in the present experiments changed the pattern of food consumption during gestation but not the total amount of food eaten. Total food intakes of protein-restricted dams during lactation were smaller than those of adequately fed females. However, food eaten per pup weaned did not differ between adequately nourished and protein-restricted females. Net weight gains for rats restricted in protein intake during gestation was 67 to 80% smaller than those for rats adequately nourished during pregnancy. Both adequately nourished and protein-restricted females lost weight during lactation, an average of 35 and 33 g,

respectively, in the studies reported here. Weight loss was related to number of pups weaned as well as diet, however; and when the number of pups nursed to weaning was similar (Experiment II, litter 2), adequately nourished females lost less weight (32 g) than those restricted in protein (56 g).

Development of the young

Birth weight Average birth weight for litters of live-born pups was significantly reduced by protein restriction in each of the 3 experiments (Tables 5 and 6). Adequately nourished neonates weighed 6.22 to 6.61 g on the average while offspring of protein-restricted females weighed 5.53 to 5.97 g. In Experiment III, newborns whose mothers had undergone prolonged protein restriction prior to mating were not smaller than progeny in Experiments I and II when restriction was begun at mating, however. Reduced birth weight among progeny of protein-restricted dams also was observed by Macomber (1933), Thompson (1937), Goettsch (1949), Curtiss (1953), Nelson and Evans (1953), Wang et al. (1966), Zeman (1967), Kenney (1969), Stewart and Sheppard (1971), Barton (1973), Turner (1973), and Younoszai and Ranshaw (1973). Turner (1973) reported that nonviable litters occurred among both adequately and poorly nourished animals but made up a larger porportion of the litters from dams fed restricted amounts of protein. Small birth weights perhaps indicate smaller stores of nutrients and may be a factor in the reduced survival of the progeny.

Mortality at birth and perinatal survival Nelson and Evans (1953) reported increased numbers of stillbirths when casein was decreased to 6% in gestation. Similarly, mean birth mortality rates were increased among protein-restricted compared with adequately nourished animals in the current studies. However, group averages approached being significantly dif-

ferent ($0.05 < P < 0.10$) only in Experiment I when the percent stillbirths for HP was 1 and for LP was 14 and in Experiment III when 2 and 16% of the HP and LP pups, respectively, were stillborn.

Perinatal survival rates of pups born of females fed restricted quantities of protein during gestation have often been lower than those of pups born to well nourished dams (Macomber, 1933; Thompson, 1937; McCoy, 1940; Goettsch, 1949; Cowley and Griesel, 1959, 1963; Venkatachalam and Ramanathan, 1964; Wang et al., 1966; Zeman, 1967; Kenney, 1969; Adeyanju, 1971; Stewart and Sheppard, 1971; Widdowson and Cowen, 1972; Barton, 1973; Turner, 1973). Survival rates on day 4 among pups born of adequately nourished dams were 79, 81, and 71%, respectively, in Experiments I, II, and III, values which were $1\frac{1}{2}$ to 2 times larger than those of pups born to protein-restricted mothers. Even so, the means approached being significantly different ($0.05 < P < 0.10$) only for HP vs LP_H in Experiment I and HP vs LP in Experiment II.

Lactation failure has been implicated in poor survival rates (Macomber, 1933; Mueller and Cox, 1946; Goettsch, 1949; Zeman, 1967). An absence of milk in the stomachs of neonates which did not survive in the current studies was noted frequently. Protein-restricted pups were also generally cold to the touch and often exhibited subcutaneous hematomas resulting from a difficult birth or maternal abuse.

Frankova (1974) observed that dams fed a diet providing 5% calories from protein contacted their young less often on day 7 than dams fed 25% calories from protein; they also exhibited less approach and more avoidance behavior during a specific test period on days 21 and 28 of lactation than the better fed females. After examining data on birth weight, birth order,

and behavior of marasmic human infants, Pollitt (1973) suggested that lethargic behavior and immature sucking response in these infants hindered milk intake and secretion. Both of these factors affected adversely the infant's ability to stimulate attention from the mother. Protein-restricted newborn rats have been described as less active than normal neonates and have failed to survive when nursed by foster mothers fed normal amounts of protein unless control pups were also present in the litter (Zeman, 1967). Therefore, interaction of characteristics of the protein-restricted dam and pup may be a significant cause of poor survival of malnourished progeny.

Barton (1973) and Turner (1973) observed that 50% or more of the pups which survived to 4 or 5 days of age survived to weaning. In Experiments II and III, respectively, 67 and 50% of those pups alive on day 4 were weaned, but only 20 to 30% of those pups surviving to day 4 in Experiment I were weaned. Because poor survival occurred in adequately nourished as well as protein-restricted progeny in Experiment I, mortality must have been due to factors other than diet. These factors may have included reduced ability to lactate due to debilitation caused by respiratory infections prevalent among laboratory animals or lack of maternal instinct among dams in Experiment I which were younger than those from Experiment II or III.

Neonatal organ weights As indicated by autopsy data of protein-restricted and adequately nourished female neonates in Experiments I, II, and III, restriction during gestation reduced carcass (11-19%), liver (15-34%), kidneys (11-30%), and spleen (7-37%) weights. These findings agree with the earlier reports of Zeman (1967) and Barton (1973). However,

relative to body weight neither carcass nor organ weights were consistently and significantly reduced among protein-restricted offspring in all of the 3 experiments. Relative liver weights were significantly smaller for protein-restricted than for adequately nourished neonates in Experiment I and in litter 2, Experiment III. Relative spleen weights in litter 2, Experiment II and relative kidney weights in litter 2, Experiment III also were significantly smaller among protein-restricted pups than among adequately nourished neonates.

Postnatal growth, survival, and organ development Protein-restricted pups were 25 to 44% smaller on the average than their adequately fed counterparts on days 7, 14, and 21 in the 3 experiments reported here. Body weights at weaning for adequately nourished and protein-restricted pups differed at the 10% level of significance in Experiment I and at the 1% level in Experiments II and III. Many previous investigators including Barnes et al. (1973) and Turner (1973) have also reported reduced body weights during suckling and at weaning among protein-restricted offspring.

Survival to weaning was significantly reduced by protein restriction of the dam during gestation and lactation in all experiments. Wang et al. (1966), Zeman (1967), Kenney (1969), Barton (1973), and Turner (1973) also observed this effect of protein restriction. In the present experiments, dams were given somewhat different treatments prior to maturity; the survival rate appeared to reflect these treatments. On the average, 6% of the protein-restricted pups compared with 30% of the adequately nourished pups from Experiment I survived to weaning. Restricted dams had been given 6% casein from mating. In Experiment III where protein restriction had been instituted at weaning, only 14% of the restricted and 56% of the adequately

nourished pups survived. Prolonged restriction in this study did not result in maternal adjustments which allowed for improved pup survival as observed by Khanam (1965). On the other hand, an average of 39% of the protein-restricted pups and 66% of the adequately nourished pups in Experiment II survived; their dams had reared one litter successfully on stock ration prior to restriction at mating for gestation 2.

The paradigm used in Experiment II was the most efficient design for producing protein-restricted subjects for subsequent growth and development or behavioral study. The question may be asked whether these pups might have been protected from the effects of protein restriction due to larger nutrient stores accumulated by their mothers while being fed stock ration. The answer to the question would appear to be no, however, because birth weights among restricted pups in Experiment II were similar to those in Experiments I and III. Average weaning weights of restricted progeny were 32.47, 33.28, and 25.71 g in Experiments I, II, and III, respectively.

Carcass, hepatic, renal, and splenic weights for protein-restricted female weanlings in Experiments II and III were smaller than those of adequately nourished weanlings. (These data were not collected on weanlings from Experiment I.) During rapid growth which occurred during the first 3 weeks of life, the effect on carcass, liver, kidney, and spleen weight of pups whose dams were fed a low protein ration was similar to the effect on total body weight. Although relative weight of these organs did not appear to be affected unduly by preweaning protein restriction, evidence from various investigations (Winick and Noble, 1966; Shrader and Zeman, 1969; Zeman and Stanbrough, 1969; Younoszai and Ranshaw, 1973) have indicated that the cell populations of these tissues differ both quantitatively and qualitatively from those of well nourished weanlings.

Livebirths, perinatal survival, and survival at weaning were adversely affected by protein restriction as were birth weight and body weight at 7, 14, and 21 days of age in the present studies. Absolute carcass, hepatic, renal, and splenic weights of protein-restricted newborn and weanling pups were smaller than those of their adequately nourished counterparts in these investigations, but relative carcass and organ weights were not consistently affected by protein restriction in these experiments.

Postweaning Growth, Development, and Metabolic Efficiency

Growth and organ development

Protein restriction before or after weaning or during both periods resulted in markedly decreased body weight for animals in each experiment at 20 weeks of age (Table 53). As expected, animals restricted in protein both before and after weaning exhibited the largest weight deficit compared with animals fed adequate protein throughout life; the deficits amounted to 91, 84, and 129 g in Experiments I, II, and III, respectively. Restriction either before or after weaning only in Experiments II and III resulted in similar body weights at 20 weeks of age; these weights fell between those for treatments which imposed restriction before and after weaning (LP/LP and LP_p/LP) and those in which rats were adequately fed throughout life (HP/LP and HP_p/HP).

