SOME EFFECTS OF A PROTEIN DEFICIENT DIET ON THE ABILITY OF THE ALBINO RAT TO LEARN A SIMPLE MAZE

A RESEARCH PAPER

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by

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Approved! 5/15/67 Vola Simpson

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INTRODUCTION

Research indicates that depriving the body of certain nutrients, or varying combinations of them, may be expected to result in some detrimental effects upon the physical stature, general activity level and performance in learning situations of both experimental animals and human beings. In fact, deficiencies of vital nutrients at an early age have been shown to have long-range consequences on maturation though it is still not known if learning is permanently influenced. Therefore, more detailed studies of selected nutrients have been undertaken to determine possible outcomes in relation to this area of development.

The building blocks of the body, amino acids, are contributed by protein. In fulfilling this function protein furnishes the materials necessary for the construction of various tissues, for the formation and maintenance of cells and for production of antibodies, hormones, and enzymes. In addition, protein is a source of energy. It might be expected that any deficiency or imbalance of such an important nutrient would have some detrimental effects on maturation and learning.

Definite lack of protein may be found even in our own affluent society in diets of individuals with poor food habits, the true vegetarian, the elderly, the weight-watcher and, especially, the children of low-income families. Since insufficient amounts or quality of protein may have far-reaching results in children and adults throughout the world, it seems necessary to increase the body of knowledge we have about protein in connection with growth and learning.

The purpose of this study was two-fold. It was undertaken, first, to learn how to do a simple research project and, second, to determine to what extent a protein deficiency would affect the ability of albino rats to learn a simple maze.

PROBLEM

The problem was to investigate some effects of a protein deficient diet on the ability of male albino rats to learn a simple maze. In addition differences in physical developments as well as activity levels were to be noted.

To accomplish this study a control group of albino rats was maintained on a diet providing sufficient amounts of protein as well as necessary nutrients. In order to rule out other deficiencies the experimental group was placed on a diet providing all the essential nutrients with the exception of protein.

It has been indicated that inadequate amounts of protein may affect ability to learn a simple behavior; therefore, a learning design to test this theory was devised in cooperation with Dr. J. H. Mennear, Pitman-Moore, and Dr. Arno Wittig, Ball State University.

REVIEW OF LITERATURE

According to Cravioto (13), it is well known that underfeeding affects the weight and size of different organs to a varying extent; however, the ultimate pattern depends upon the period of growth at which undernutrition begins. This concept of development is that the body's chemical make-up at each age brings about the changes which cause tissues to progress one step further toward maturation. If one step is retarded, growth and development also may be. Novákova (24) feels this is substantiated when he says that Dénéberg has demonstrated Scott's theory of critical periods in development by the fact that the same impulse applied at various ages evokes different responses. Each critical period provides the necessary growth that leads to the next step in development.

Research indicates that most of the changes in biochemical growth and development produced in experimental animals are present in infants, children, and adults affected by severe chronic malnutrition. It has been noted that retardation in development of rats on a low protein diet occurs at an early age and if this finding may be generalized to other species, this early period may well be critical for the establishing of certain response patterns. Failure to establish these patterns, as in a child, may lead to permanent disabilities in later life (12).

According to Bevan and Freeman (8,p.75), "changes in nutritional status are generally regarded to be an important parameter of efficiency of performance in problem solving." A study of the effect of inanition on rats (12) found differences in maze learning ability among first-filial-generation albino rats present at an early age, though they were not permanent. In experimental animals undernutrition has been shown to retard certain aspects of the chemical development of the central nervous system, as well as impairing learning potential; therefore, Cravioto and Martin (1) have stressed the importance of studying mental development in relation to dietary deficiency.

Studies such as psychomotor tests have led to the belief that severe malnutrition, especially the type described as kwashiorkor, causes a retardation in mental development (7). When rehabilitation is attempted, one may get the same ultimate weight but will end with an undersized subject who is abnormal in shape and composition and may possibly be of a lower mental capacity (13). However, it was noted by Barnes, et al. (7) that it is not known whether this is an actual reduction of capacity or just a retardation of learning.

The indications are that nutritional deprivation in adults may cause some loss of mental activity though the final capacity may not be affected. According to Martin it does appear that intelligence or mental capacity of adult humans is not affected by nutrition status. Still, in the case of poor nutrition, mental performance may be measurably inferior due to depleted energy, inability to concentrate and fatigue. However, Riess and Black (26) state that certain deficiencies in rats retard learning, but that the subsequent stunting may also result in increased activity and an acceleration of learning.

While Cowley and Griesel (12) have noted that results show that a low-protein diet has little effect upon intelligence of the parent generation, but the first-filial-generation rats are retarded in intelligence, thus indicating the importance of the mother's diet.

