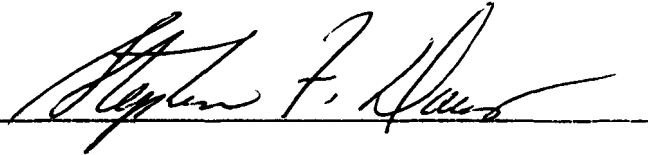


AN ABSTRACT FOR THE THESIS OF

Angela H. Becker for the Master of Science
in Psychology presented on May 1988
Title: GROWTH PATTERNS AND COMPLEX MAZE PERFORMANCE
AS A FUNCTION OF EXPOSURE TO A PROTEIN- AND
TRYPTOPHAN-DEFICIENT DIET

Abstract approved:



Stephen F. Davis

Numerous correlational studies have reported that malnutrition experienced by humans during early development results in retarded physical growth and reduced mental functioning (Montagu, 1962; Kaplan, 1972; Winick, 1976; Dent & Caster, 1971; Cabak & Najdanvic, 1965; Cravioto & Robles, 1965). In response to the ethical restrictions on empirical evaluation of deficient diets upon humans, animal models of malnutrition have been developed. Several such studies have focused specifically upon protein- and tryptophan-restriction (Fernstrom & Hirsch, 1977; Segall & Timiras, 1976; Remley, Armstrong, Gilman, & Mercer, 1980). Diets most commonly employed in these studies utilized casein or corn as the main source of protein. Such diets have consistently produced animals with

retarded brain weights (Forbes, Tracy, Resnick & Morgane, 1977) and body weights (e.g., Segall & Timiras, 1976; Remley et al. 1980). However, a complete picture of the growth curve for these tryptophan deprived animals does not exist. Effects of protein- and tryptophan-deficient diets upon learning ability in animals have been less conclusive, with deprived animals performing as well as or better than control animals on some tasks, and performing more poorly on others (Zimmerman, Geist, & Strobel, 1975; Remley et al. 1980; Cowley & Griesel, 1959; Caldwell & Churchill, 1967). In the present two studies a protein- and tryptophan-deficient corn grits diet was used to establish a more complete picture of growth and learning effects. Experiment 1 concluded that animals raised on the deficient diet from birth to 75 days of age were significantly lighter than animals exposed to this diet from weaning until 75 days of age. Both groups were significantly lighter than animals raised on a normal diet. Administering a normal diet to the grits-exposed animals resulted in weight gain. Experiment 2 concluded that differences in complex maze performance between grits-fed and normal animals existed for number and type of errors made, with grits-fed animals making fewer and less repetitive errors.

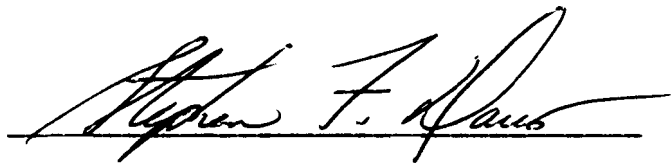
GROWTH PATTERNS AND COMPLEX MAZE PERFORMANCE AS
A FUNCTION OF
EXPOSURE TO A PROTEIN- AND TRYPTOPHAN-DEFICIENT DIET

A Thesis
Presented to
the Department of Psychology
EMPORIA STATE UNIVERSITY

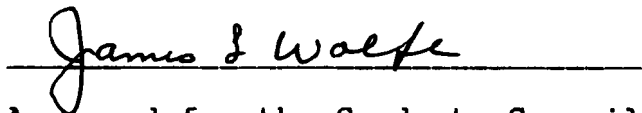
In Partial Fulfillment
of the Requirements for the Degree
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by
Angela H. Becker
May, 1989

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Approved for the Major Department

A handwritten signature in cursive script, reading "James J. Wolfe", written over a horizontal line.

Approved for the Graduate Council

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CHAPTER ONE

GENERAL INTRODUCTION

It has long been recognized that malnutrition affects both physical and mental development. While physical development of humans has often been assessed in terms of the effects of malnutrition in general, some researchers have attempted to determine the physical effects associated with low-protein diets specifically. Moreover, the majority of empirically adequate human studies of possible mental deficiency associated with poor diet have focused almost exclusively upon protein deficiencies. Unfortunately, ethical considerations prevent the degree of empirical control necessary to firmly conclude that physical and mental effects are due solely to dietary factors. Therefore, much of the human research in this area is correlational in nature and must be interpreted with caution. Nevertheless, it is worthwhile to review the findings of research on malnutrition in general and protein-deficiencies in particular upon human subjects' physical and mental development before examining reports of more controlled animal studies of dietary deficiencies.

Researchers have attempted to substantiate the relationship between poor nutrition and physical development in humans with correlational studies of

people on poor diets due to wartime restrictions or poverty. For example, Montagu (1962) reviewed approximately 29 studies which took place in Europe either during World War I or in the several years immediately following when living conditions were at their worst, and found that in many cases, children born during World War I had significantly lower birth weights than those born during peacetime. Laporte (1946) compared Paris grade-school-age children during WWII with same-age children prior to the war and found a sizeable decrease in average height and weight of those growing up during the war. However, such effects might be attributed to a combination of malnutrition and wartime stress rather than insufficient nutrition alone (Kaplan, 1972).

Kaplan (1972) reviewed a number of studies that assessed nutritional deficits by comparing people from different socioeconomic backgrounds. The majority of these studies found that women of lower socioeconomic status had poorer diets and were more likely to give birth prematurely or had low birth weight babies that were more likely to have serious health problems. To further substantiate the correlation between low socioeconomic status, poor diet, and poor health of offspring, several studies provided "dietary

supplements" for poor pregnant women. They found that the health of babies born to those women receiving supplements was significantly improved (Dieckmann et al., 1951; Ebbs, Brown, Tisdall, Moyle & Bell, 1942). Furthermore, the child suffering severe malnutrition has been reported to be behind well-fed age peers matched for ethnicity in both height and skeletal development (Dean, 1960).

To this point, the studies discussed have assessed the physical effects of malnutrition in general. However, it might be beneficial to focus upon more specific dietary deficiencies. A number of researchers have reported the effects of protein-deficient diets in particular. Among humans, two dietary disorders associated with protein deficiencies have been described. Marasmus is a disorder caused by insufficient amounts of both calories and proteins. It appears to occur most often in children under two years of age, and is characterized by severe loss of weight and failure to grow, swollen joints, apathy, and hyperirritability (Winick, 1976). In referring to this same disorder, Kaplan (1972) indicated that "mental apathy" was quite common. Winick (1976) stated that although some symptoms were relieved by proper dietary treatment, there appeared to be some permanent stunting of brain and

organ growth. The second protein-deficiency disorder, Kwashiorkor, is produced by a diet that is adequate in carbohydrates but low in protein. Kwashiorkor occurs most often in children two or three years of age, after they have been weaned from breastmilk. This disorder also is characterized by retarded growth and apathy (Winick, 1976). Although there is some weight loss, there is not the severe wasting of tissue that accompanies Marasmus (Kaplan, 1972). Differentiating characteristics include discoloration and loss of hair (one of the earliest features) (Kaplan, 1972); accumulation of fluids (edema) producing swollen legs, arms, belly, or face; skin lesions; and excessive deposits of fat in the liver (Winick, 1976).

In relation to those studies comparing diet and development of upper versus lower socioeconomic levels, McCance, Widdowson, and Verdon-Roe (1938) found that while intake of fats, carbohydrates, and calories was not related to income level of the women studied, intake of proteins, vitamins, and minerals was related to socioeconomic status. Burke, Harding, and Stuart (1943) found a positive correlation between the amount of protein consumed during the latter months of pregnancy and the subsequent height and weight of babies born, with those that received more protein being heavier and

longer. Dieckman et al. (1951) and Ebbs et al. (1942) found that if impoverished pregnant women were given protein supplements, their babies were born healthier. From these reports, it would appear that protein deficiency plays a particularly important role in producing growth deficiencies as well as other abnormal physical characteristics among humans.