Results of investigations by Winick and Noble (1966), Dickerson et al. (1972), and Knittle (1972) have indicated that the tissues represented in the final weights of adequately nourished and protein restricted animals in the current studies might differ widely. In their experiments, restriction before weaning tended to result in decreased numbers of normal cells in all

Table 53. Mean body weights of male offspring in Experiments I, II, and III at 3 and 20 weeks of age

	n	3 wk (g)	20 wk (g)
Experiment I ^a			
HP/HP	7	40	397
LP/LP	6	36	306
Experiment I ^b			
St/St	6	54	517
4 _g 15 ₁ /15	7	49	455
Experiment I ^c			
HP/HP	13	48	441
LP/HP	5	32	413
Experiment II			
HP/HP ^a	23	52	457
Litter 2	16	53	478
Litter 3	7	49	406
HP/LP ^a	23	51	410
Litter 2	17	51	418
Litter 3	6	53	387
LP/HP ^a	26	32	404
Litter 2	17	32	423
Litter 3	9	31	369
LP/LP ^a	25	34	373
Litter 2	17	34	375
Litter 3	8	34	370
Experiment III			
HP _p /HP ^a	8	48	454
Litter 1	2	44	470
Litter 2	6	50	448
HP _p /LP ^a	6	47	406
Litter 1	1	38	417
Litter 2	5	49	404
LP _p /HP ^a	5	25	382
Litter 1	1	28	410
Litter 2	4	24	375
LP _p /LP ^a	5	24	325
Litter 1	1	27	340
Litter 2	4	24	322

^aArithmetic mean for litters 2 and 3 in Experiment II; for litters 1 and 2, Experiment III.

tissues evaluated while restriction after weaning resulted in a normal number of cells which were decreased in size. Neither cell number nor cell size were measured in the present experiments; so the reduced weights of adipose, hepatic, and renal tissues which were observed cannot be ascribed to specific changes in these parameters.

Perirenal and epididymal fat deposits were reduced significantly by protein restriction before weaning but were not affected significantly by restriction instituted after weaning (Tables 18 and 19). In Experiment II, adipose deposits of LP/LP + LP/HP rats in litter 2 weighed only 71% as much as those of HP/HP + HP/LP animals on the average. Deposits of LP/LP + HP/LP rats weighed 83% as much as those of HP/HP + LP/HP rats. In Experiment III fat deposits of LP_p/LP + LP_p/HP rats weighed 56% as much as those of HP_p/HP + HP_p/LP rats while deposits of LP_p/LP + HP_p/LP rats weighed 88% as much as those of HP_p/HP + LP_p/HP animals. Knittle (1972) found that protein restriction of lactating dams decreased adipose cell number in weanling pups; hence adipocyte numbers may have been smaller at weaning in rats restricted during gestation and lactation in Experiments II and III than in those adequately nourished. This reduction was not corrected by an adequate protein supply after weaning. For example, in litter 2 of Experiment II, average adipose deposit weight for LP/HP rats was 13.62 g compared with 12.03 g for LP/LP and 20.50 g for HP/HP rats. Comparable values in Experiment III were 10.50 g for the LP_p/HP treatment and 9.25 and 18.26 g for the LP_p/LP and HP_p/HP treatments, respectively.

Both the preweaning and postweaning diet influenced hepatic and renal weights (Tables 18 and 19). However, protein restriction before weaning more consistently resulted in slightly smaller livers than that instituted

after weaning. Kidney weight, on the other hand, was consistently decreased more by postweaning than preweaning protein restriction. Lee (1973) fed 6 and 24% soy protein supplemented with methionine during gestation than foster-suckled pups on dams fed a stock ration containing about 24% protein from mixed sources. She found that renal function of the offspring of females fed 6% soy protein in gestation was normal even though the kidneys remained significantly smaller on day 14 than those of pups born of dams fed 24% soy protein in gestation. Therefore, the adequate postweaning diet which contributed to increased renal size in rats restricted before weaning in Experiments I, II, and III also may have contributed to improved renal function.

Rats restricted in protein before and after weaning were smaller at 20 weeks of age than those restricted only during preweaning or postweaning periods. Body weight of rats restricted only before or only after weaning were similar but were smaller than those of rats adequately fed before and after weaning. Adipose deposits were reduced by preweaning protein restriction but were not affected by restriction begun after weaning. Hepatic weights also were more seriously reduced by preweaning protein restriction while renal weights were reduced to a greater extent when protein was restricted after weaning than when it was limited before weaning.

Food consumption and utilization

During the first 2 weeks after weaning (pups were 4 to 5 weeks of age), rats restricted in protein before, after, or before and after weaning ate more food per g metabolic weight than those adequately nourished before and after weaning (Tables 23, 24, and 25). Food consumption relative to

metabolic weight of animals restricted only before weaning (LP/HP or LP_p/HP) was 7 to 18% higher in week 4 and 3 to 19% higher in week 5 on the average than that of HP/HP or HP_p/HP rats in Experiments I, II, and III. Rats restricted only after weaning (HP/LP or HP_p/LP) ate an average of 23 or 24% more food per g metabolic weight in week 4 and 24 to 32% more during week 5 than HP/HP or HP_p/HP rats in Experiments II and III. (No HP/LP group was included in Experiment I.) Relative intakes of rats restricted before and after weaning (LP/LP or LP_p/LP) were 16 to 26% larger in week 4 and 3 to 39% more in week 5 than those of HP/HP or HP_p/HP animals in Experiments I, II, and III. The increased food intake among rats restricted during some period of life disappeared during week 6 in Experiments II and III and during week 7 in Experiment I. Intakes for HP/HP groups in Experiments I and II increased during weeks 7 and 6, respectively, while those of the other groups remained constant or decreased; intakes of HP_p/LP and LP_p/LP animals decreased while those for HP_p/HP and LP_p/HP rats remained steady for week 6 in Experiment III.

Rate of growth increased for HP/HP and LP/HP rats in Experiments I and II and HP_p/HP and LP_p/HP animals in Experiment III simultaneously with the change in the relative food intake patterns during weeks 6 and 7. In week 8 in Experiment I and week 7 in Experiments II and III, however, relative intakes for animals restricted after weaning (HP/LP and LP/LP or HP_p/LP and LP_p/LP) were again higher than those of the HP/HP or HP_p/HP groups and remained so through week 16 in Experiment I, through week 20 in Experiment II, but only through week 8 in Experiment III.

Barnes et al. (1968, 1973) fed 12% casein during lactation and then continued or initiated restriction after weaning by feeding 3% casein to

weanling pups for 4 weeks. Rats were fed a ration supplying 25% casein after weaning or after 7 weeks of age. They observed increased food intake relative to metabolic weight among animals restricted during suckling and after weaning and among those restricted only after weaning; the difference in intakes persisted until the study was terminated when rats were 16 weeks old. De Castro and Boyd (1968) and Kirsch et al. (1968) observed increased absolute food intake among animals fed restricted quantities of protein for 28 and 60 days after weaning, respectively. These intakes would also have been larger on the basis of metabolic weight since restricted rats were smaller than those fed adequate amounts of protein.

Intakes per g metabolic weight for LP/HP or LP_p/HP rats were similar to those of HP/HP or HP_p/HP animals during weeks 7 to 20 in each of the 3 experiments; therefore, increased relative food consumption resulting from preweaning protein restriction did not endure under conditions of the present experiments. In contrast, Barnes et al. (1973) and Hsueh et al. (1974) have observed increased food intakes relative to metabolic weight among rats born of and nursed by dams who were restricted in protein or food energy; when they were fed adequate rations after weaning, the increased relative intakes persisted through 14 to 16 weeks of age at which time the studies terminated. Hsueh et al. (1974) used rats of the McCollum strain, restricted their total food to approximately 50% of an ad libitum intake in gestation and lactation, then fed weanlings a balanced laboratory chow. Barnes et al. (1973) fed Holtzman rats 7% casein in gestation, 12% casein in lactation, and 25% casein after weaning. These 2 regimens differed from each other, but the latter was quite similar to the experimental procedure used in the present study, i.e., rats were fed 6 and 10% casein in gesta-

tion and lactation, respectively, and 24% casein after weaning. However, rats of the Wistar strain were used in the experiments reported here. It is not known if differences in strain of albino rats or in experimental procedure are responsible for the variation in results.

The reason for increased food intake demonstrated by protein-restricted animals has not been clarified. Barnes et al. (1973) measured body composition and oxygen consumption in adult males that had been nursed for 3 weeks by dams fed 12% casein then fed a diet containing 3% casein for 4 weeks after weaning. From 7 weeks of age, diets containing 25% casein were fed. A small but significant decrease in body fat could have accounted for the small significant increase in O_2 consumption observed under fasting conditions in the experimental animals when compared with animals adequately nourished during lactation and after weaning. A much larger and more significant increase in O_2 intake of rats restricted from birth to 7 weeks of age compared with that of rats adequately fed throughout life was observed when O_2 consumption was measured while rats were fed the 25% casein diet *ad libitum*; this increase was attributed to an increase in specific dynamic action (SDA) resulting from a larger than expected food intake observed in these animals. The increased SDA was believed to be the result of rather than cause of increased food consumption, however. Although neither O_2 consumption nor body composition was measured in the current experiments, perirenal and epididymal adipose deposits were decreased significantly in animals restricted in protein before weaning (LP/HP and LP/LP) in Experiments II and III. Hence, total body fat may have been decreased.

Food utilization, indicated by food efficiency ratios, was depressed in rats subjected to postweaning protein restriction when compared with those of similar age which were adequately fed after weaning in each of the 3 experiments. This finding agreed with those of De Castro and Boyd (1968), Kirsch et al. (1968), Stead and Brock (1972), and Musten et al. (1974).

Food intake per unit metabolic weight was consistently increased in rats restricted in protein after weaning. This behavior persisted through 20 weeks of age in Experiment II, 16 weeks in Experiment I, but only 8 weeks in Experiment III. Immediately after weaning, rats restricted in protein before weaning tended to eat slightly more food per unit metabolic weight than those adequately nourished during lactation. However, the tendency persisted only through week 6 under the conditions of Experiments I, II, or III. Food efficiency ratios were depressed by postweaning protein restriction in each of the 3 experiments.