Most literature suggests that parental factors and heredity must be considered in any learning study; therefore, experimental animals should be of the same sex and littermates, if possible. It was also found in several studies that male albino rats do grow more rapidly and show differences in learning that may not be apparent in the females of the species. (5, 7, 19, 24)

Protein is directly related to growth in children, and growth is indicated in humans and experimental animals by an increase in bulk. Since an increase in weight is a measure of growth for the rat it may be used as an objective evaluation. (26, 34)

Since white rats grow thirty times as fast as humans, results may be obtained in a shorter period of time. A rat on a good diet will gain from 15 to 20 grams per week (25), while one on a poor diet will either fail to grow or show only a little weight gain (25, 34). Weight means for control rats have been shown to be higher than for experimental diet animals (17). Some body functions such as length, organ weights and sexual development are related to body weight also (12). For instance, the white male rat normally reaches sexual maturity at about 80 days. Though a few of the developmental processes do continue, food restrictions not only arrest some of the changes associated with aging, but may even reverse some of these so that at the end of the restrictive period the chemical structure of the organs may resemble those of a much younger animal (13).

According to Bevan and Freeman "deficient animals show increased resistance to handling, cowering, and more violent diffuse reactions to ordinarily mildly distracting stimulation" (8, p.79). This suggests that affective factors are major determinates of maze performance in animals so treated. Further, a recent experiment noted that deficient diet rats exhibit a generally heightened activity more obviously cyclic in character than that noted for normal controls. Though strength, speed, and endurance may be expected to fail when undernutrition is present, Riess and Black state that the stunting of growth may result in increased activity (26).

Albino rats are hardy animals, resistant to infection. However, malnutrition predisposes the body to infection (1) and research does support the theory that protein deficiency results in a reduced ability to resist disease. This could be due to a decreased capacity to form antibodies to overcome infection. Therefore, it is necessary to watch the experimental animals carefully, especially for pulmonary malfunction. Mennear suggested that this may be checked by placing the palm of the hand on the ventral side of the rat, so that any breathing difficulty may be observed.

Characteristics that might be observed in rats on poor diets are: shaggy, dull and possibly thinning fur; rough, dry and scaly ears, feet and tail; eyes not clear; a pinched look in their faces; short whiskers; susceptibility to sniffles and possible breathing difficulty; and restlessness, irritability and crossness. (25, 31)

The following would be expected to be characteristic of the rats on good diets: clean, sleek fur; bright and clear eyes; pink nose, ears, feet and tail; smooth tail, free from roughness; clean, tidy habits; and alertness, tameness and ease in handling. (25, 31)

It was suggested that best results in feeding experiments are obtained if the diet is begun at four weeks of age, since growth demands are great at this time. An omnivorous animal, the albino rat eats the same kind of foods that human beings eat. However, since it does not overeat, food may be kept in the cages at all times. Dry food is thought to be better for experimental purposes because it is easy to control the ingredients, preparation and storage. Since ascorbic acid is produced in the animal's body it is not necessary to add this nutrient to the diet. During the experiment fresh food should be put underneath the previous day's food.

In some of the literature it was indicated that feeding protein deficient diets to rats invariably leads to reduced food consumption under ad libitum conditions, while the more a rat eats of a given diet the more water it will drink (9). In relation to learning, Riess and Black state that rats maintained on a powdered diet will seek some sort of roughage to supplement their diet; therefore, paper toweling, shredded and soaked in water was used as a non-nutritional motivation.

EXPERIMENTAL PROCEDURE

The experiment involved observation of the effects of a protein deficient diet on the following:

1) learning a simple maze
2) measurable physical growth
3) observable physical characteristics
4) observable activity levels

Albino rats were used for purposes of this study since research indicates that some correlation can be made between their dietary habits and those of humans. Also, since the rats grow at a much faster rate than people, results may be obtained in a much shorter period of time. Male albino's were specifically chosen because their growth pattern and learning behavior differences show results that may not be apparent in the female of the species.

The procedure involved placing six albino rats on a balanced control diet (Diet A) and six albino rats on an experimental diet deficient in protein (Diet B) for a period of 55 days; the last two weeks of the period were used for testing the learning of a simple discrimination in an uncomplicated double T maze.

Physical growth was measured by length and weight gains, while observations were made of the outward physical characteristics. Activity level also was determined by observation of overt signs of irritability, nervousness such as starting at sudden noises, or just generalized activity.

Population

Two groups of white male rats, were ordered from Sprague-Dawley, Incorporated. Group I (25-30 grams) littermates were born on June 5, 1966 while Group II (125-130 grams), also littermates, were born May 17, 1966. Each major group (I or II) was then subdivided with three of each group (I or II) placed on Diet A (control) and the remaining animals on Diet B (experimental). During the course of the experiment one of the twelve rats (Group II, Diet B) died.

Care

The subjects were kept in individual cages, elevated above a pan containing newspapers. Each cage was provided with a jar for food and a self-feeding water bottle.

Each day the rat's weight in grams was noted and recorded with the other pertinent information, while the length measurements were made weekly. Observations of physical characteristics as indicated in the review of literature, as well as general activity level were also recorded.

All food was removed from each cage before any rat was weighed or given fresh food. The fresh food from each diet was weighed, placed in the respective dishes, and the old food placed on top of it. Weighing was done in order to estimate the amount of food to be increased or decreased (especially for Diet B).