While some researchers have focused upon physical effects of malnutrition, others have assessed the extent to which poor nutrition affects mental development. When given the Gesell test, undernourished African children exhibited ability levels lower than would be expected for their age in "language, adaptive, personal-social, and psychomotor behavior" (Geber & Dean, 1957). Dent & Caster (1971) reported that there appeared to be a relationship between mental retardation and diets in which corn grits and cheap cuts of meat were the main sources of protein. The authors maintained that such diets, which are both low in protein and of poor quality protein content, were common among low-income southerners in the United States.

Kaplan (1972) delineated six criteria which must be met to ensure that malnutrition is the only variable affecting mental development. These criteria included: keeping close check on the length of time the person is

malnourished and the consequent physical and psychological effects; consistency of age at which malnutrition takes place; control of extraneous variables such as prematurity, serious illness or deformity, social and economic variables, education level of parents, and diet; assessment of mental development with several different types of measures; as well as periodic reassessment to determine duration of any effects. Although human studies have a great deal of difficulty meeting all of these criteria, those that come closest are studies of protein deficiencies. Cabak and Najdanvic (1965) used a modification of the Stanford-Binet intelligence test to evaluate 36 Yugoslavian children ages 4 months to 2 years. While one-third were from a higher socioeconomic level, all were being treated for marasmus. When tested, half of the children obtained scores that were borderline or in the mentally retarded range. Birch and Cravioto (1968, cited in Kaplan, 1972) found essentially the same results in their study of malnourished Guatemalan children. Cravioto and Robles (1965) studied three age groups of children (3-6 months, 15-29 months, and 37-42 months) admitted to a hospital in Mexico for treatment of severe protein-calorie malnutrition. All were given the Gesell test to determine developmental quotients at

the beginning of treatment. The children were retested every two weeks for the duration of their hospital stay. Throughout their hospital stay, children under six months of age remained at the original level of deficiency. This study suggested that a short-term mental deficit existed even after several months of adequate diet.

A somewhat better answer to the question of long-term effects of malnutrition was provided by Chase and Martin (1970). They included a number of measures, including "height, weight, head circumference, and developmental quotients" for American children hospitalized at less than one year of age due to undernutrition. Approximately half of the undernourished group received treatment prior to four months of age, while the other half received help sometime later in their first year of life. When tested approximately three and a half years after their hospitalization, those children not receiving help until after four months of age scored significantly below both the control group and the early-treatment, undernourished children on all measures.

Due to the fact that intentional restriction of essential nutrients for research purposes is an ethically unacceptable practice with human subjects,

verification of its effects upon growth and learning has been limited to correlational data. In response to these difficulties, researchers have developed models of malnutrition based on animal studies. The most common techniques for producing nutrient deficiency in animals are: restriction of the pups' access to the lactating dam, re-distribution of pups among dams to produce unusually large litters which decreases the amount of food available per pup, and restriction of the lactating dam's diet (Forbes, Resnick, & Morgane, 1977).

A number of studies have restricted the essential amino acid tryptophan (e.g., Fernstrom & Hirsch, 1977, Segall & Timiras, 1976, Remley, Armstrong, Gilman, & Mercer, 1980). "It is hypothesized that tryptophan deficiency delays growth, development and maturation of the central nervous system (CNS), in particular, by decreasing the levels of the neurotransmitter serotonin, for which tryptophan is the necessary precursor" (Segall & Timiras, 1976, p. 109). Fernstrom and Hirsch (1977) described their animal model of malnutrition as being "characterized by substantial reductions in the brain levels of tryptophan, serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA)" (p. 877).

In many cases, tryptophan- and protein-deficiency was accomplished through use of a corn-based diet. In

justification of the use of corn-based diets, Fernstrom and Hirsch (1977) reported that "corn proteins are among the most deficient in tryptophan (and lysine) of all the proteins normally ingested by man" (p. 877).

Furthermore, "corn-based diets are consumed by a significant portion of the world's population, including people in Central and Latin American countries"

(Fernstrom & Lytle, 1976, p. 258) and in the rural south in the United States (Dent & Caster, 1971). The present paper reports data from two experiments employing grits-based protein and tryptophan restriction. A longitudinal evaluation of growth-related deficits is considered in Experiment 1, while possible cognitive (learning) deficits are the focus of Experiment 2.

CHAPTER TWO

EXPERIMENT 1

A number of studies have addressed the effects of low-protein diets upon the growth of rats (Fernstrom & Hirsch, 1977; Forbes, Tracy, Resnick & Morgane, 1977; Remley, Armstrong, Gilman & Mercer, 1980; Segall & Timiras, 1976). Forbes, Tracy, Resnick, and Morgane (1977) administered a low-protein diet (8% casein) to pups born of dams which had also been maintained on a low-protein diet beginning five weeks prior to mating. Control dams and litters received a 25% casein diet. Although protein-deprived and control pups had similar brain and body weights at birth, with age the protein-deprived animals' body growth was severely retarded while brain growth was mildly restricted as compared to controls.

In contrast to the 25% casein control diet used by Forbes et al. (1977), Fernstrom & Hirsch (1977) utilized an 18% casein control diet. They reported that after six weeks, juvenile male rats fed the control diet were six to seven times heavier than at the onset of the diet, while those fed a corn-based diet were only about two times heavier than at onset of the diet. Thus, even the lower protein percentage in the control diet used by these investigators was sufficient to allow substantial

weight gain in the control animals. Unfortunately, only one weighing was accomplished and the exact age at which the weighing was conducted was not reported.

Segall and Timiras (1976) maintained groups of rats on tryptophan-deficient diets beginning either at weaning, at 3, 13-14, 24-26, or 36 months. The primary source of protein and tryptophan in their diet was ground yellow corn. Those 3 groups beginning the restricted diet at weaning, 3 months, and 13-14 months were maintained on the protein-deficient diets for 2.5-23, 17, and 26-27 months, respectively, and were then placed on Purina Lab Chow until death. The two later-starting groups were maintained on special diets until death. As compared to two groups of controls (one fed Purina Lab Chow, the other fed the tryptophan-deficient diet to which tryptophan had been added), they found that the tryptophan-deprived animals gained less weight and showed general signs of delayed aging, such as better coat quality. When deprived animals were placed on a diet with sufficient levels of protein (i.e., Lab Chow), they eventually reached normal adult body weight.

Up to this point, the studies reviewed have used some form of complex dietary mixture in which protein content was altered by varying the amount of casein or

ground corn to be included. A more parsimonious procedure employed by Remley et al. (1980) involved placing the selected animals on an exclusive diet of corn grits at weaning. Corn grits was selected because it would simulate a more natural diet that was deficient in the desired nutrients. Littermates, that formed the control groups, were maintained on the normal diet of Purina Laboratory Chow. Similar to the previous studies, while no weight differences existed at 30 days of age, pronounced differences had developed by 60 days. The average weight of the normal males was 3.16 times more than that of the grits males, while normal female animals weighed 2.23 times that of their grits counterparts. This difference was exacerbated by the third and final weighing at 90 days of age.

Unfortunately, only a limited number of weighings, or weights taken at inconsistent intervals, were reported by Fernstrom and Hirsch (1977), Forbes et al. (1977), Remley et al. (1980), and Segall and Timiras (1976). Hence, a complete picture of the growth curve of animals raised under these conditions has yet to be developed. Additionally, the effects of reversing the experimentally imposed dietary deficiency, as reported by Segall and Timiras (1976), have yet to be replicated. The present experiment was designed to address both of

these issues.