Brain Development and Behavior

Brain weight

Newborn and weanling females Absolute brain weights of newborn females were decreased by protein restriction in each of the 3 experiments, but the values for protein-restricted and adequately nourished neonates were not always significantly different. However, brain weight relative to body weight was increased in protein-restricted neonates indicating that brain weight enjoyed a greater degree of protection from the effects of in utero protein restriction than did body weight. Weanling females, born of and suckled by protein-deficient females, also has smaller absolute brain

weights than those of adequately fed offspring. Brain weight relative to body weight, as for newborns, was increased among protein-restricted compared with adequately fed weanlings.

These findings agree with those of a number of earlier investigations in this laboratory and elsewhere (Winick and Noble, 1966; Culley and Lineberger, 1968; Guthrie and Brown, 1968; Zamenhof et al., 1968, 1971, 1972; Winick, 1970; Chou, 1970; Puar, 1972). The smaller brains of the protein-restricted animals probably represented permanently reduced cell populations (Winick and Noble, 1966) as well as permanently altered concentrations of various cell types in different regions of the brain (Winick, 1970).

Adult males Protein restriction before weaning resulted in permanently smaller brains in adult males while restriction after weaning appeared to have little effect on final brain weight. Restriction before or after weaning resulted in relatively large brains per unit body weight, demonstrating the relatively protected status of the brain during protein restriction in the experimental design used here.

Protein restriction before and/or after weaning did not exert any consistent effect on cortical or subcortical weight nor on the ratio of cortical to subcortical weights. No significant differences in these parameters were observed in Experiment I. In Experiment II, subcortical sections of animals subjected to protein restriction before weaning (LP/LP + LP/HP) were lighter than those of rats supplied adequate protein during that period (HP/HP + HP/LP). In contrast, in Experiment III, cortical sections of animals born of and suckled by adequately nourished dams ($HP_p/HP + HP_p/LP$) were heavier than those of rats ($LP_p/LP + LP_p/HP$) whose mothers had

consumed restricted protein for a prolonged time, i.e., from their own weaning through 2 reproductive cycles. Separation of the rat brains into cortical and subcortical sections was not a precise procedure due to the small size and soft consistency of the tissue. Consequently, experimental error as well as biological variation may have contributed to the inconsistency of the results from one experiment to the other.

Brain cholinesterase activity

Newborn and weanling females Among newborn females, in utero protein restriction generally resulted in reduced specific and total cholinesterase (ChE) activity though the mean values for adequately nourished and protein-restricted neonates were significantly different in Experiment II only. Specific ChE activity increased at a slightly faster rate during suckling among restricted than among well fed animals so that at weaning values for the 2 groups were similar. Because the brains of adequately nourished weanlings were larger than those of restricted pups, total ChE activity remained significantly higher for the well fed group.

Reports in the literature about the effect of dietary restriction in gestation and lactation on ChE activity in neonatal and weanling brains are conflicting. Sereni et al. (1966) observed lower specific ChE activity in brains of rats suckled in litters of 16 than in brains of rats suckled in litters of 4. Significant differences were observed on days 6, 8, and 14 postpartum but not on day 21. Restriction was continued after weaning by feeding 50% of the ad libitum intake of well nourished animals to the pups that had been suckled in large litters. However, ChE activities for the well nourished and restricted rats continued not to be different on

days 35 and 45. Adlard et al. (1970) underfed dams during gestation and lactation to the extent that the mean weaning weight of their young was only 34% of that of controls. Specific ChE activity of brain tissue from these pups averaged 14% below that of the controls at weaning; in addition, their brain weight was 27% below that of controls. On the other hand, Im et al. (1970) found that specific ChE activity in the brains of pups adequately nourished prenatally then nursed by dams fed 12% casein ad libitum was significantly higher on day 9 postpartum than that of pups nursed by dams fed 25% casein. At weaning (21 days), specific ChE activity for control and restricted pups was similar, but the larger brains of the control groups resulted in total ChE activity that was significantly higher than that of the restricted group.

Of the studies on ChE activity cited previously, only Adlard et al. (1970) combined gestational and lactational restriction. Their restriction, however, was achieved by underfeeding rather than explicit protein limitation; perhaps this procedural difference could account for the larger reduction in specific ChE activity among restricted weanlings in their study (14%) than was observed in Experiment II (0%) and III (5%) in the present studies. The findings of Sereni et al. (1966) and Im et al. (1971) who employed restriction during suckling only were comparable to those of the present experiments at 21 days, i.e., no differences among groups with respect to specific ChE activity; since no measurements of brain ChE activity were made between birth and weaning in Experiments II and III whether perinatal ChE activity in these studies was similar to that in Sereni's (decreased in restricted pups at 6, 8, and 14 days) or Im's (increased in restricted pups at 9 days) investigation cannot be predicted.

Adult males In the present studies, ChE activity in the brains of adult males was not consistently affected by protein restriction before or after weaning (Tables 54a and 54b). Since the limbic system and corpus striatum which possess high concentrations of acetylcholinesterase are located in the subcortex (Krnjevic, 1969), specific subcortical ChE activity among rats in the current studies was generally higher than specific cortical ChE activity. When such was not the case in individual samples, error in separation of the brain section may have been a reasonable explanation.

Im et al. (1971) reported significantly higher specific ChE activity in the brains of 7 and 39 week old males restricted during lactation and 4 weeks after weaning compared with those of control animals. Im et al. (1971) used Holtzman rats whereas Wistar rats were chosen for the current series of experiments. They restricted casein to 12% of the diet for lactating dams and to 3% for 4 weeks after weaning while 6 and 10% casein were fed to pregnant and lactating dams, respectively, to achieve preweaning restriction in the present studies; from the age of 3 weeks to autopsy following behavioral training, 10% casein was fed. It is not known which of these procedural differences accounted principally for the difference in experimental results. In addition, brains in the present studies had been held at -20°C for 18 to 24 months prior to ChE assay and were thawed once during this period due to freezer failure. Although activity may have been altered under these conditions, ChE activity in the brains of 4 freshly autopsied stock neonates was similar to that in the brains of 4 stock neonates which had been frozen more than 24 months and had thawed once during the freezer failure; therefore, the assay values were assumed to be valid.

Table 54a. Mean specific cortical, subcortical, and total brain ChE activities of adult male rats in Experiment I

Experiment Group	I _a		P ^c (R ^c x 10 ⁶)	I _b		P
	HP/HP 7	LP/LP 6		St/St 5	4 ^g 15 ¹ / ₁₅ 8 ^g 6 ¹	
Cortex	8.18	7.34	NS ^d	8.26	7.23	NS
Subcortex	8.36	9.31	NS	8.33	8.35	NS
Whole brain	8.29	8.53	NS	8.33	7.90	NS

^aHP/HP_{nt} vs HP/HP.

^bHP/HP vs LP/HP.

^cRate in moles ASCh hydrolyzed per minute.

^dNS = not significant at least at 0.10 level.

Table 54b. Mean specific cortical, subcortical, and total brain ChE activities of adult male rats in Experiments II and III

	HP/HP	HP/LP (R ^b x 10 ⁶)	LP/HP	LP/LP
Experiment II				
Cortex	6.85(22) ^c	6.83(23)	7.23(26)	7.33(25)
Subcortex	8.12	7.69	7.96	7.90
Whole brain	7.64	7.36	7.67	7.65
Experiment III				
Cortex	HP _p /HP 8.15 (8)	HP _p /LP 8.73 (6)	LP _p /HP 7.59 (5)	LP _p /LP 7.97 (5)
Subcortex	8.52	8.22	8.65	8.49
Whole brain	8.37	8.44	8.24	8.26

^aDiet before weaning x diet after weaning.

^bRate in moles substrate hydrolyzed per minute.

^cNumber of rats.

^dNS = not significant at least at 0.10.

^e0.05 < P < 0.10.

P ^a	HP/HP _{nt}	I _c		LP/HP	P ^b
		(R ^c x 10 ⁶)			
NS	7.79	NS	7.84	7.78	NS
NS	7.56		7.90	7.84	NS
NS	7.68		7.90	7.80	NS

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P (R ^b x 10 ⁶)	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^d	6.84(45)	7.28(51)	NS	7.06(48)	7.09(48)	NS
NS	7.90	7.93	NS	8.03	7.80	NS
NS	7.50	7.66	NS	7.66	7.51	NS
	HP /HP + HP ^p /LP	LP /LP + LP ^p /HP		HP /HP + LP ^p /HP	LP /LP + HP ^p /LP	
NS	8.40(14)	7.78(10)	<0.10 ^e	7.94(13)	8.38(11)	NS
NS	8.39	8.57	NS	8.57	8.34	<0.10
NS	8.40	8.25	NS	8.32	8.36	NS

Behavior

Performance on the visual discrimination problem employed in Experiments I, II, and III was not consistently nor significantly affected by preweaning protein restriction. Rats restricted in protein before weaning only (LP/HP or LP_p/HP) averaged as many or more correct choices daily in acquisition, reversal, and extinction training as those given adequate protein before and after weaning (HP/HP or HP_p/HP). Also, a similar number of trials was required by both groups to reach criterion.

In contrast, rats given a restricted protein supply after weaning (HP/LP or HP_p/LP and LP/LP or LP_p/LP) made more incorrect choices and required more trials to achieve criterion on the average than either rats well nourished before and after weaning or those restricted before weaning and adequately fed afterwards. Rats restricted after weaning (HP/LP or HP_p/LP and LP/LP or LP_p/LP) committed a similar number of errors in daily training irrespective of their preweaning treatment. Rats in the HP/LP group required the most trials to reach criterion in Experiment II, but in Experiment III, LP_p/LP rats required more trials to achieve criterion than any other group.