Papers beneath the cages were changed daily and cages and dishes cleaned if necessary; otherwise, once weekly the cages were scrubbed with soda and soap. Once a week, the diet was prepared and stored for the following week.

Diet

The control diet was a balanced powdered diet selected from several suggested protein diets and mixed by the author. The contents of this diet were dried milk, whole wheat flour, white flour, sugar, salt, butter, cod-liver oil, dried meat, dried alfalfa leaf and raw carrot. (See Appendix A for specific details)

A protein free diet "the biological test diet" prepared by Nutritional Biochemicals Corporation, "containing the fat soluble vitamins A and D," was used as the base for Diet B. The required supplementation of the vitamin B complex was provided by the addition of four grams of Brewer's yeast to every 100 grams of the "protein free diet." (See Appendix B for specific content percentages of corn starch, Alphacel, vegetable oil, salt mixture, and cod-liver oil.)

Food was kept in the cages at all times for ad lib feeding. Both diets were prepared and stored in the refrigerator to prevent rancidity and loss of vitamins.

The subjects were maintained on their respective diets until the 23rd day; at this time blood was noticed at the nostrils of Diet B rats, and there was some indication of difficulty in breathing. Beginning on the 25th day, these rats were put on a maintenance diet of one gram of Diet A to every ten grams of Diet B for the remainder of the experiment.

Learning

The behavior chosen for the rats to learn was a discrimination between black and white in a simple, double T maze. Motivation was non-nutritional in the form of small pieces of water-soaked paper toweling.

Maze

The double T maze was constructed in 8" modular units of unfinished plywood. Sliding doors were placed at each choice point (see Appendix C for drawing). The floor was constructed so that interchangeable 8" squares of cardboard in either, gray, black or white might be used. Neutral gray was chosen to be used at all choice points, while black was associated with a wrong choice and white with reward. An error constituted by the paws over a black line or a retrace to a white square before the doors were closed. The door was closed behind the rat after he made the choice to reinforce the white square as the correct choice.

The maze drawing was duplicated many times and patterns IA, IB, IIC and IID were set up (see Appendix D). A relatively random selection of patterns was made for the 14 day period (see Appendix E). A stop watch was used to determine time in seconds; both seconds and errors were then recorded on the duplicated maze as well as with the other information gathered.

Procedure of the Learning Experiment

The testing period for purposes of this study was set up for a 14 day period starting with day 42 and ending with day 55. Three trials were run for ten days and four trials for the remaining four days.

On the 40th day the subjects were exposed to the shredded water-soaked paper toweling. The 41st day they were given 5 minutes each to explore the completely open maze minus the colored squares. The water bottles were removed from all of the cages at 12:15.

Beginning with day 42 and ending on day 55 of the experiment all food dishes were removed from each cage and labeled before running any trials. At this time freshly weighed food was put in the dishes. Then the rats were weighed, always in the same order, one to twelve. On the first day of testing the non-nutritional motivation in form of water-soaked paper towels was presented for three minutes at the end of the first trial and one minute at the end of each succeeding trial. However, it was noted that the Diet B rats seemed to drink their fill before the last trial; for the remainder of the experiment the paper toweling reward was given for 30 seconds for each of the first trials and one minute at the last trial. At the end of the testing period each rat was removed to its respective cage for an additional 15 minutes with a water bottle. After the alloted time period the bottle was removed until the next day's testing.

RESULTS

Using the following formula, the Fisher's "t" test for reliability was employed to determine significant differences and amount of change in learning between means for seconds and errors between Diet A and Diet B subjects:

$$t = \frac{M_1 - M_2}{\sqrt{\left[\frac{\Sigma x^2 + \Sigma y^2}{N_1 + N_2 - 2}\right]\left[\frac{N_1 + N_2}{N_1 N_2}\right]}} \qquad d.f. = N_1 + N_2 - 2$$

Fisher's table of P values for "t" scores was used to determine probability of occurrence.

In a comparison of Diet A, groups I and II, and Diet B, groups I and II, for seconds and errors, only one significant "t" value was found for seconds on day 12 (p .05).

Tests were also conducted to determine the significance of changes for each group in selected day comparisons (Table I). Significant changes in both measures occurred for both groups from day 42 to day 55, 42 to 48, 42 to 49, 48 to 52; however no significant difference was found for day 49 to 53. It may be seen from Figures I and II that peaks on the graphs for seconds and for errors occur more frequently for Diet B rats and more often at points of pattern changes.