Method

Subjects, Housing, and Maintenance

Four, sperm positive female rats were purchased from the Holtzman Company. Upon arrival in the laboratory, the pregnant dams were individually housed in a 10-gal. aquarium having a floor of San-I-Cel animal bedding material. Purina Laboratory Chow and water were available during gestation. All litters were maintained in the aquariums until weaning (21 days) when all animals were transferred to individual, suspended, wire-mesh cages in the animal vivarium.

The protein- and tryptophan-deficient grits diet consisted of Quaker Instant Grits mixed with warm water to a thick soupy consistency. Instant grits were employed in order to avoid the ingestion of raw starch. In order to insure adequate and consistent consumption of the grits diet, .30 grams of sodium saccharin were added to each 200 grams of dry grits. According to Orr and Watt (1957), corn grits contains .053 grams of tryptophan per 100 grams and is composed of 8.70% protein. Hence, it clearly meets the requirements for a tryptophan- and protein-deficient diet. All foods were presented on a free-feeding basis during all phases of the experiment.

Procedure

At birth, one litter ($n = 11$) was randomly selected as the GRITS/BIRTH group. By switching the diet of the nursing dam to grits at the birth of the pups and by providing this diet to the pups in their individual cages upon weaning, this group was maintained on the tryptophan- and protein-deficient diet from birth to 75 days of age.

The remaining three dams (and litters) were maintained on Purina Laboratory Chow until weaning. At this time two groups, GRITS/WEANING (8 males, 5 females) and CONTROL (7 males, 7 females) were randomly formed from these litters. Subjects in the GRITS/WEANING group were placed on the grits diet when they were transferred to their individual cages at weaning. Subjects in the CONTROL group were maintained on laboratory chow for the duration of the experiment.

In order to evaluate the effects of reversing the growth defects engendered by the grits diet, all but 8 of the GRITS/WEANING animals were placed on the normal, laboratory chow diet at 75 days of age. Due to the death of 9 of the 11 GRITS/BIRTH animals between 85 and 110 days of age, it was not possible to produce comparable and reliable data concerning the effects of diet reversal for this group. The experiment was concluded

when the animals were 183 days old. All animals were individually weighed every two days.

Results

The mean weight of each litter from 3 days until 21 days (weaning) is shown in Figure 1 on the following page. While all subjects gained weight, the GRITS/BIRTH animals appeared to be significantly lighter than the litters nursed by dams maintained on a normal diet. Analysis of variance (ANOVA) performed on these data yielded significance for the Groups, $F(3, 34) = 2574.60$, $p < .001$; Blocks, $F(8, 272) = 1860.93$, $p < .001$; and Groups by Blocks, $F(24, 272) = 149.37$, $p < .001$, effects. Subsequent Newman-Keuls tests used to probe the interaction indicated that while Litters 1, 2, and 3 did not differ reliably from each other, they were significantly ($p < .01$) heavier than the GRITS/BIRTH animals from Block 5 through Block 9.

The mean weights of the CONTROL and GRITS/WEANING animals, as a function of gender, from weaning until 75 days of age are shown in Figure 2. While it is clear that all groups gained weight during this period, it also is evident that the CONTROL animals gained more weight than the GRITS/WEANING animals. Moreover, the male CONTROL subjects appeared to gain more weight than their female counterparts during this period. This

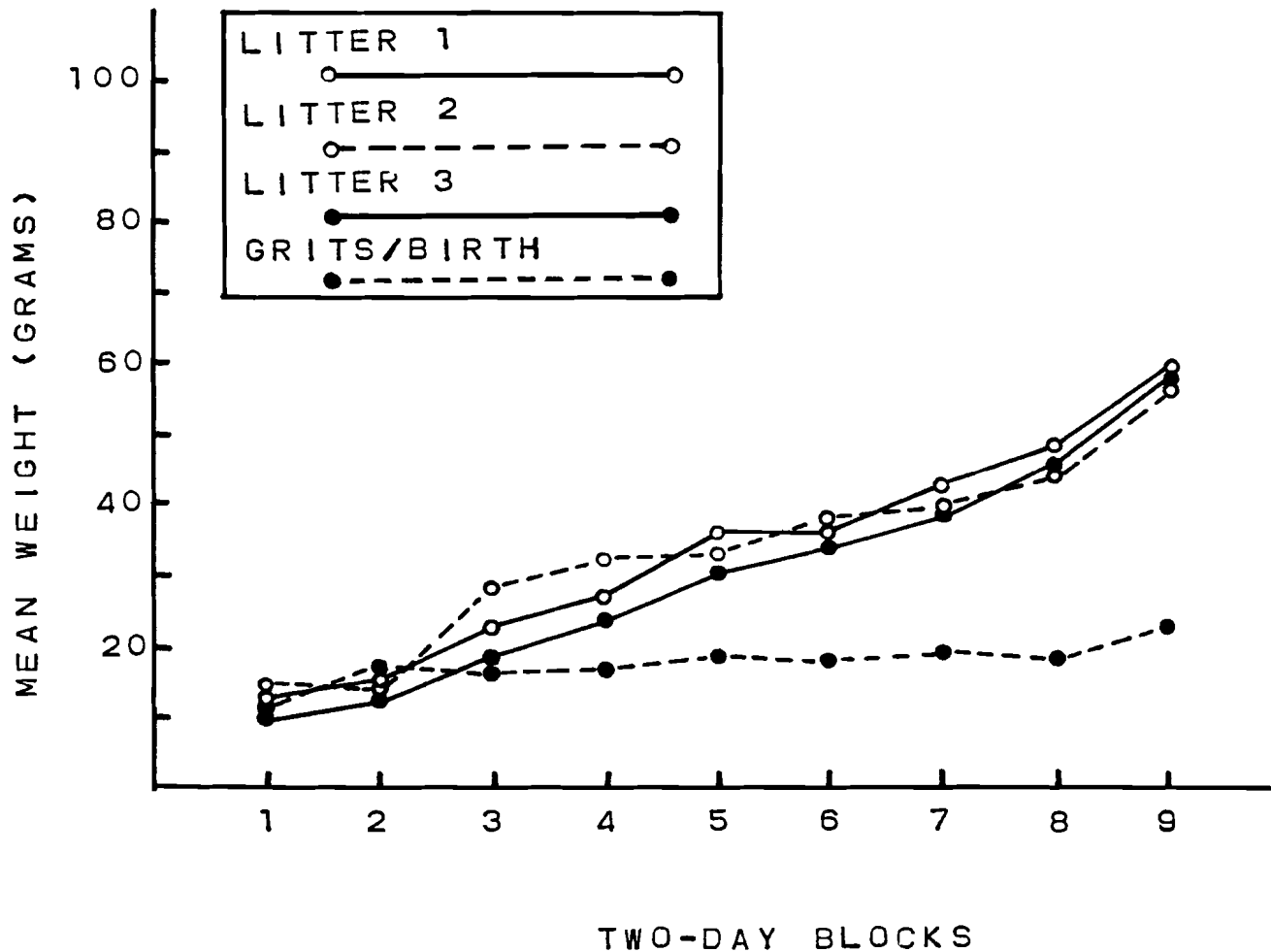


FIGURE 1: Group mean weight (grams) of the GRITS/BIRTH and three normally maintained litters from 3 days of age until 21 days of age (weaning). Experiment 1.