Latency, the time required from entry into the maze to completion of a choice, was not significantly affected by preweaning protein restriction. Of the 4 experimental groups, LP/HP rats generally had the shortest latencies during acquisition; however, latencies associated with both acquisition and extinction tended to be longer for animals restricted after weaning (LP/LP or LP_p/LP and HP/LP or HP_p/LP) than those for rats fed 24% casein after weaning (HP/HP or HP_p/HP and LP/HP or LP_p/HP).

Several investigators have observed significant behavioral effects of preweaning protein or caloric restriction. Simonson and Chow (1970) examined performance of rehabilitated male rats in an elevated T maze. The rats had been born of and nursed by dams fed about 50% as much food as that eaten by control rats during gestation and lactation and were given a stock ration from weaning to termination of the study. Behavioral tests in the elevated T maze were initiated at 10 weeks of age, and water deprivation was employed to motivate rats to solve a problem. During acquisition, rehabilitated rats exhibited longer starting and running times and made more errors than rats which had been well nourished throughout life. During extinction when water was withdrawn as a reward for correct choices, the previously malnourished rats ran more trials and continued to make more errors than the control group. Barnes et al. (1966) reported that 6 to 9 month old rats, which had been suckled in large litters (14 to 16 pups) then fed 3 or 4% protein for 8 weeks after weaning, made significantly more errors when escaping from a water maze than control rats suckled in litters of 8 and fed 25% casein after weaning. In contrast to these 2 studies, Rajalakshmi et al. (1967) observed no impairment of discrimination ability following rehabilitation on stock ration among adult rats whose access to lactating females had been limited so that they weighed about half as much as controls when weaned at 28 days of age.

Although rats restricted in protein during gestation and lactation only (LP/HP or LP_p/HP) performed as well as control rats (HP/HP or HP_p/HP) on the visual discrimination maze in the present studies, one may not conclude that protein restriction before weaning did not affect behavior nor that feeding an adequate ration from weaning resulted in complete rehabili-

tation by 20 weeks of age. Barnes¹ has reported that rats restricted in protein during early life consumed larger quantities of water under ad libitum conditions even after nutritional rehabilitation than animals fed a control ration throughout life.

Water intake was not measured in the current experiments, but weight change under conditions of water deprivation was monitored. Most rats lost some weight (from 3.05 to 6.50% of their pre-deprivation weight) over the first 8 days of restriction before training began. Weight change, in percent, from days 1 through 25 are presented in Tables 55a and 55b. In Experiment II, rats restricted in protein before weaning only (LP/HP) lost more weight or gained less during the 25 days of training than any other group, i.e., they were less able to maintain body weights under conditions of water deprivation than the other 3 groups. This finding could indicate an increased need for water which could have increased motivation for LP/HP rats under the training conditions employed in the present studies. In Experiment III, which produced a more severe protein deficit in dams, the weight change during training for LP_p/HP rats did not differ from that of the other 3 groups; nor did the weight changes in LP/HP rats in Experiment I_c differ from those in the HP/HP group. Therefore, an increased need for water in rats restricted in protein before weaning only compared with other groups was not confirmed in Experiments I_c and III. Without direct measurements of ad libitum intake, the hypothesis that rats restricted before

¹R. H. Barnes, Graduate School of Nutrition, Cornell University, Ithaca, New York. Unpublished data. Private communication. 1972.

Table 55a. Mean percent weight change of adult male rats when subjected to water deprivation in Experiment I

Experiment Group	I _a		P
	HP/HP	LP/LP	
Number of rats	7	6	
% wt. change days 1 to 25 training	6.91	6.79	NS ^a

^aNS = not significant at least at 0.10 level.

*P<0.05.

Table 55b. Mean percent weight change of adult male rats when subjected to water deprivation in Experiments II and III

Experiment II	HP/HP	HP/LP	LP/HP	LP/LP
% wt. change days 1 to 25 training ^b	4.36(22) ^c	8.44(22)	1.44(26)	4.45(25)
Litter 2	4.89(15)	9.30(17)	1.74(17)	5.13(17)
Litter 3	3.22 (7)	6.02 (6)	0.87 (9)	3.10 (8)
Experiment III	HP _p /HP	HP _p /LP	LP _p /HP	LP _p /LP
% wt. change days 1 to 25 training ^b	3.99 (8)	5.62 (6)	4.63 (5)	3.77 (5)
Litter 1	3.50 (2)	2.97 (1)	-3.41 (1)	-8.96 (1)
Litter 2	4.15 (6)	6.15 (5)	6.64 (4)	6.95 (4)

^aDiet before weaning x diet after weaning.

^bArithmetic mean for litters 2 and 3 in Experiment II; for litters 1 and 2 in Experiment III.

^cNumber of rats.

^dNS = not significant at least at 0.10 level.

^eInsufficient observations for regression analyses.

*P<0.05.

*P<0.01.

St/St	I _b		P	I _c		P
	4	15 ¹ /15		HP/HP	LP/HP	
5	8	7 ¹		6	4	
6.06	1.31		--*	-0.24	-1.66	NS

Inter- action ^a	Diet before weaning			Diet after weaning		
	HP/HP + HP/LP	LP/LP + LP/HP	P	HP/HP + LP/HP	LP/LP + HP/LP	P
NS ^d	6.45(45)	2.92(51)	NS	2.78(48)	6.36(42)	NS
NS	7.23(32)	3.44(34)	--**	3.22(32)	7.21(34)	--**
NS	4.52(13)	1.88(17)	--*	1.90(16)	4.30(14)	NS
	HP /HP + HP ^p /LP	LP /LP + LP ^p /HP		HP /HP + LP ^p /HP	LP /LP + HP ^p /LP	
NS ^e	4.62(14)	4.20(16)	NS	4.23(13)	4.78(11)	NS
-- ^e	3.32 (3)	-6.19 (2)	--	1.20 (3)	-2.99 (2)	--
NS	5.06(11)	6.75 (8)	NS	5.14(10)	6.51 (9)	NS

weaning consume more water under normal laboratory conditions than rats adequately nourished throughout life cannot be refuted, however.

Rats subjected to postweaning restriction (LP/LP or LP_p/LP and HP/LP or HP_p/LP) in Experiments I through III made more errors and required more trials to achieve criterion than rats well nourished after weaning (HP/HP or HP_p/HP and LP/HP or LP_p/HP). These results may have been predictable since the animals were maintained on the restricted ration (10% casein) through the training periods. Cowley and Griesel (1959, 1963, 1966) and Rajalakshmi et al. (1965) also have reported increased errors on maze problems in rats maintained with diets containing low protein.

In summary, behavior measured in the current studies may have been confounded by 2 conditions. Because rats deprived of protein early in life have been reported to consume more water ad libitum than normally nourished animals,¹ malnourished rats in the present experiments may have been motivated more by water deprivation and given more attention to the location of the reward than the adequately nourished animals. Their increased motivation may have compensated for less than complete rehabilitation and accounted for the performances of the LP/HP and LP_p/HP groups which were similar to those of HP/HP and HP_p/HP animals, respectively.

Performances of HP/LP or HP_p/LP and LP/LP or LP_p/LP rats were probably confounded because they continued to consume the low protein diet during training. Other investigators have shown that behavioral performance varied with food being consumed at the time measurements were made. Brožek

¹R. H. Barnes, Graduate School of Nutrition, Cornell University, Ithaca, New York. Unpublished data. Private communication. 1972.

and Vaes (1961) reviewed a number of investigations on conditioned reflexes and reported that doubling the control intake of protein by dogs increased the animals' responsiveness to environmental stimuli while a drastic decrease in dietary protein elicited sluggish responses and decreased sensitivity to electric current. Brožek and Vaes' report combined with those of Cowley and Griesel (1959, 1963, 1966) and Rajalakshmi et al. (1965) would indicate that the increased number of discrimination errors and larger number of trial required to reach criterion by HP/LP or HP_p/LP and LP/LP or LP_p/LP rats compared with those of rats well nourished after weaning was promoted by the low protein diet consumed during training in addition to the effect, if any, of treatment prior to training.

Correlations

No parameters of brain weight or brain ChE activity were consistently correlated with trials to criterion in acquisition for any experimental group in the current series of experiments. Krech et al. (1962) found strong correlations between reversal scores in a visual discrimination maze and 1) cortical:subcortical ratios of ChE activity and 2) brain weight in animals exposed to a complex environmental stimuli for 30 days after weaning. In consideration of these studies, it had been hypothesized that protein restriction might exert its effect on behavior through ChE activity. If so, behavioral scores and ChE activity would be significantly correlated. Such was not the case under the conditions of these studies, however.

SUMMARY

The effects of protein restriction on reproductive performance, growth, development and metabolic efficiency, and brain development and behavior were investigated in the rat. Three studies were designed to compare reproduction in rats fed 6% casein from weaning, in rats fed 6% casein beginning day 0 of pregnancy, and in rats who had successfully weaned 1 litter on an adequate stock ration prior to restriction on day 0 of their second gestation. For lactation, dietary casein was increased to 10% in all experiments. Adequately fed rats received 24% casein in gestation. All diets were supplemented with DL-methionine equivalent to 0.83% of the casein.

Each female was allowed to complete 2 reproductive cycles on the assigned experimental diet. Maternal food intake and weight change during gestation and lactation were monitored as were number of pups born, gestation length, survival, and weight gain of the offspring. Litter size was limited to 8 or 10 pups for lactation; males were preferentially retained, and females were chosen at random to complete the litter. Excess female neonates were sacrificed so that carcass and organ weight data could be obtained. Female weanling body and organ weight data were also collected in Experiments II and III. Brain ChE activity was evaluated for both newborn and weanling females.

Male progeny were assigned to a postweaning diet of either 10 or 24% methionine-supplemented casein when weaned so that the effects of protein restriction before or after weaning or during both periods could be

assessed. Growth, food intake, and food efficiency were monitored in these rats from weaning through about 6 months of age.