The objective measure of growth, weight, is pictorally represented on Figure III. From this it may be seen that there was a rapid continued growth of Diet A rats until the testing began; at that time a slight decrease in weight occurred. Diet B rats lost slowly

TABLE 1

SELECTED DAY COMPARISONS OF SECONDS AND ERRORS FOR DIETS A AND B

	Diet A t - value		Diet B t - value	
Comparison	Seconds	Errors	Seconds	Errors
42 - 55	3.75**	3.64**	4.42**	5.31**
42 - 48	3.6 **	2.97**	3.75**	2.69**
42 - 49	3.7 **	3.45**	4.25**	4.7 **
48 - 55	4.4 **	2.9 *	4.3 **	5.3 **
49 - 55	1.52	.34	1.08	.97
48 - 49	2.75*	3.02*	3.06*	1.8

* $p < .05$
** $p < .01$

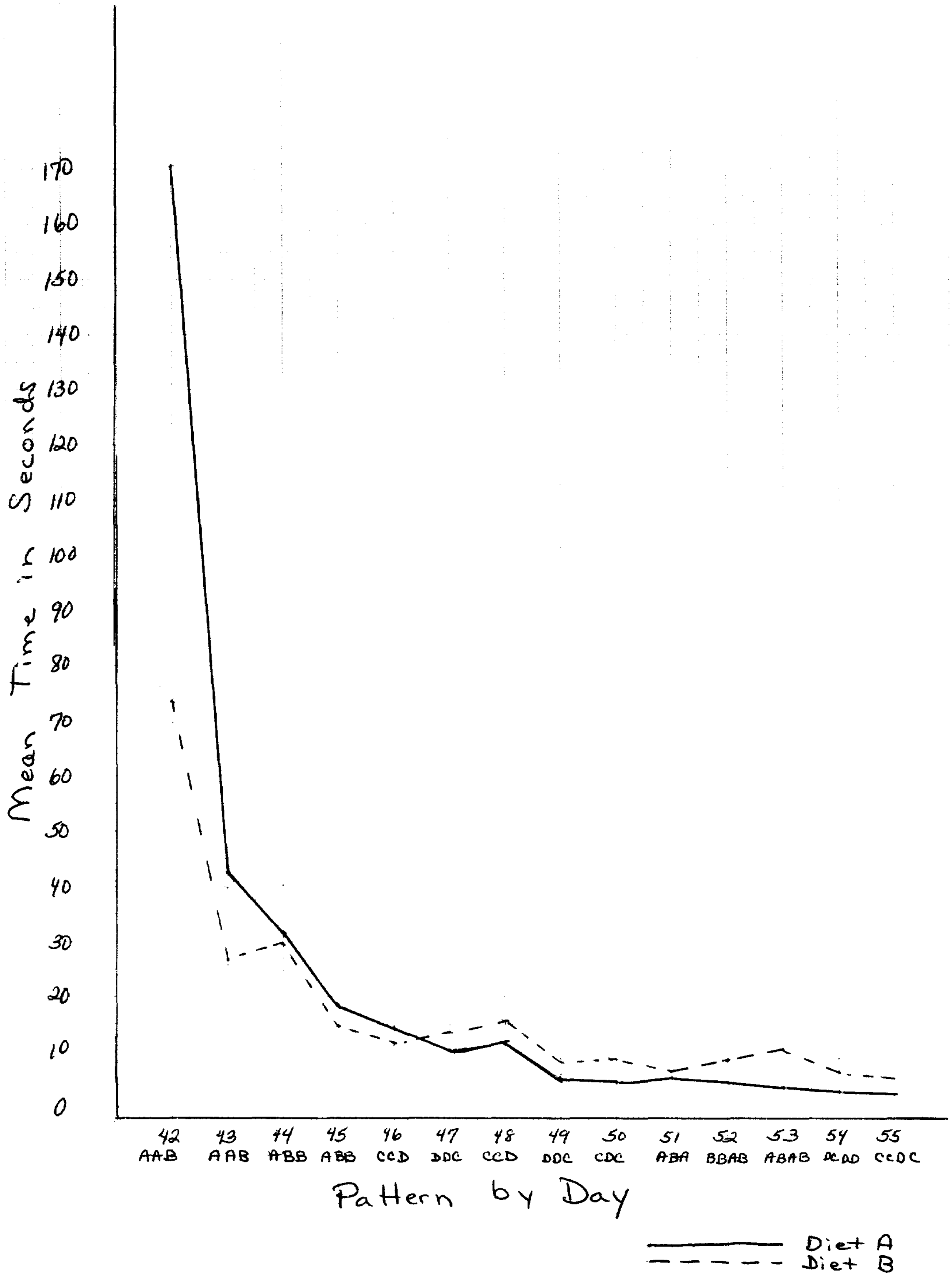

Mean Time in Seconds
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
42 AAB
43 AAB
44 ABB
45 ABB
46 CCD
47 DDC
48 CCD
49 DDC
50 CDC
51 ABA
52 BBAB
53 ABAB
54 DCDD
55 CCDC
Pattern by Day
Diet A
Diet B