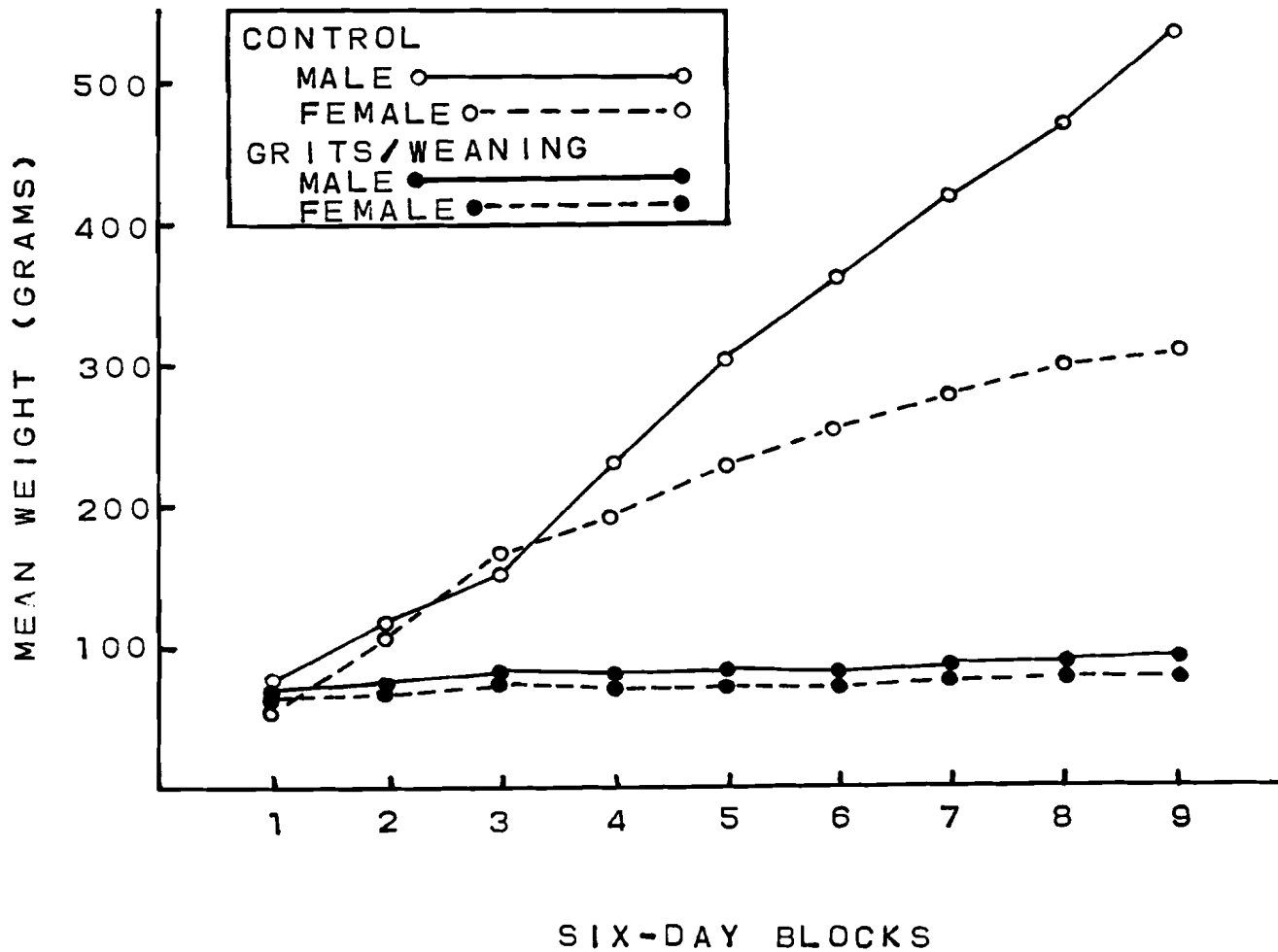


FIGURE 2: Group mean weights (grams), as a function of gender, for the GRITS/WEANING and CONTROL animals from 21 days of age (weaning) until 75 days of age. Experiment 1.

male-female difference was suggested only very weakly for the GRITS/WEANING animals. Analysis of variance, which yielded significance ($p < .001$) for all factors, including the Groups by Gender by Blocks interaction, $F(8, 184) = 10.82$, $p < .001$, provided statistical support for the graphical impression created by Figure 2. The results of subsequent Newman-Keuls tests indicated that the CONTROL animals were significantly ($p < .01$) heavier than the GRITS/Weaning animals by Block 3. Moreover, the CONTROL males were found to be significantly ($p < .01$) heavier than the CONTROL females by Block 5.

Figure 3 compares the growth of the GRITS/BIRTH, GRITS/WEANING, and CONTROL groups from weaning until 75 days of age. As the GRITS/BIRTH animals remained sexually immature during this period, it was difficult to determine gender. Hence, comparisons including these animals were collapsed over the gender factor. Despite the additional error variance created by pooling over gender, the analysis of these data yielded significance for the Groups, $F(2, 31) = 139.00$, $p < .001$; Blocks, $F(8, 248) = 52.99$, $p < .001$; and Groups X Blocks, $F(16, 248) = 41.36$, $p < .001$, effects. Newman-Keuls tests indicated that CONTROL animals were significantly ($p < .01$) heavier than the GRITS/BIRTH animals on all blocks