When rats were about 5 months old, a water deprivation schedule in which water was withheld for $23\frac{1}{2}$ hours out of each 24-hour period was initiated in preparation for behavioral training. After 5 to 8 days of adjustment to water deprivation, rats were introduced to the training device, a simple Y maze, in groups of 4 to 5 for 5 minutes each. Then each animal was placed singly in one of the goal boxes or in the start box and allowed to enter either goal box at will. He was confined in the goal box until he drank from the water cup. This shaping procedure was repeated for 2 to 3 successive days. No visual cues were presented during this period.

Training was divided into 3 phases: 1) acquisition, 2) reversal, and 3) extinction. Rats were trained to enter the lighted or dark arm of the maze for a reward of 0.1 to 0.2 ml water in acquisition. An average of 10 correct choices out of 12 daily trials for 2 successive days was established as the criterion of success in Experiment I; 9 correct choices out of 10 daily trials on 2 successive days was the criterion in Experiment II. Reversal training was begun the day after an individual rat achieved criterion in acquisition in Experiment I. During reversal, the location of the reward was changed so that choices that had been wrong in acquisition became correct, i.e., if the reward had been located in the lighted arm in acquisition, it was located in the dark arm in reversal. When rats achieved criterion in the reversal regimen, extinction training in which no rewards were given was begun. Extinction training was conducted for 20 days in Experiments I_a and I_b and 12 days in I_c.

Number of correct choices in daily trials, number of trials required to achieve criterion, and latencies, the time required from entry into the maze to completion of the choice for selected days, were observed in each experiment. Reversal training was omitted in Experiments II and III. Acquisition training was limited to 15 days and extinction training to 10 days in the final 2 studies.

Upon completion of behavioral training, rats were allowed water ad libitum for at least 1 week in Experiments I_a and I_b and 2 days in I_c, II, and III. Then they were sacrificed by decapitation. Carcass, adipose deposits, brain, hepatic, renal, and splenic weights were determined. Brains were divided also into cortical and subcortical sections which were weighed and analyzed for ChE activity. Correlations of ChE activity and brain weight with trials required to achieve criterion in acquisition were assessed.

Food intake and weight change during gestation and lactation varied between adequately nourished and protein-restricted females. During gestation, total food intake was similar for the 2 groups, but the patterns of intake differed. Restricted dams ate more food than adequately fed females during the first 2 weeks but less during the final week of gestation. Total food intakes of protein-restricted dams during lactation were smaller than those of adequately fed females. However, food eaten per pup weaned did not differ between the 2 groups. Net weight gains for rats restricted in protein intake during gestation were 67 to 80% smaller than those for rats well nourished during pregnancy. Both adequately nourished and protein-restricted females lost weight during lactation. Weight loss was related to number of pups weaned as well as diet, but when the number of

pups nursed to weaning was similar, adequately nourished females lost less weight than those restricted in protein.

Reproductive performance was adversely affected by protein restriction in each of the 3 experiments. Stillbirths and perinatal mortality tended to be higher among restricted than among adequately nourished progeny. When restriction was introduced on day 0 of pregnancy, mean survival rate at weaning was only 6% among restricted pups compared with 30% for the offspring of adequately nourished females. Restriction of the dams from weaning resulted in 14% survival of their pups at weaning compared with 56% among adequately nourished progeny. Therefore, in the present studies, prolonged restriction did not result in maternal adjustments which allowed for improved pup survival. An average of 39% of the restricted progeny and 66% of the adequately nourished pups were weaned from dams which had been allowed to rear one litter on stock ration before being given 6% casein beginning day 0 of their second pregnancy. This paradigm was the most efficient design for producing protein-restricted subjects for subsequent growth and development or behavioral study.

Restricted offspring from all 3 experiments were significantly smaller than well nourished neonates at birth and continued to be so at 7, 14, and 21 days of age. Absolute carcass, hepatic, renal, and splenic weights of protein-restricted newborn and weanling females were smaller than those of their adequately nourished counterparts, but relative carcass and organ weights were not consistently affected by protein restriction.

Male offspring restricted in protein before and after weaning were smaller at 20 weeks of age than those restricted only before or after weaning. Body weight of rats restricted only before or only after weaning were

similar at 20 weeks of age; their weights were intermediate to those of rats adequately nourished and those of rats restricted throughout life. Adipose deposits, absolute amounts, were reduced by preweaning protein restriction but were not affected by restriction begun after weaning. Hepatic weights also were more seriously reduced by preweaning than postweaning restriction while renal weights were reduced to a greater extent when protein was restricted after than when it was limited before weaning.

Food intake per unit of metabolic weight was consistently larger for rats restricted in protein after weaning than for those adequately fed after weaning. Immediately after weaning, rats restricted before weaning only tended to eat slightly more food relative to metabolic weight than those adequately nourished both before and after weaning. This effect of preweaning restriction disappeared in 2 or 3 weeks, however, while the increased relative intakes due to postweaning restriction persisted for a longer time, 5 to 17 weeks. Food efficiency ratios were depressed by postweaning but not by preweaning protein restriction.

Absolute brain weights of newborn and weanling females were decreased by protein restriction, but brain weight relative to body weight was larger among protein-restricted than among adequately nourished offspring. In adult males, protein restriction before weaning resulted in permanently smaller brains while restriction after weaning appeared to have little effect on final brain weight. Restriction before or after weaning resulted in relatively large brains per unit body weight. Neither protein restriction before or after weaning exerted any consistent effect on cortical or subcortical brain weight nor on the ratio of cortical to subcortical weights.

Specific and total ChE activities were generally lower in the brains of protein-restricted than in those of adequately nourished neonates. At weaning, specific ChE activities in the brains of the 2 groups were similar, but because the brains of adequately nourished weanlings were larger than those of restricted pups, total ChE activity was higher for the well fed group. Neither total, cortical, subcortical, nor the ratio of cortical to subcortical ChE activity in the brains of adult males was affected by protein restriction before or after weaning.

Performance on a visual discrimination problem was not affected by preweaning protein restriction, but those rats fed 10% casein after weaning consistently made more errors, required more trials to achieve criterion, and exhibited longer latency times than rats fed 24% casein after weaning. It is believed that motivational factors may have confounded the experimental results. Rats restricted in protein early in life may have an increased need for water and, therefore, rats restricted before weaning may have been more motivated by the water reward than those rats adequately nourished during gestation and lactation. Rats consuming a restricted diet have performed poorly in behavioral test situations; therefore, performance of rats fed the 10% casein ration during training may have been mediated by the current as well as previous dietary treatment.

Behavioral performance was not correlated with any parameters of brain weight or brain ChE activity.

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APPENDIX

Table A1. Composition of Steenbock XV and XVII diets^a

Ingredients	Steenbock XV (%)	Steenbock XVII (%)
Corn meal ^b	42.1	48.3
Skim milk ^c	18.3	10.3
Linseed meal ^d	12.0	13.8
Wheat germ ^e	7.5	8.6
Yeast, brewers ^b	7.1	8.6
Casein, high protein ^b	3.8	4.3
Alfalfa meal ^f	1.5	1.7
NaCl (iodized) ^g	0.4	0.4
CaCO ₃ + trace elements ^h	0.4 ⁱ	0.4 ^j
Yeast + Ca-pantothenate ^k	0.4	--
Corn oil ^l	6.4	3.5
Corn oil + vit. D ₃ ^m	0.1	0.1
	100.0	100.0

^aPercent N₂: Steenbock XV 3.80 x 6.25 = 23.75% protein; Steenbock XVII 3.66 x 6.25 = 22.88% protein; analyzed Aug., 1974.

^bGeneral Biochemicals, Inc., Chagrin Fall, Ohio.

^cDes Moines Cooperative Dairy, Des Moines, Iowa.

^dFroning and Deppe Elevator, Ames, Iowa.

^eGeneral Mills, Inc., Minneapolis, Minnesota.

^fNational Alfalfa, Lexington, Nebraska.

^gLocal grocer.

^hMatheson Coleman & Bell Div. of Matheson Company, Inc., Norwood, Ohio.

ⁱKI, 0.400 g; MnSO₄, 1.584 g; K₂Al₂(SO₄)₄, 0.490 g; CuSO₄, 2.035 g; and CaCO₃ to make 500 gm.

^jKI, 0.200 g; MnSO₄, 0.790 g; K₂Al₂(SO₄)₄, 0.245 g; CuSO₄, 1.018 g; and CaCO₃ to make 500 gm.

^k6 gm Ca-pantothenate per 1 kg yeast to give 24 mg Ca-pantothenate per kg diet.

^lMazola, Best Foods Division Corn Products Company, New York, New York.

^mCrystalline vitamin D₃ (cholecalciferol) diluted with corn oil to give 2,000 IU or 50 mcg vitamin D₃ per kg diet.

Table A2. Composition of diets for Experiments I_b

Component	% diet		
	15 ^a	15 ₁ ^b	4 _g ^c
Casein, vitamin free, test ^d	17.8	17.8	4.7
Cornstarch ^d	66.0	65.0	78.2
Cottonseed oil ^e	5.0	5.0	5.0
Vitamin mix ^f	5.0	5.0	5.0
Hawk-Oser mineral mix ^{dg}	3.0	3.0	3.0
NaCl	--	1.0	1.0
CaHPO ₄ ^{dg}	1.0	1.0	1.0
DL-methionine ^d	0.200	0.200	0.0534
Nonnutritive fiber ^d	2.0	2.0	2.0

^a15 ≈ 15% protein ration fed to rats after weaning for growth and maintenance.

^b15₁ ≈ 15% protein ration fed to lactating dams.