Figure II. - Mean Errors by Days for Both Diet Groups

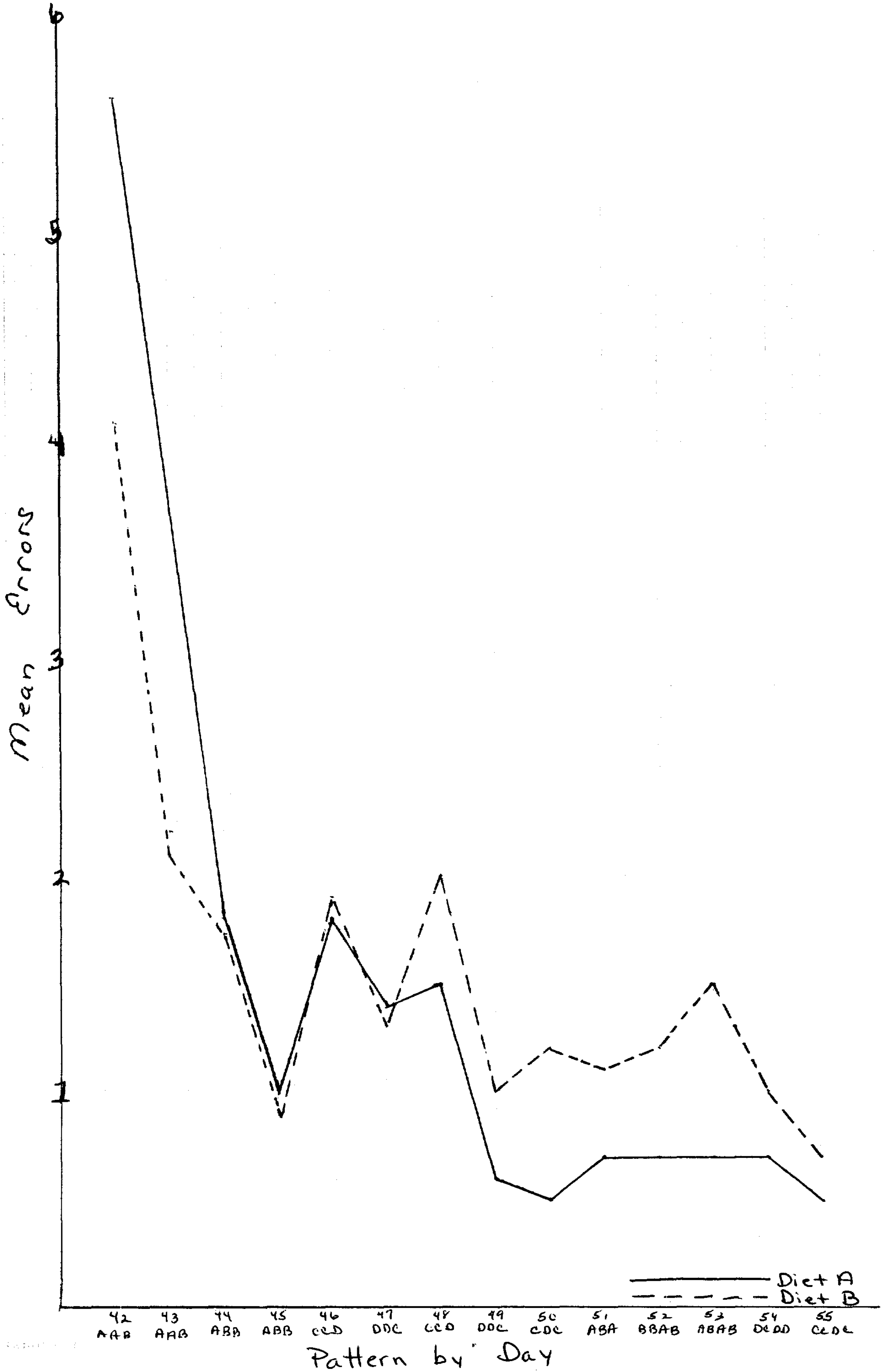
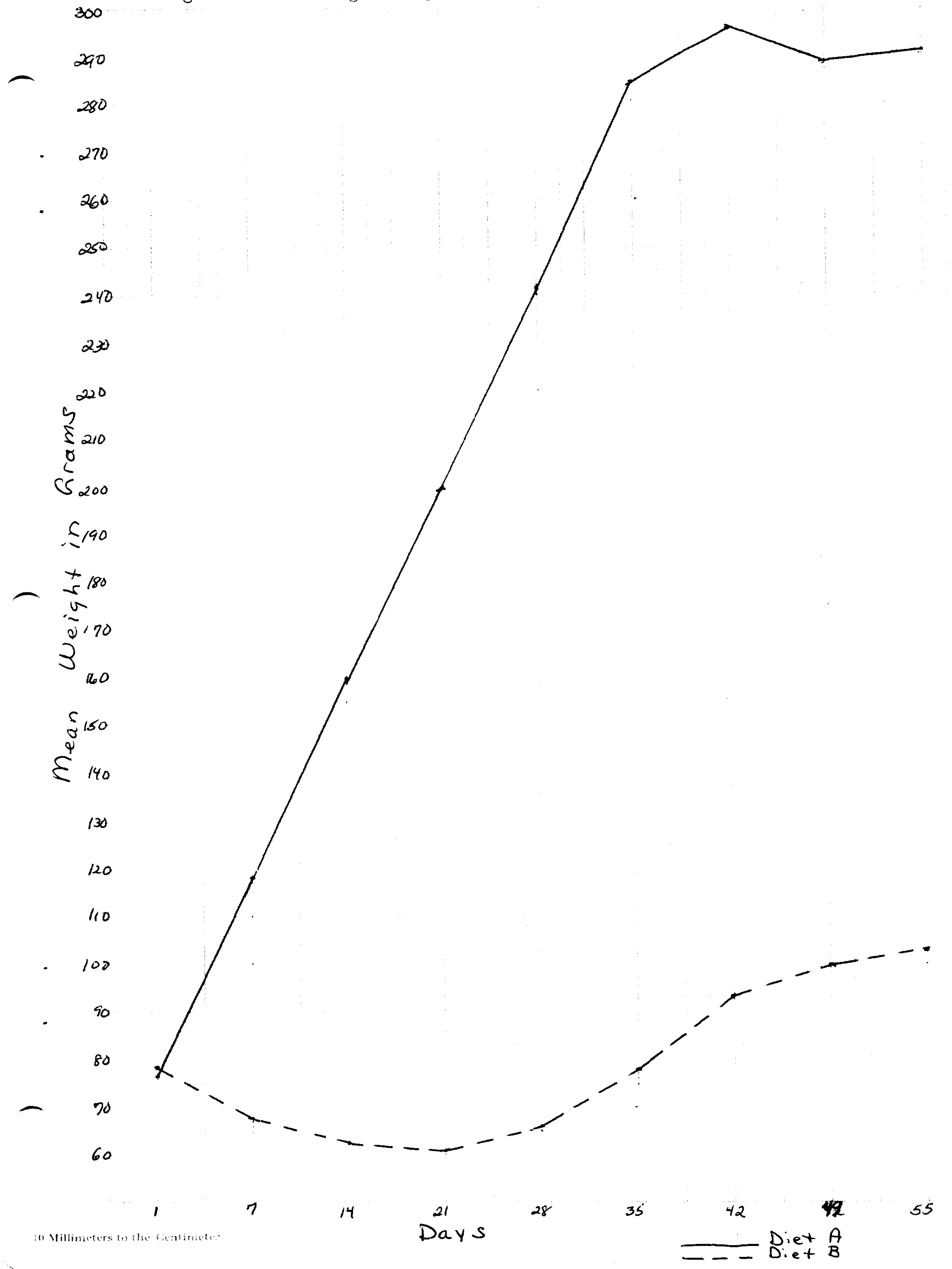