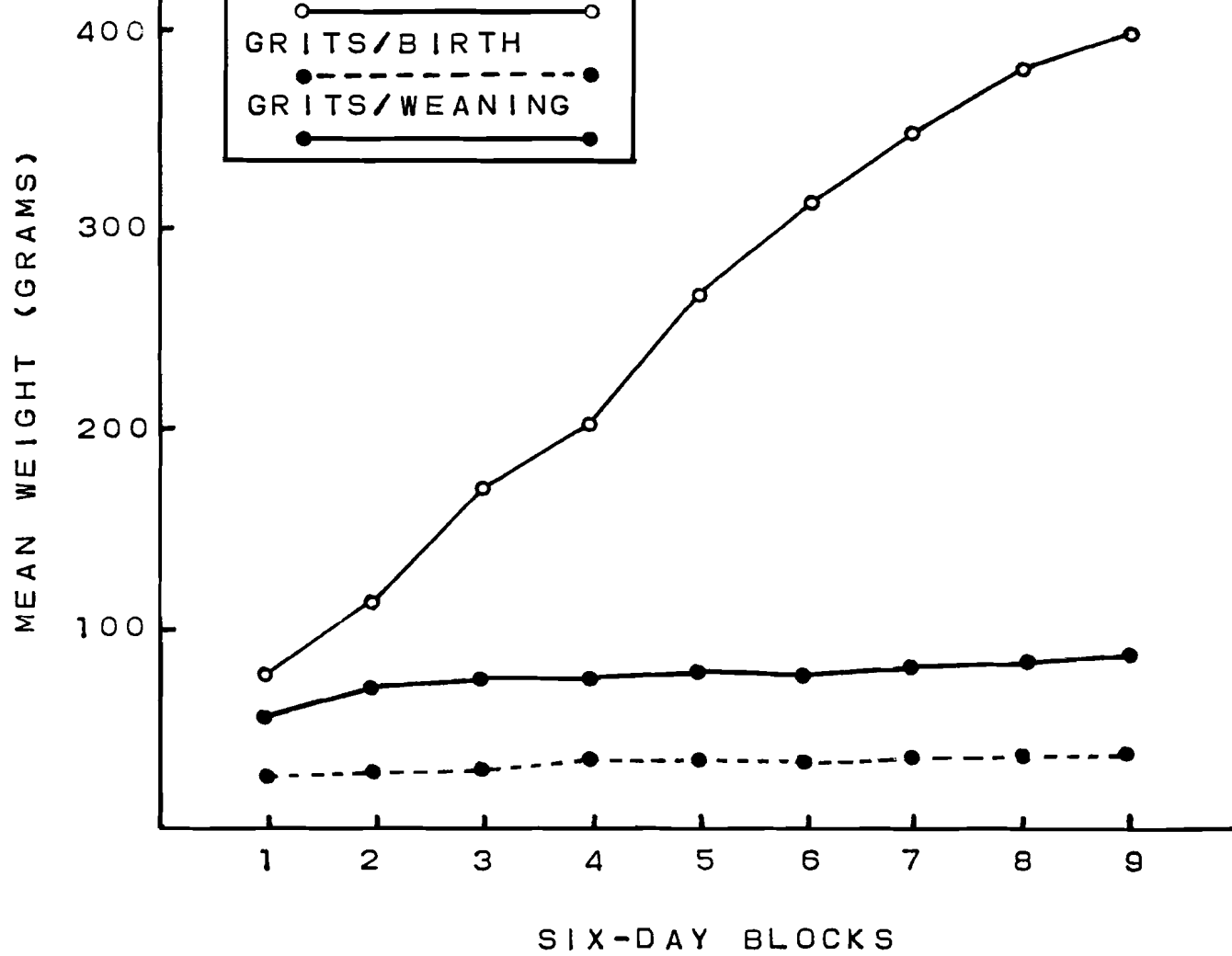


FIGURE 3: Group mean weight (grams) of the GRITS/BIRTH, GRITS/WEANING, and CONTROL groups from 21 days of age (weaning) until 75 days of age. Experiment 1.

and the GRITS/WEANING animals from Blocks 2-9. The GRITS/WEANING animals were significantly ($p < .01$) heavier than the GRITS/BIRTH animals on all blocks. Thus, the impression that curtailing the receipt of tryptophan and protein from birth on had a more pronounced effect upon growth than the restriction of these substances from weaning on was statistically reliable.

Turning to Figure 4, it clearly can be seen that the growth deficit induced by the grits diet was not permanent. Introduction of the normal diet lead to a gradual increase in weight by both the males and females in the GRITS/WEANING group. Moreover, the anticipated male-female weight differences can be seen to develop during Blocks 4 through 9. Statistical support for these observations was provided by analysis of variance which yielded significance for all factors, including the Groups x Gender x Blocks, $F(8, 184) = 4.94$, $p < .001$ interaction. Newman-Keuls tests indicated that the CONTROL males were significantly ($p < .01$) heavier than all other subjects on all blocks. The CONTROL females were significantly ($p < .01$) heavier than the GRITS/WEANING females on all blocks. While the GRITS/WEANING males and females did not differ reliably on Blocks 1-3, the males were significantly ($p < .01$)

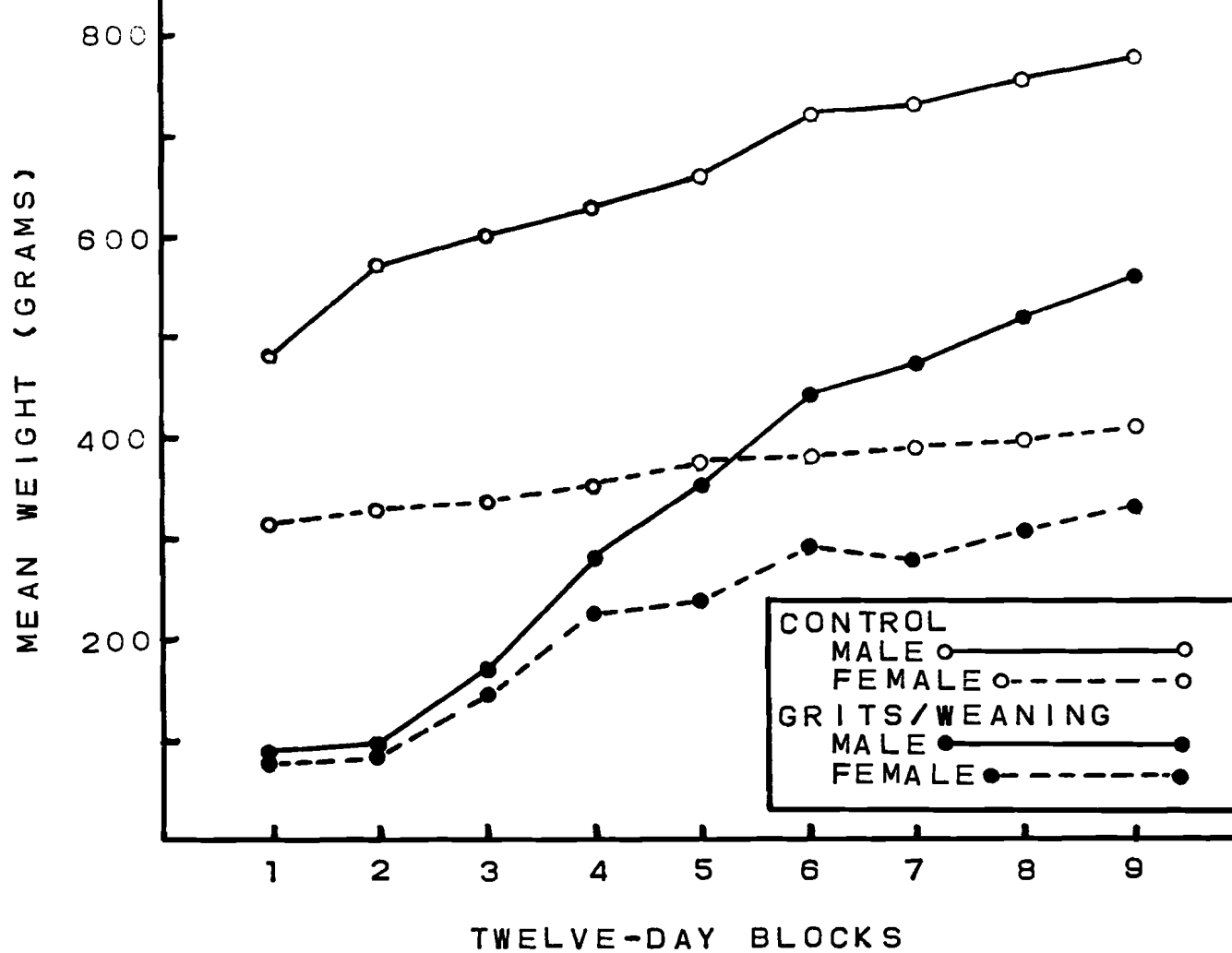


FIGURE 4: Group mean weight (grams), as a function of gender, of the CONTROL and GRITS/WEANING groups from 75 through 183 days of age. Experiment 1.

heavier than the females on Blocks 4-9. On Blocks 6-9 the GRITS/WEANING males were significantly ($p < .05$) heavier than the CONTROL females.

Discussion

What conclusions are prompted by the results of Experiment 1? First, they are in agreement with data reported by investigators whose deficient diets were based upon casein (Fernstrom & Hirsch, 1977; Forbes et al., 1977), or corn (Remley et al., 1980; Segall and Timiras, 1976). The animals exposed to the tryptophan- and protein-deficient grits diet showed substantial retardation in growth when compared with the normally reared animals. In fact, our differences appear to be even more pronounced than those reported by Remley et al. (1980). Clearly, this dietary regimen produces major growth differences.

Second, the present data indicate that the development of sexual dimorphism was retarded in the GRITS/WEANING group. Whereas sexual dimorphism had begun to assert itself by approximately 40 days of age in the CONTROL animals, it was a non-significant factor in the GRITS/WEANING animals, even by 75 days of age.

Third, a comparison of the growth pattern of the GRITS/BIRTH and GRITS/WEANING groups indicates that the earlier this diet is imposed, the more severe its

effects appear to be. The fact that the majority of the GRITS/BIRTH animals did not survive past 80 days of age underscores this point.