^c4_g ≈ 4% protein ration fed to pregnant females.

^dGeneral Biochemicals Inc., Chagrin Falls, Ohio.

^eWesson Oil, Wesson Sales Co., Fullerton, California.

^fThe cornstarch-based mix provided, in mg/kg diet: thiamin-HCl, 1.88; riboflavin, 3.75; pyridoxine-HCl, 1.80; folic acid, 1.5; niacin, 22.5; Ca-pantothenate, 12.0; choline Cl, 1125; vitamin B₁₂, 0.075; biotin, 0.30; ascorbic acid, 750; para-aminobenzoic acid, 30.0; menadione, 0.15; dl-alpha-tocopheryl acetate powder (250 I.U./g), 360; retinol palmitate (water-dispersible beadlets 0.41 I.U./μg), 43.3. Vitamins A and E were added immediately before incorporation of the mix into the diet.

^gHawk-Oser formulation plus CaHPO₄ with sulfates of Mn, Zn and Cu provided in mg/kg diet: Ca, 6200; P, 4250; Na, 920; K, 4900; Mg, 500; Mn, 50; Fe, 127; I, 0.9; F, 7.0; Cu, 5.0; Zn, 0.04.

Table A3. Ranges of maternal food intake and net weight change in gestation and lactation; age and weight at mating and length of gestation of female rats in Experiments I, II, and III

Experimental group	Age at mating days	Weight at mating g	Total food	
			Gestation g	Lactation g
<u>Experiment I</u>				
HP				
Litter 1	77 to 84	224 to 249	331 to 443	575
Litter 2	118 to 140	263 to 330	342 to 389	560 to 731
LP				
Litter 1	76 to 80	214 to 280	353 to 498	567
Litter 2	115 to 135	266 to 314	321 to 449	502
LPM				
Litter 1	75 to 81	231 to 281	379 to 516	544 ^a
Litter 2	113 to 134	302 to 328	304 to 406	-- ^a
LP _H				
Litter 1	77 to 86	240 to 284	382 to 461	--
Litter 2	122 to 142	285 to 333	313 to 449	406
<u>Experiment II</u>				
HP				
Litter 2	131 to 196	290 to 370	371 to 454	576 to 738
Litter 3	197 to 258	309 to 395	334 to 442	548 to 703
LP				
Litter 2	128 to 191	290 to 391	339 to 570	477 to 695
Litter 3	184 to 280	295 to 418	292 to 586	447 to 573
<u>Experiment III</u>				
HP _p				
Litter 1	143 to 161	284 to 370	298 to 443	520 to 726
Litter 2	201 to 220	301 to 395	246 to 421	545 to 727
LP _p				
Litter 1	145 to 188	220 to 285	239 to 392	332 to 454
Litter 2	190 to 225	235 to 333	299 to 385	430 to 487

^aNo group member completed lactation.

<u>intake</u> Lactation/No. weaned	<u>Net weight change</u>		Length of gestation days
	Gestation g	Lactation g	
72	39 to 82	-37	21.25 to 21.75
80-95	39 to 60	-25 to -34	21.25 to 21.50
142	-2 to 48	5	20.75 to 22.75
125	-23 to 43	-7	21.25 to 23.25
109	-1 to 54	-18	21.25 to 21.75
--	-13 to 43	--	21.25 to 23.25
--	12 to 48	--	21.25 to 22.00
101	-17 to 46	-15	21.25 to 21.75
84 to 115	45 to 81	-13 to -70	21.50 to 21.75
91 to 184	31 to 82	-9 to -52	21.25 to 22.50
79 to 238	-38 to 56	-80 to 35	20.50 to 22.75
71 to 152	-40 to 65	-19 to -98	21.50 to 23.00
94 to 130	22 to 76	-20 to -71	21.25 to 21.75
91 to 115	60 to 76	-6 to -48	21.50
91 to 332	-45 to 47	-15 to 39	21.25 to 22.75
54 to 70	-17 to 32	-20 to -25	21.25 to 22.50

Table A4. Ranges of number in litter, average birth weight, percent stillbirths, perinatal and weaning survival rates of rat litters in Experiments I, II, and III

Experimental group	Litter size	Average birth wt. g/pup	Stillbirths %	No. pups selected to nurse	Survival rate day 4 %	Survival rate at weaning %
<u>Experiment I</u>						
HP						
Litter 1	6-15	5.52-6.97	0	5-10	0-100	0-80
Litter 2	10-14	5.79-6.61	0-8	1-8	0-100	0-100
LP						
Litter 1	10-16	4.63-6.70	0-60	4-10	0-100	0-40
Litter 2	5-14	5.17-5.85	0-40	1-8	0-100	0-50
LPM						
Litter 1	12-17	5.07-6.01	0	10	0-100	0-50
Litter 2	7-14	5.51-6.23	0-100	4-8	0-62	0
LPH						
Litter 1	10-15	4.99-6.01	0-15	10	0-100	0
Litter 2	4-13	4.86-6.87	0	4-8	0-100	0-57
<u>Experiment II</u>						
HP						
Litter 2	8-15	6.19-7.25	0-18	7-8	86-100	71-100
Litter 3	1-17	5.20-7.29	0-33	1-8	0-100	0-86
LP						
Litter 2	8-15	4.95-6.89	0-25	8	0-100	0-100
Litter 3	6-13	4.30-6.76	0-100	1-8	0-100	0-89
<u>Experiment III</u>						
HP _p						
Litter 1	6-14	6.00-6.92	0-12	6-8	0-100	0-100
Litter 2	7-13	5.90-7.32	0	7-8	0-100	0-88
LP _p						
Litter 1	6-11	4.00-6.63	0-100	4-8	0-100	0-71
Litter 2	4-14	5.14-6.88	0-56	3-8	0-100	0-100

Table A5. Ranges of body and organ weights of newborn female rats in Experiments I, II, and III

Experimental group	Body wt. g	Carcass g	Liver mg	Kidney mg	Spleen mg
<u>Experiment I</u>					
HP	5.44-6.80	3.71-4.79	215-332	40-69	5.2-13.2
LP	4.68-5.97	3.21-4.09	132-222	39-62	4.6-11.8
LP _M	4.42-6.43	3.50-4.50	178-279	40-59	4.6-12.0
LP _H	4.62-6.78	3.18-4.88	126-324	34-66	5.9-14.6
<u>Experiment II</u>					
HP					
Litter 2	5.40-7.00	3.77-4.92	163-337	44-84	8.8-22.2
Litter 3	5.28-7.01	3.62-4.87	242-390	43-79	9.0-18.0
LP					
Litter 2	4.31-6.88	2.91-4.85	162-324	37-89	5.4-20.2
Litter 3	5.35-6.73	3.71-4.78	235-314	55-72	7.8-25.2
<u>Experiment III</u>					
HP _p					
Litter 1	5.35-7.13	3.73-5.03	233-356	50-80	8.2-15.2
Litter 2	5.62-7.21	3.87-5.02	239-321	55-77	12.1-26.4
LF _p					
Litter 1	4.73-6.40	3.30-4.44	188-256	48-76	8.0-13.2
Litter 2	4.78-5.72	3.38-3.98	172-245	40-56	8.0-15.8

Table A6. Ranges of average preweaning body weights, of pups in Experiments I, II, and III

Experimental group	Average body wt. (g)			
	Day 0	Day 7	Day 14	Day 21
<u>Experiment I</u>				
HP				
Litter 1	6.30	10.16	20.58	33.60
Litter 2	5.99-6.61	12.18-13.81	28.07-29.63	44.10-49.14
LP				
Litter 1	6.70	8.02	21.58	37.18
Litter 2	5.51	9.65	21.00	29.43
LPM				
Litter 1	6.01	9.39	21.41	32.64
Litter 2	-- ^a	--	--	--
LPH				
Litter 1	--	--	--	--
Litter 2	6.22	9.48	19.85	30.65
<u>Experiment II</u>				
HP				
Litter 2	6.19-7.25	12.36-17.96	20.88-36.99	35.00-57.84
Litter 3	6.42-7.29	9.86-16.23	20.87-37.37	38.14-60.77
LP				
Litter 2	5.28-6.89	6.72-14.31	15.38-30.24	23.38-44.36
Litter 3	5.85-6.76	8.06-12.51	17.90-23.22	30.12-37.61
<u>Experiment III</u>				
HP _p				
Litter 1	6.00-6.78	10.59-14.29	21.49-29.85	35.90-50.80
Litter 2	5.90-7.32	9.68-12.22	23.28-29.48	38.34-51.19
LP _p				
Litter 1	6.15-6.63	5.85-9.31	11.40-18.61	22.13-35.16
Litter 2	6.10-6.61	6.65-10.21	12.92-16.38	20.39-25.02

^aNo pups survived to weaning.