Figure III. - Weight Comparisons for Experimental Period



until placed on the maintenance diet. They then began a gradual weight gain.

Diet A rats exhibited clean, sleek fur; bright eyes, pink noses, ears and tails. Their urine was yellow and their stools were dark in color. During the experiment, the testes of Group II, Diet A rats descended.

Diet B rats generally had rather shabby fur, with some loss; rather dry, scaly, feet and tails; and shorter whiskers. Their urine lacked the definite yellow color of the Diet A rats and the stools were also much smaller and very light in color. On the 23rd day blood was noticed for the first time at the nostrils of all Diet B subjects. Also during the deprivation period the testes of the older rats ascended. When placed on the maintenance diet the stools remained small but became darker, yellow appeared in the urine, and Group IB rats' testes descended. However, Group IIB rats remained very small and did not exhibit this characteristic of maturation.

In relation to diet it was noted that Diet B rats ate less food and drank less water than the Diet A rats. They also seemed to "dig out" the protein in the maintenance diet.

The drawings of paths through the maze for each rat for each trial showed more moves made by Diet B rats than by the groups maintained on the control diet. On the basis of experimental observation Diet A subjects did show a high level of activity while the experimental groups showed increased resistance to handling, as well as starting at small noises. Toward the end of the experiment, Diet A rats seemed to move more surely to the reward, while Diet B rats moved quickly but sporadically.

SUMMARY AND CONCLUSIONS

This study was conducted to determine a few effects of protein depletion on the ability of the albino rat to perform a simple behavior. It encompasses the physical characteristics and general activity level as well. During the 55 day period, two groups of rats were placed on Diet A (control) and two groups on experimental Diet B (low protein). Testing was accomplished the last fourteen days using the learning of a simple discrimination in a double T maze. Fisher's "t" was used to test differences in performance.

As indicated, there was only one significant "t" score found for the comparison of the groups by days; however, the values indicate that the learning was occurring in the expected direction. Significant improvement in performance occurred for both groups during the 14 day test period. The values suggest that most learning for both groups occurs during the first week, with a leveling off during the second week. The "t" tests comparing day 42 to day 48 show significant change, while the gain of the later period (day 49 to day 55) is not significant. It would appear then that the learning affect for both groups occurs during the first week and the second week is spent in refining techniques of performance. The more uneven performance of the experimental groups (see Figures I and II) in relation to pattern changes suggests differences may be a result of diet.