The final phase of the experiment indicates that the growth deficit imposed by the grits diet is reversible. In fact, a projection of the trends depicted in Figure 4 suggests that the GRITS/WEANING animals would have attained weights similar to those of their CONTROL counterparts if the duration of the experiment had been extended. Indeed, Segall and Timiras (1977) reported just such results when they placed their protein-deprived animals back on a normal diet.

Other physical characteristics associated with the deficient diet were also noted among both the GRITS/BIRTH and GRITS/WEANING groups. Similar to the report by Segall and Timiras (1976), after an extended period of time on the restricted diet our animals also developed tremors and exhibited a substantial number of cataracts. By the end of the experiment, well over half of the GRITS/BIRTH animals had developed cataracts as compared to no such obstructions among CONTROL subjects.

CHAPTER THREE

EXPERIMENT 2

The experimental evaluation of the effects of low-protein diets upon retarded mental capacities has been of interest for nearly 25 years. Several earlier studies (e.g., Cravioto & Robles, 1965; Kaplan, 1972; Winick, 1976) reported data supporting the contention that if such diets are experienced during development, reduced mental functioning may result.

However, such data were based upon correlations gathered from field studies of human subjects. When such effects were subjected to more rigorously controlled experiments using animals as subjects, differences in learning ability were not always forthcoming. For example, Zimmerman, Geist, and Strobel (1975) failed to report differences in delayed response, learning set, object discrimination, and simple reversal learning tasks in monkeys. However, protein-deprived animals exhibited poorer performance than controls on conditional discrimination, embedded-figures, and cross-string tasks. It was concluded that such inferior performance may have been due to "attentional and emotional" differences rather than true cognitive deficits. Likewise, Remley et al. (1980) reported that rats raised on a protein-deficient, grits diet from

weaning to maturity did not differ from normal littermates in terms of two-way active avoidance, and actually were superior to controls in black-white visual discrimination performance. As it has been reported that tryptophan-deficiencies produce low pain tolerance (Fernstrom & Lytle, 1976; Spring, Chiodo & Bowen, 1987), potential differences in active avoidance learning ability may have been masked by the deprived animals' heightened attention to painful stimuli. Remley et al. (1980) suggested that superior performance shown by the deprived animals on the black-white discrimination task possibly was due to differences in motivation for the water-sucrose reward rather than superior learning capacity. It was suspected that control animals were not sufficiently water deprived, and indeed, a subsequent group of normally fed animals placed on 23.5 hour deprivation (as opposed to the original 7-hour deprivation period) exhibited a learning curve similar to the grits-fed animals.

In contrast to those reporting no difference between learning ability of protein-deprived and non-deprived animals, Cowley and Griesel (1959) found learning deficits among the first generation offspring of female rats fed a low-protein diet based on fish meal (a diet of 12.97% protein) from 21 days of age. After

weaning at 35 days of age, the offspring also were maintained on the low-protein diet. The apparatus used was a Hebb and Williams enclosed field test. Animals were tested on a total of 12 different barrier patterns with no pattern being tested more than once. Thus only first-trial learning, not retention, was tested.

Low-protein animals chose less efficient routes to the goal and spent significantly more time traversing the test field than did controls. Subsequent tests of exploratory behavior revealed no differences, indicating that differences on the Hebb-Williams task were not due to variations in exploratory behavior. Cowley and Griesel (1959) also noted a higher death rate among low-protein rats up to 18 months of age.

Caldwell and Churchill (1967) maintained pregnant dams on a protein-deficient diet between the 11th day of gestation and delivery. Upon delivery, the dams were returned to a regular laboratory diet. Between 30-35 days of age, pups were given 3 test trials per day for 3 consecutive days in a Lashley III water maze. They found that protein-deprived animals were significantly slower and made more blind alley errors than controls, but only on the first day of testing. Between 50-55 days of age, two randomly selected groups of pups also were tested on a pole-jump-avoidance learning task that

involved shock. In contrast to the Remley et al. (1980) report of no difference, Caldwell and Churchill (1967) found better performance by controls on the avoidance task.

In an attempt to diminish motivational and motor differences between protein-deprived and control animals, Turkewitz (1975) used a modified Lashley jumping stand which required subjects to learn to choose between jumping onto one of two doors, each displaying a different pattern. One door opened to allow a safe fall to a platform below, while the other door was secured such that the animal first would hit its nose on the door and then fall through the door into a net. When comparing sixth generation protein-deprived animals with controls fed a normal laboratory chow diet, Turkewitz found no significant difference in the number of animals who reached criterion nor in the number of trials to criterion on a simple black-white discrimination. Significant superiority for the normal animals emerged on the horizontal-vertical test and the most difficult circle-triangle discriminations.

Turkewitz (1975) provides a reminder that motivational differences, motor differences, and attentional differences can all affect learning without necessarily reflecting any differences in

learning ability per se. In fact, . . . when proper attention is paid to such factors, it is extremely difficult to find differences in learning ability between well nourished and poorly nourished animals. (p. 113)

However, the fact that Cowley and Griesel (1959, 1963) and Turkewitz (1975) himself reported significant learning differences would indicate a marked discrepancy among the research findings. Hence, the question of diminished cognitive capacities awaits further experimental verification.

The present study attempted to assess learning differences due to a tryptophan- and protein-deficient diet by using a learning task which did not involve the application of painful stimuli and which was more complex than the simple black-white discrimination. More specifically, a modified version of the Hampton Court maze was used. The floor plan for this maze was adapted from Woodworth and Schlosberg (1961, p. 614) and is shown in Figure 5. The Hampton Court maze differs from the Hebb-Williams enclosed field test used by Cowley and Griesel (1959) in two main respects (Hebb & Williams, 1946). In the Hebb-Williams maze the startbox and goalbox are positioned in opposing corners, while in the Hampton Court maze the startbox is in one corner and the

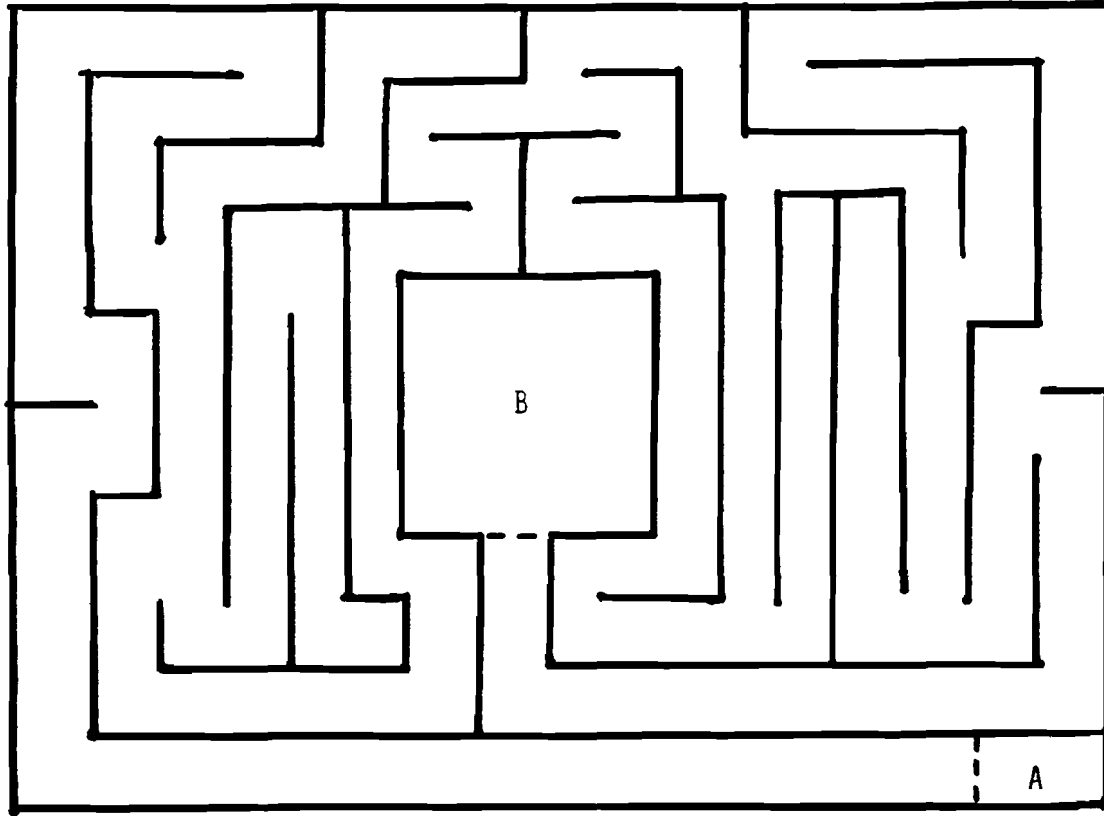


FIGURE 5: Floor plan of the Hampton Court Maze showing the location of the startbox (A) and the goalbox (B). Experiment 2.