Table A7. Ranges of body and organ weights of weanling female rats in Experiments II and III

Experimental group	Body wt. g	Carcass g	Liver g	Kidney g	Spleen g
<u>Experiment II</u>					
HP					
Litter 2	40.23-56.90	28.25-44.30	1.34-2.29	0.46-0.90	0.09-0.33
Litter 3	30.10-64.58	22.38-48.61	1.05-2.54	0.35-0.86	0.16-0.27
LP					
Litter 2	25.65-55.63	18.70-41.59	0.70-1.97	0.28-0.57	0.09-0.38
Litter 3	28.10-41.90	20.42-31.17	0.96-1.50	0.29-0.48	0.10-0.20
<u>Experiment III</u>					
HP _p					
Litter 1	41.04-52.17	30.10-37.87	1.43-2.06	0.46-0.67	0.16-0.26
Litter 2	34.98-53.18	25.37-39.46	1.25-2.05	0.40-0.59	0.17-0.31
LP _p					
Litter 1	22.13-35.34	15.33-26.73	0.72-1.40	0.23-0.37	0.06-0.20
Litter 2	14.10-23.46	9.91-16.70	0.48-1.06	0.17-0.29	0.04-0.11

Table A8. Ranges of body weights of male offspring at intervals from 3 to 20 weeks of age in Experiments I, II, III

Experi- mental group	Body weight						
	3 weeks g	6 weeks g	9 weeks g	12 weeks g	15 weeks g	18 weeks g	20 weeks g
<u>Experiment I</u>							
^I _a HP/HP	32-49	100-152	218-299	266-362	310-433	324-456	324-465
LP/LP	30-43	86-106	163-198	227-246	268-302	290-333	283-332
^I _b St/St	33-75	114-182	280-314	352-426	400-509	432-514	456-558
4 ^I _g 15 ₁ /15	37-58	150-192	255-354	341-446	337-451	376-506	382-528
^I _c HP/HP	44-54	101-144	232-289	263-391	363-436	387-474	404-487
LP/HP	24-41	101-143	220-282	290-356	338-406	356-444	377-455
<u>Experiment II</u>							
HP/HP							
Litter 2	46-60	114-191	238-362	298-447	350-513	375-571	392-612
Litter 3	36-62	97-175	146-288	205-382	223-444	311-478	317-498
HP/LP							
Litter 2	42-55	91-167	151-297	236-381	297-435	335-468	350-492
Litter 3	31-61	83-157	125-229	216-328	273-411	318-460	333-464
LP/HP							
Litter 2	21-47	83-192	185-314	238-418	253-462	287-500	314-527
Litter 3	25-34	88-159	141-307	208-385	239-426	264-434	289-455
LP/LP							
Litter 2	22-45	58-147	123-271	189-333	265-387	269-432	285-458
Litter 3	30-40	72-119	133-228	206-307	255-373	284-390	307-395
<u>Experiment III</u>							
HP _p /HP							
Litter 1	43-44	144	228-278	338-377	395-417	418-468	449-490
Litter 2	40-62	133-162	248-278	307-370	365-428	396-468	415-480
HP _p /LP							
Litter 1	38	117	221	293	361	396	417
Litter 2	46-52	99-142	177-254	268-325	310-392	332-417	345-446
LP _p /HP							
Litter 1	28	133	231	306	354	389	410
Litter 2	23-27	105-118	219-247	268-304	297-375	331-397	347-399
LP _p /LP							
Litter 1	27	113	217	277	317	343	340
Litter 2	20-27	80-88	157-184	220-270	267-305	299-345	301-359

Table A9. Ranges of body, adipose deposit, and organ weights of adult male rats in Experiments I, II, and III

Experimental group	Body wt. g	Carcass g	Adipose deposit g	Liver g	Kidney g	Spleen g
<u>Experiment I</u>						
I ^a HP/HP	395-542	331-433	-- ^a	9.77-14.16	2.04-3.26	0.52-1.02
LP/LP	345-424	279-344	--	8.21-9.93	1.50-1.91	0.49-0.68
I ^b St/St	544-640	440-532	--	12.27-18.40	3.00-3.64	0.56-0.83
4 15 ₁ /15 _g	400-588	345-470	--	9.40-15.40	2.33-3.11	0.47-0.84
I ^c HP/HP	457-565	359-438	--	12.86-16.09	2.83-3.21	0.67-0.98
HP/HP ^{nt}	456-522	366-402	--	11.76-16.40	2.67-3.06	0.61-0.83
LP/HP	380-515	299-420	--	6.14-13.90	2.51-3.12	0.56-0.79
<u>Experiment II</u>						
HP/HP						
Litter 2	417-624	319-481	9.25-30.11	12.86-20.82	2.47-4.32	0.66-1.07
Litter 3	333-507	276-414	5.56-18.37	9.88-16.34	2.19-3.36	0.61-0.88
HP/LP						
Litter 2	389-555	305-432	8.04-32.50	10.14-17.14	2.06-3.05	0.55-0.85
Litter 3	343-521	279-428	11.04-13.55	9.27-16.06	1.72-2.96	0.63-0.86
LP/HP						
Litter 2	268-537	230-421	1.81-19.53	5.69-17.66	1.64-3.98	0.47-1.34
Litter 3	281-456	238-367	3.16-23.27	8.02-13.93	1.85-3.08	0.49-0.82
LP/LP						
Litter 2	307-462	252-361	6.22-24.53	7.56-13.17	1.49-2.68	0.47-0.97
Litter 3	320-427	260-348	7.57-20.36	8.59-11.99	1.56-2.49	0.50-0.69

^aNo adipose deposits were measured in Experiment I.

Table A9. (Continued)

Experimental group	Body wt. g	Carcass g	Adipose deposit g	Liver g	Kidney g	Spleen g
<u>Experiment III</u>						
HP _p /HP						
Litter 1	448-507	355-397	20.36-27.80	12.89-15.36	2.76-3.09	0.72-0.90
Litter 2	440-499	351-406	11.03-20.08	10.65-14.80	2.48-2.98	0.70-1.12
HP _p /LP						
Litter 1	425	343	17.05	10.79	2.38	0.57
Litter 2	362-462	297-376	11.59-20.02	9.78-13.74	2.04-2.65	0.57-0.73
LP _p /HP						
Litter 1	393	319	10.31	12.05	2.73	0.60
Litter 2	371-449	297-359	8.13-12.95	10.99-14.47	2.49-3.12	0.49-0.77
LP _p /LP						
Litter 1	331	264	11.67	9.33	2.02	0.49
Litter 2	325-363	263-299	7.27-11.01	9.48-10.20	1.82-2.44	0.55-0.70

Table A10. Ranges of postweaning food intake of male offspring in Experiments I, II, and III

Experimental group	Week 4	Week 5	Week 6	Week 7	Week 8	Week 12	Week 16	Week 20
	Food intake ((g/g body wt. ^{0.75})/day) x 100							
<u>Experiment I</u>								
I ^a HP/HP	28-36	34-41	30-36	30-38	27-34	17-27	17-21	16-22
LP/LP	26-46	30-45	36-40	27-36	28-35	22-29	19-23	17-20
I ^c HP/HP	23-44	27-38	21-38	29-36	29-35	20-28	19-22	15-21
LP/HP	31-36	32-42	27-40	29-34	28-32	18-25	18-20	17-19
<u>Experiment II</u>								
HP/HP								
Litter 2	23-35	25-45	27-50	26-39	14-32	17-26	16-21	13-20
Litter 3	27-34	26-41	21-35	17-32	17-34	16-23	17-23	16-20
HP/LP								
Litter 2	24-44	36-47	25-41	24-39	23-39	20-29	17-22	14-22
Litter 3	28-44	30-42	25-34	12-33	24-38	25-27	18-22	18-20
LP/HP								
Litter 2	22-37	28-43	30-43	27-40	23-35	19-28	11-27	14-20
Litter 3	26-36	26-40	21-41	19-34	16-33	18-26	14-19	11-20
LP/LP								
Litter 2	24-45	40-51	23-44	23-42	18-37	15-29	17-27	15-23
Litter 3	32-40	36-50	19-42	16-41	16-38	24-27	16-22	12-20
<u>Experiment III</u>								
HP _p /HP								
Litter 1	31	32-33	31-33	25-31	23-29	23-28	19-20	17-19
Litter 2	29-32	24-38	25-35	25-32	25-32	19-29	15-19	13-17
HP _p /LP								
Litter 1	40	41	32	38	32	24	19	16
Litter 2	34-41	34-47	21-41	35-43	31-36	21-27	14-20	14-22
LP _p /HP								
Litter 1	36	37	37	33	30	22	19	19
Litter 2	30-38	33-40	34-38	23-34	27-31	19-22	16-19	13-18
LP _p /LP								
Litter 1	42	49	33	39	35	24	21	15
Litter 2	29-43	39-44	25-38	33-43	26-41	20-28	16-20	15-19

Table All. Ranges of postweaning food efficiency ratios of male offspring in Experiments I, II, and III

Experimental group	Food efficiency ratio x 100			
	Weeks 3-6	Weeks 6-9	Weeks 9-20 ^a	Weeks 3-20 ^a
<u>Experiment I</u>				
I ^a				
HP/HP	43-56	35-45	10-15	21-24
LP/LP	32-37	18-35	11-15	18-20
I ^c				
HP/HP	33-50	34-45	9-16	18-22
LP/HP	49-53	36-45	11-15	20-24
<u>Experiment II</u>				
HP/HP				
Litter 2	33-59	33-52	6-20	18-27
Litter 3	46-54	16-44	13-19	21-24
HP/LP				
Litter 2	28-39	29-34	10-19	18-22
Litter 3	31-40	17-37	12-20	18-23
LP/HP				
Litter 2	47-62	32-46	11-17	21-25
Litter 3	49-59	26-41	10-17	21-24
LP/LP				
Litter 2	32-42	25-41	12-19	19-23
Litter 3	33-44	23-37	11-20	19-22
<u>Experiment III</u>				
HP _p /HP				
Litter 1	52	32-39	15-16	22-23
Litter 2	45-56	31-43	14-18	22-26
HP _p /LP				
Litter 1	39	32	15	21
Litter 2	29-40	26-32	12-15	18-21
LP _p /HP				
Litter 1	54	33	15	22
Litter 2	48-62	40-45	12-14	22-23
LP _p /LP				
Litter 1	45	30	10	18
Litter 2	35-44	30-34	12-15	19-21

^aFER wk. 9-19 and wk. 3-19 in Experiment I_a.