Protein depletion does appear to have some affect upon the activity level as evidenced by nervousness and starting at small noises in the experimental group. The starting may also be a factor in their inability to cope with the changes in pattern situations.

Marked differences in weight gain, physical appearance and general well-being are indicated in the rats maintained on a diet lacking sufficient protein. This would seem to suggest that such deprivation has some affect upon these areas of development.

It should be noted that in general food consumption was related to body size as well as water consumption.

Ascension of the testes in the adult rats and the failure of the testes to descend in the young rats indicates that physical growth may not only be slowed down but may also result in stunting and reversal of some body processes.

In summary, it may be seen that though weight gains and losses on the respective diets were as expected, significant effect was not shown upon performance. Both groups showed significant learning during the first week and a leveling off after this time; however the difference favored the groups maintained on the diet providing sufficient protein. Physical characteristics revealed effects of protein depletion upon appearance, while overt signs of nervousness and starting were associated with this.

In future research a further study of emotionality in relation to learning performance may prove to be very beneficial in determining effects of protein depletion upon the ability to learn. Since, general well-being may also affect learning, a method to determine actual loss of learning potential would be beneficial.

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APPENDIX

APPENDIX A

DIET A*

FOODS	WT. IN GRAMS	APPROXIMATES HUMAN DAILY DIET OF
Dried milk	400	4 cups of milk
Whole wheat flour	200	4 slices of bread or
White flour	200	cereal or pudding
Sugar	40	small amount of jam, or sugar in dessert
Salt	15	small amt. of seasonings
Butter	50	2 tablespoons butter
Cod-liver oil	40	3 teaspoons cod-liver oil
Dried meat	80	1 serving meat
Dried alfalfa leaf	15	2 servings vegetables, potatoes, one serving
Raw carrot	any amount	of fruit

* *Animal Feeding Demonstrations for the Classroom.* National Dairy Council, 1961.

APPENDIX B

DIET B

THE BASE*

A "protein free" biological test diet containing the fat soluble vitamins A and D. It requires supplementation with the B complex vitamins.

COMPOSITION:

Corn starch	70%
Alphacel (cellulose)	15%
Vegetable oil	10%
Salt mixture U.S.P. XIV	4%
Cod-liver oil	1%

THE SUPPLEMENT

The supplement was four grams of Brewer's yeast per 100 grams of diet to supply the required B complex vitamins. Ten grams of the yeast contained the following ingredients:

Thiamine	2.0	mg.		
Riboflavin (B_2)	.8	mg.		
Niacin	6.0	mg.		
Pantothenic acid	.6	mg.		
Pyridoxine (B_6)	.17	mg.		
			IN 100 GRAMS	
Calories	37.0	calories	(14.8	calories
Protein	4.5	grams	(1.8	grams
Carbohydrates	4.0	grams	(1.6	grams
Fat	.1	grams	(.04	grams
Ash	.5	grams	(.02	grams

Four grams would supply 2/5 of these ingredients in 100 grams of diet.

* "Protein Free Diet," Nutritional Biochemicals Corporation Diet Manual, pp. 11. (n.d.)