goalbox in the center of the maze. Moreover, only four of the movable barriers are employed at any one time to form the Hebb-Williams maze pattern. The Hampton Court maze features 40 non-movable barriers. The increased number of barriers and the set nature of the task suggests that the Hampton Court maze is better suited to evaluate the progressive acquisition of a single complex maze task.

Method

Subjects

Eight of the GRITS/WEANING (Group GRITS) and 17 of the CONTROL (NORMAL) animals tested in Experiment 1 served as subjects in Experiment 2. Housing and dietary conditions were the same as in Experiment 1.

Apparatus

The Hampton Court maze was approximately 182.88 cm in width, 243.84 cm in length, and 11.43 cm in depth, with alleys approximately 10.16 cm wide. The floor and walls of the maze were constructed of 3.175 mm sheet metal. The hinged lid was a heavy gauge steel mesh with two doors, one serving as an entrance (see Figure 5, point A) and the other positioned directly over the central goal box. At point B (see Figure 5) was a removable, solid metal door which, when in place allowed the first 8" of the maze to function as a start box. In

order to confine subjects to the goal area, a second removable door was positioned at its entrance.

The reward was presented in a 5 cm diameter, 1 cm deep dish which was placed on the goalbox floor and centered along the wall directly opposite the goalbox entrance. Reward consisted of access to a .15% saccharin solution.

Procedure

As the GRITS animals had been raised on a saccharin-sweetened diet, it was deemed appropriate to provide preexposure to saccharin for the NORMAL animals to avoid the display of a neophobic response. Hence, they were administered a saccharin-adulterated diet for one week prior to training.

Twelve hours prior to the first training trial, animals were placed on both water and food deprivation. Although a fluid reward was used, it was determined that food deprivation also was necessary because of the high water content of the grits diet. The procedure of depriving the animals for 12 hours prior to each training trial remained in effect for the duration of the experiment.

Training consisted of 8 maze trials that were administered when the subjects were between 70 and 102 days of age. A 7-day period separated Trials 1 and 2,

and 2 and 3; while Trials 3 and 4, 4 and 5, 6 and 7, and 7 and 8 each were separated by one day. A 14-day period separated Trials 5 and 6.

On Trials 1 and 2 each subject received preexposure to the saccharin solution in the goalbox approximately four hours prior to the administration of the maze trial. Preexposure consisted of directly placing the subject in the enclosed goalbox. Each subject was removed following a 3-second saccharin-consumption period, or at the end of 10 minutes if saccharin was not consumed.

To administer a Training Trial, the designated subject was placed in the start box. Following a three-second confinement time, the start door was removed and the subject allowed to traverse the maze. The subject's route, errors, and latency (seconds between removal of the start door and when the subject's hind quarters were completely within the goalbox) were recorded on each trial. The subject was removed from the goalbox and returned to the home cage when it had consumed the saccharin solution for three seconds. Subjects not completing the maze on Trials 1 and 2 were removed after two hours; the completion criterion was reduced to 30 minutes on Trials 3 - 8. Damp-sponging and vaccuming the maze between the testing of each subject

was implemented to preclude the presence of extraneous olfactory cues. The order for running subjects was randomized for each trial.

Results

It should be noted that all subjects drank saccharin during at least one of the preexposure periods. A split-plot ANOVA with diet as the between-groups factor and trials as the within-groups factor was performed on the latencies. While the Groups effect failed to achieve significance, $F(1, 23) = 2.369$, $p = .134$, the Trials effect was found to be reliable, $F(7, 161) = 9.595$, $p < .001$. Newman-Keuls tests used to probe the significant Trials effect indicated that although latencies on Trials 1 and 2 did not differ from each other, they were significantly ($p < .05$) longer than those for Trials 3-8, which did not differ reliably among themselves. Mean latencies of the GRITS and NORMAL animals for the eight training trials are shown in Figure 6 on the following page.

A similar ANOVA performed on the error data yielded significance for the Groups, $F(1, 23) = 5.759$, $p = .023$, and Trials, $F(7, 161) = 2.742$, $p = .01$, main effects. Inspection of the group means indicated that the GRITS group made significantly fewer errors than did the NORMAL group. Subsequent Newman-Keuls revealed that