Table A12. Ranges of absolute brain weights and specific brain ChE activities of newborn and weanling female rats in Experiments I, II, and III

Experimental group	Newborn females		Weanling females	
	Brain mg	ChE/g brain $R^a \times 10^6$	Brain g	ChE/g brain $R \times 10^6$
<u>Experiment I</u>				
HP	207-275	1.42-2.10	-- ^b	--
LP	195-255	1.25-2.03	--	--
LP _M	209-244	1.46-1.97	--	--
LP _H	212-266	1.33-1.89	--	--
<u>Experiment II</u>				
HP				
Litter 2	224-279	1.51-2.16	1.33-1.53	5.98-7.84
Litter 3	212-264	1.33-1.99	1.18-1.61	4.49-7.37
LP				
Litter 2	209-283	1.07-2.03	1.19-1.52	5.53-7.81
Litter 3	223-269	1.37-1.95	1.27-1.38	5.73-8.33
<u>Experiment III</u>				
HP _p				
Litter 1	226-284	1.49-2.15	1.33-1.50	5.42-7.40
Litter 2	226-267	1.41-1.75	1.24-1.50	6.23-8.30
LP _p				
Litter 1	210-246	1.54-1.61	1.12-1.31	5.51-6.02
Litter 2	225-251	1.33-1.67	0.97-1.27	6.05-7.31

^aR = rate in moles ASCh hydrolyzed per minute.

^bNo weanlings sacrificed in Experiment I.

Table A13. Ranges of absolute brain weights and brain ChE activities of adult males in Experiments I, II, and III

Experimental group	Total brain g	Cortex g	Subcortex g	ChE/g cortex R ^a x 10 ⁶	ChE/g subcortex R x 10 ⁶	ChE/g brain ⁶ R x 10 ⁶
<u>Experiment I</u>						
I ^a HP/HP	1.959-2.206	0.697-1.026	1.035-1.230	7.35-9.44	7.02-10.58	7.15-9.62
LP/LP	1.960-2.073	0.658-0.950	1.004-1.201	5.76-8.81	8.10-10.89	8.07-8.94
I ^b St/St	2.128-2.368	0.846-1.019	1.180-1.347	7.15-10.59	7.03-9.27	7.61-8.68
4 15 ₁ /15 _g	2.152-2.419	0.842-1.089	1.077-1.423	5.13-8.71	6.56-10.10	7.17-8.89
I ^c HP/HP	2.152-2.343	0.840-1.054	1.152-1.304	6.28-9.57	6.72-8.27	7.26-8.08
HP/HP ^{nt}	2.095-2.338	0.845-0.975	1.089-1.316	5.77-9.05	7.01-8.51	7.20-8.27
LP/HP	1.978-2.181	0.811-0.951	1.074-1.221	6.95-8.73	7.25-8.56	7.56-8.19
<u>Experiment II</u>						
HP/HP						
Litter 2	2.080-2.369	0.852-0.955	1.117-1.505	5.20-7.41	6.11-10.48	6.58-8.82
Litter 3	2.030-2.133	0.683-0.968	1.022-1.258	6.26-9.51	6.90-8.80	6.87-9.07
HP/LP						
Litter 2	2.024-2.395	0.779-0.983	1.070-1.374	6.08-7.74	6.46-10.19	6.63-9.21
Litter 3	2.060-2.303	0.814-1.055	1.083-1.326	5.14-8.25	6.12-8.55	6.06-7.32
LP/HP						
Litter 2	1.844-2.375	0.743-1.169	0.965-1.337	5.80-9.33	6.55-10.56	7.12-9.57
Litter 3	1.998-2.220	0.784-0.916	1.057-1.271	5.20-8.56	6.27-8.89	6.46-7.84
LP/LP						
Litter 2	1.816-2.267	0.723-1.044	0.941-1.351	4.73-10.28	5.98-9.29	6.15-8.98
Litter 3	2.034-2.202	0.781-0.865	1.152-1.290	5.20-8.72	7.27-8.65	6.71-8.15

^aR = rate in moles ASCh hydrolyzed per minute.

Table A13. (Continued)

Experimental group	Total brain g	Cortex g	Subcortex g	ChE/g cortex $R^a \times 10^6$	ChE/g subcortex $R \times 10^6$	ChE/g brain $R \times 10^6$
<u>Experiment III</u>						
HP _p /HP						
Litter 1	2.135-2.160	0.750-0.928	1.134-1.229	5.77-8.22	9.04-9.56	8.13-8.67
Litter 2	2.151-2.321	0.808-0.954	1.140-1.323	7.97-9.47	7.49-9.31	7.91-8.75
HP _p ^r /LP						
Litter 1	2.166	0.872	1.186	8.65	8.62	8.63
Litter 2	1.933-2.328	0.845-0.976	1.031-1.265	7.60-9.79	7.65-8.44	7.81-8.95
LP _p /HP						
Litter 1	2.018	0.801	1.120	6.98	9.92	8.70
Litter 2	1.983-2.112	0.778-0.902	1.005-1.201	6.71-9.87	7.25-8.93	8.00-8.49
LP _p /LP						
Litter 1	2.067	0.810	1.155	7.15	9.31	8.42
Litter 2	1.952-2.120	0.806-0.925	1.051-1.125	7.63-8.74	6.97-9.46	7.74-8.73

Table A14. Ranges of trials to criterion and latencies during acquisition and reversal training and percent weight change when subjected to water deprivation of adult male rats in Experiment I

Experimental group	TCA ^a	TCR ^b	Initial latency acquisition sec.	Final latency acquisition sec.	Final latency reversal sec.	% wt. change days 1-25 training
I ^a HP/HP	60-240	240-492	7.8-33.1	3.9-15.4	4.4-6.2	3.42 to 12.93
LP/LP	120-372	192-456	6.7-41.5	3.2-9.2	5.0-8.3	4.38 to 9.44
I ^b St/St	156-324	264-481	23.0-114.6	4.7-19.6	3.8-8.3	-0.71 to 11.16
I ^c 4 _g /15 ₁ /15	156-324	264-588	12.9-40.1	5.0-16.8	4.8-10.3	-1.45 to 4.30
I ^c HP/HP	84-252	144-444	6.1-69.9	4.4-8.7	3.3-5.4	-4.19 to 3.05
LP/HP	36-180	144-348	4.4-27.2	2.6-7.1	2.5-3.8	-4.90 to 0.40

^aTCA = trials to criterion in acquisition.

^bTCR = trials to criterion in reversal.

Table A15. Ranges of trials to criterion and latencies during acquisition and extinction training and percent weight change when subjected to water deprivation of adult male rats in Experiments II and III

Experimental group	TCA ^a	Acquisition				Extinction		% wt. change days 1-25 training
		Latency day 1 sec.	Latency day 5 sec.	Latency day 10 sec.	Latency day 15 sec.	Latency day 5 sec.	Latency day 10 sec.	
<u>Experiment II</u>								
HP/HP								
Litter 2	70-190 ^b	5.7-64.9	2.6-24.8	2.9-43.1	2.4-7.0	9.0-35.2	8.0-67.2	-1.17 to 12.04
Litter 3	20-150	4.4-20.8	2.7-10.2	4.0-8.4	2.2-7.1	14.2-40.4	21.2-109.5	0.52 to 6.06
HP/LP								
Litter 2	50-259	6.1-44.0	4.2-117.1	4.0-10.8	4.0-9.7	8.8-120.0	8.0-120.0	2.03 to 17.79
Litter 3	110-204	4.8-53.4	6.8-15.1	4.7-57.6	2.8-9.4	14.6-31.2	19.7-91.5	0.00 to 13.56
LP/HP								
Litter 2	40-140	6.7-54.4	2.5-47.8	3.5-17.5	2.0-12.8	12.3-95.6	8.8-120.0	-9.89 to 7.03
Litter 3	60-150	5.7-14.8	3.4-10.6	2.8-7.2	2.8-6.9	4.4-23.5	7.3-64.0	-8.16 to 8.00
LP/LP								
Litter 2	70-285	7.9-39.6	4.6-41.6	4.1-14.4	3.8-18.6	8.4-78.1	13.9-65.2	-1.39 to 15.26
Litter 3	90-168	6.3-30.0	5.7-18.5	4.5-17.8	5.1-10.6	14.1-30.6	16.1-78.4	-4.91 to 8.88

^aTCA = trials to criterion in acquisition.

^bValues > 150 were calculated according to the formula: $150 \div (\text{mean trials correct days 14 and 15} / 0.95)$.

Table A15. (Continued)

Experimental group	TCA ^a	Acquisition				Extinction		% wt. change days 1-25 training
		latency day 1 sec.	Latency day 5 sec.	Latency day 10 sec.	Latency day 15 sec.	Latency day 5 sec.	Latency day 10 sec.	
<u>Experiment III</u>								
HP _p /HP								
Litter 1	90-130	5.8-12.5	5.8-23.6	5.3-7.2	4.2-14.9	18.3-106.2	19.6-120.0	1.64 to 5.36
Litter 2	70-158	4.9-13.2	2.5-6.7	2.6-10.3	3.2-5.7	9.7-30.3	11.2-39.4	0.00 to 7.94
HP _p /LP								
Litter 1	100	33.6	9.2	7.8	7.2	11.5	86.9	2.97
Litter 2	100-150	5.8-27.4	3.8-8.0	3.2-7.4	2.7-6.0	4.9-13.3	10.5-53.6	4.27 to 7.83
LP _p /HP								
Litter 1	80	9.1	5.8	3.2	4.4	39.3	34.7	-3.41
Litter 2	40-178	6.4-9.6	3.6-6.9	2.8-4.5	2.3-8.8	5.3-15.5	5.9-17.8	4.78 to 9.14
LP _p /LP								
Litter 1	90	6.8	4.2	6.0	58.2	22.1	32.2	-8.95
Litter 2	130-158	5.9-8.4	3.7-4.9	3.3-6.0	2.8-3.8	7.1-24.6	8.2-18.7	2.32 to 16.06