APPENDIX C

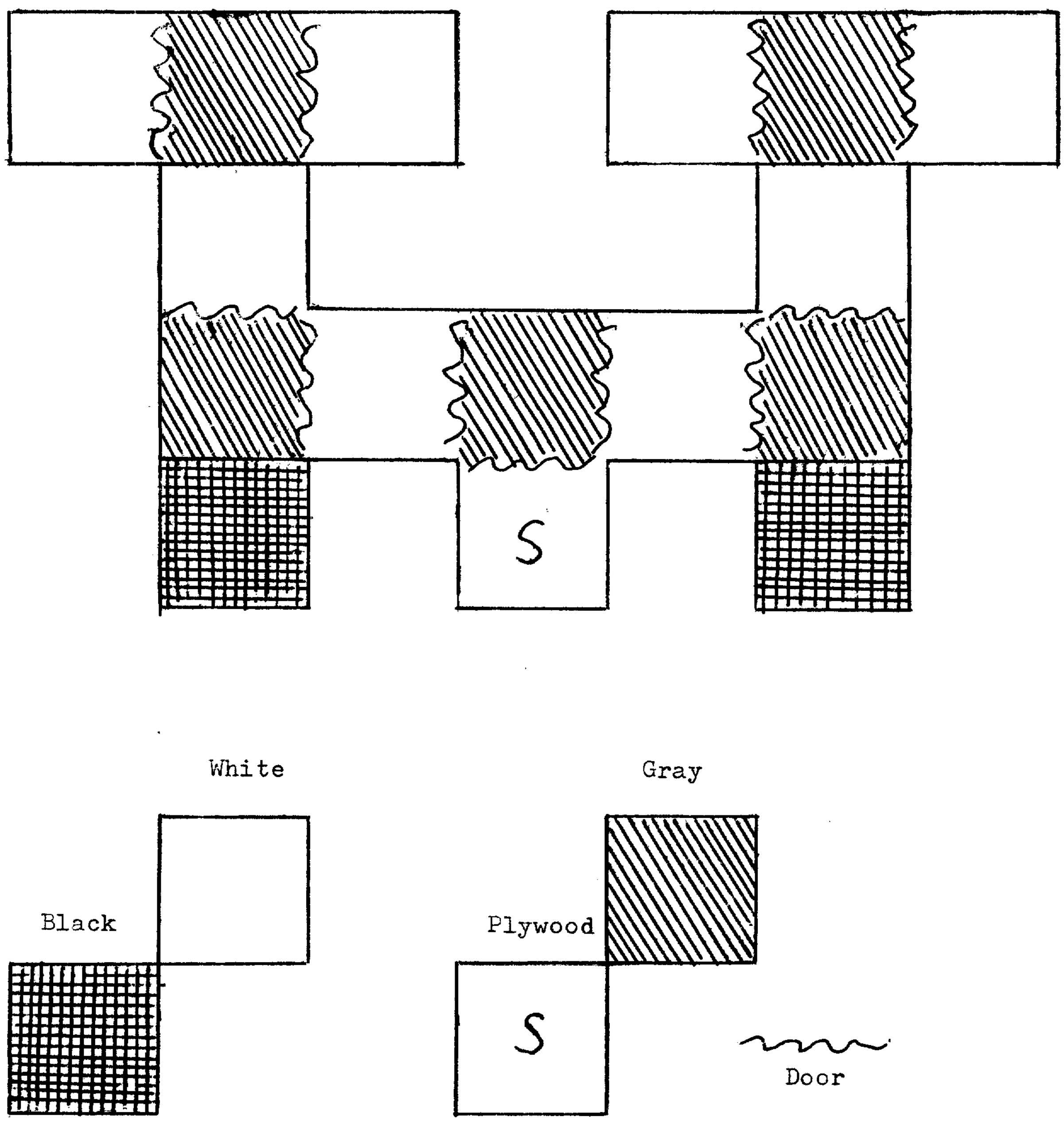

Figure IV. - Drawing Showing Doors and Constant Squares

APPENDIX D

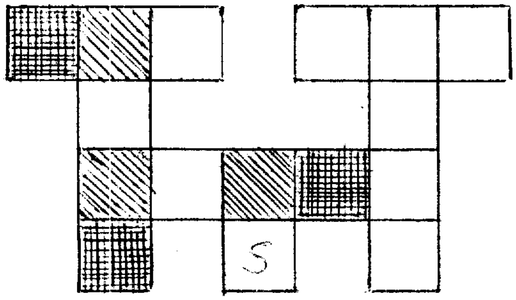

Figure V. -

Maze 1
Pattern A

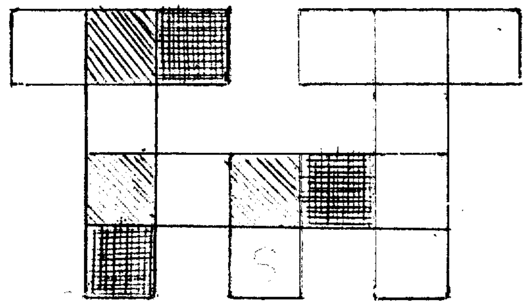

Figure VI. -

Maze 1
Pattern B

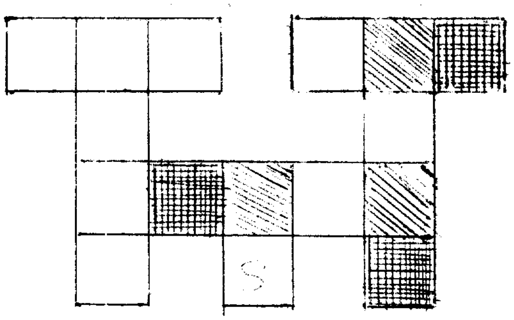

Figure VII. -

Maze 2
Pattern C

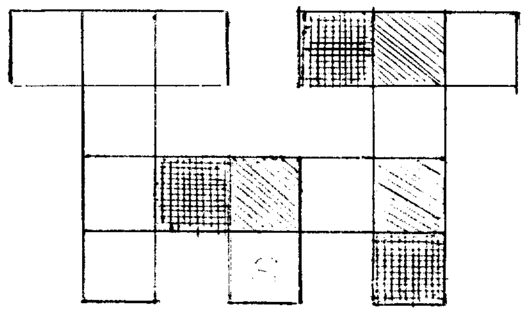

Figure VIII. -

Maze 2
Pattern C

Scale: 3/8" = 8"

APPENDIX E

SEQUENCE OF MAZE AND PATTERNS BY DAY

Day	Maze	Pattern
42.	I	AAB
43.	I	AAB
44.	I	ABB
45.	I	ABB
46.	II	CCD
47.	II	DDC
48.	II	CCD
49.	II	DDC
50.	II	CDC
51.	I	ABA
52.	I	BBAB
53.	I	ABAB
54.	II	DCDD
55.	II	CCDC