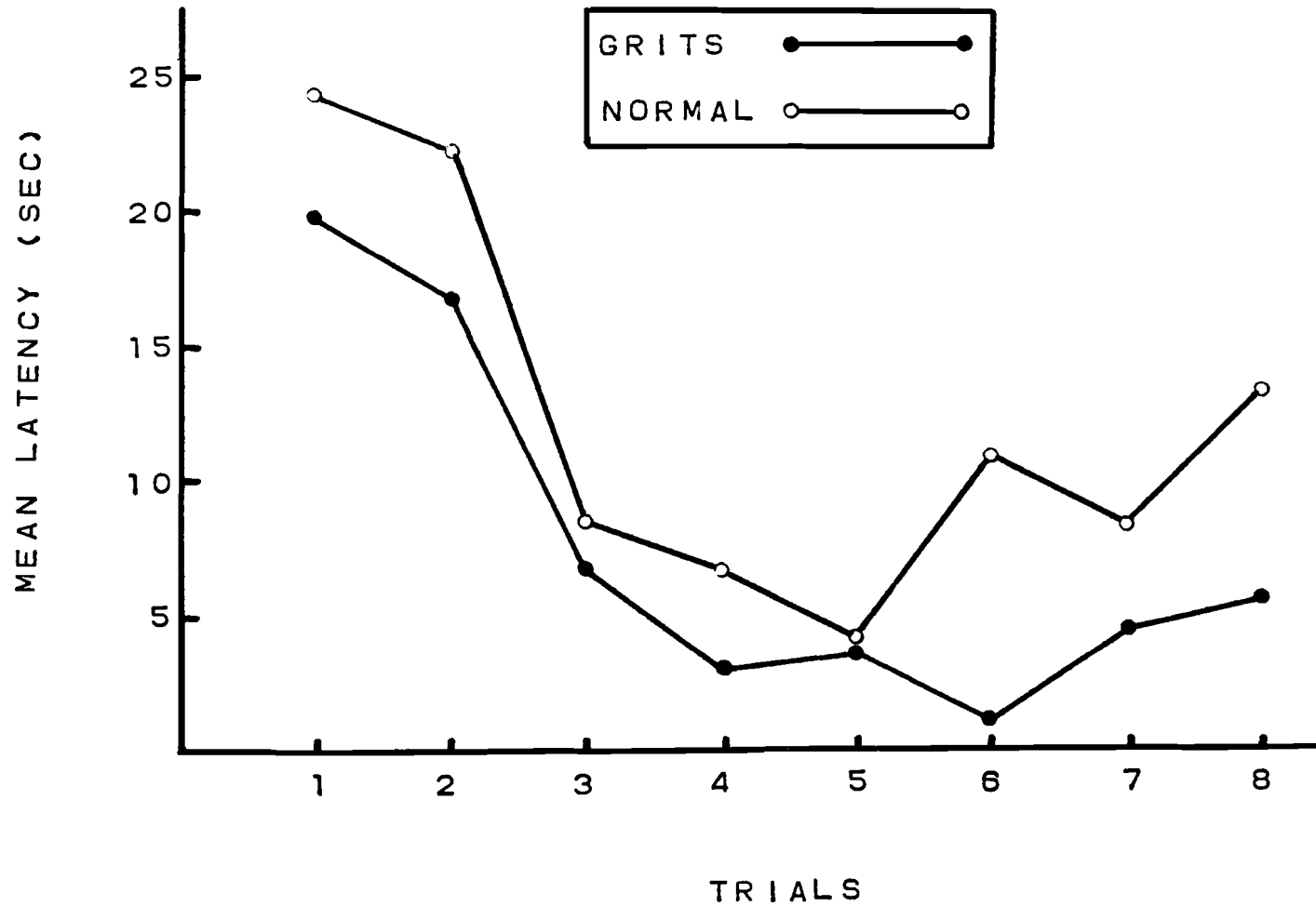


FIGURE 6: Mean latency (seconds) to complete the Hampton Court maze for the tryptophan- and protein-deficient animals (Group Grits) and the normally reared animals (Group Normal). Experiment 2.

although Trials 1 and 2 did not differ, significantly ($p < .05$) more errors were made on these trials than on Trial 5. In addition, significantly more errors were made on Trial 1 than on Trial 4 ($p < .05$). All other comparisons failed to reach an acceptable level of significance. Group mean errors are shown in Figure 7 on the following page.

Discussion

In addition to demonstrating that all animals were capable of mastering the Hampton Court maze, the data addressed differential effects of early exposure to the protein- and tryptophan-deficient diet. At the very least, the present data appears to support previous research, such as the Remley et al. (1980) study, in indicating that such early exposure may not impact negatively on learning ability. For example, analysis of the run-time scores failed to yield a significant groups effect. Likewise, analysis of the error scores failed to indicate that more errors were made by the GRITS animals.

Although the GRITS and NORMAL groups did not differ with respect to the latency required to complete the maze, the NORMAL animals made significantly more errors as they traversed the maze. Thus, the nonsignificant run-time effect might be due to the smaller, GRITS

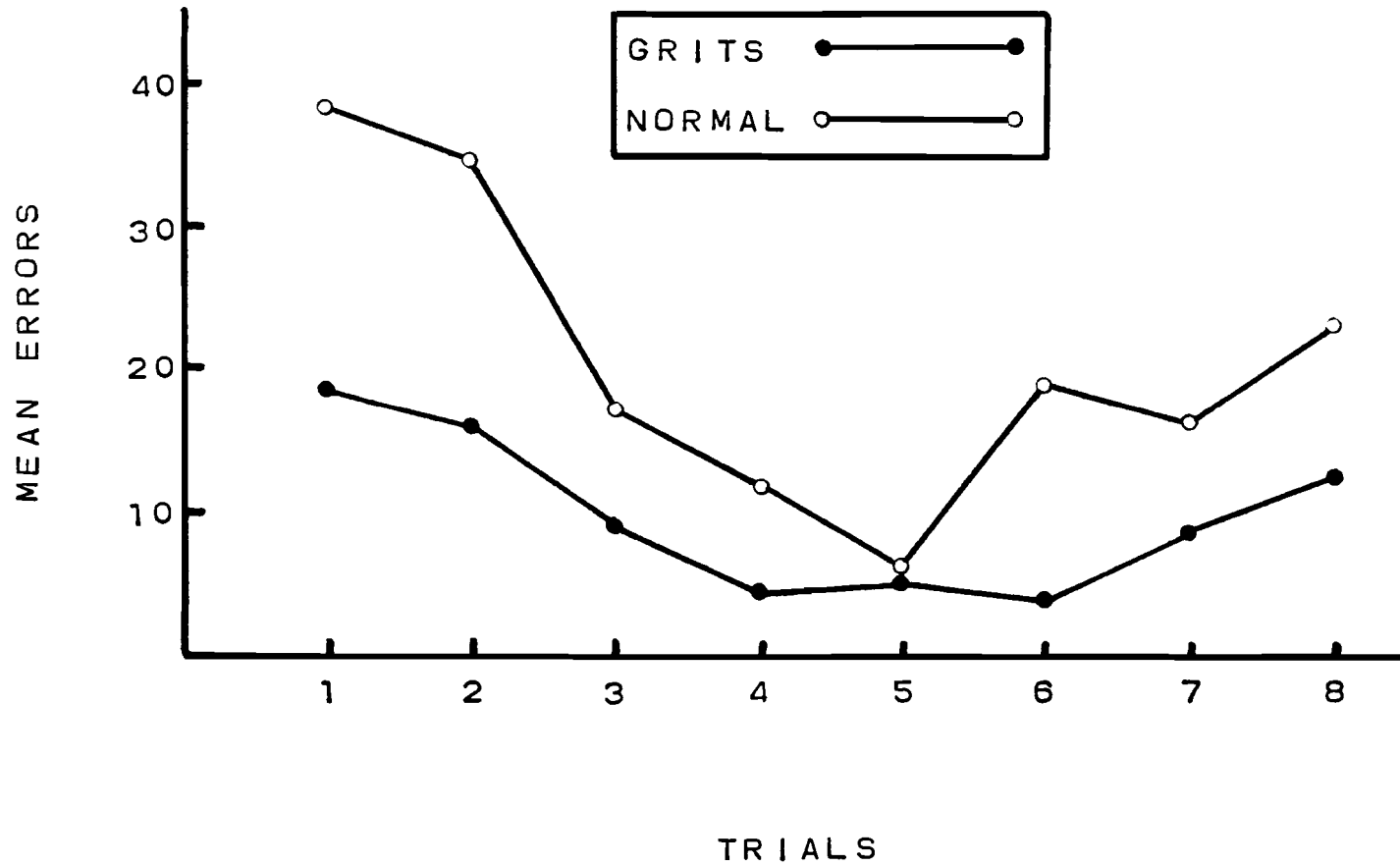


FIGURE 7: Mean errors to complete the Hampton Court maze for the tryptophan- and protein-deficient animals (Group Grits) and the normally reared animals (Group Normal). Experiment 2.

animals running slower but making fewer errors and the larger, NORMAL animals moving faster but making more errors. However, simply considering the sheer number of errors does not completely describe the situation. Visual inspection of the data sheets indicated that rather than entering numerous cul de sacs, the NORMAL animals made repetitive errors, i.e., they entered the same blind alley multiple times. In contrast, the errors made by the GRITS animals appeared to be dispersed relatively evenly across the entire maze. The repetitive nature of the errors shown by the NORMAL animals suggests the presence of a motivational factor, such as frustration, for these subjects.

The present findings stand in contrast to those reported by Cowley and Griesel (1959). It will be recalled that their deprived animals performed less well than did controls in the Hebb-Williams enclosed field test. Cowley and Griesel also indicated that their deprived animals not only ran slower, but chose less efficient routes (i.e., made more errors) to the goal box. However, one must remember that Cowley and Griesel's animals never ran the same test pattern more than once. Thus, their study implies that deprived animals may perform poorly in a non-static learning situation. The results of Experiment 2 indicate that

tryptophan and protein deprivation does not appear to impede mastery of a complex, but static, learning task. Why such dietary restrictions might provide a learning advantage is far from clear. Clearly, the data suggests this possibility. However, a more complete understanding will be based upon additional research.

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