THE EFFECT OF DIETARY ALTERATION OF PROTEIN, CARBOHYDRATE, AND FAT ON PHYSICAL PERFORMANCE OF THE ALBINO RAT

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ABSTRACT

The problem of dietary alteration and its effects on physical performance has long awaited experimental investigation. In the past, investigators endeavored to determine the adverse or beneficial effects of dietary alterations on human and animal growth, behavior, pathology, and intelligence. As athletics grew in America, the consideration of diet increased because of its inherent importance in the training of the individual for physical performance. Because of the lack of experimental evidence concerning dietary alteration and its effect on physical performance this investigation was undertaken to determine the effects of high protein, high carbohydrate, and high fat diets on the performance of forced but controlled activity in the male albino rat.

Thirty-two weaned (23-days-old) male, albino rats of the Sprague-Dawley Strain were used as experimental animals in this study. At twenty-three days of age, each animal was randomly assigned to one of the following four diet groups:

High protein - 50 per cent casein, 30 per cent dextrose, 30 per cent hydrogenated vegetable oil,

High carbohydrate - 20 per cent casein, 60 per cent dextrose, 10 per cent hydrogenated vegetable oil.

High fat - 20 per cent casein, 20 per cent dextrose, 50 per cent hydrogenated vegetable oil.

Following an adjustment period of six weeks, each animal was assigned to either the short duration or long duration activity treatment. The groups were matched according to voluntary activity, body weights, and diet treatments.

The diet treatments were administered once a day over a 14-week period. The various experimental exercise treatments were administered once a day, five days a week over a 8-week or 40-day period.

Because of its inherent value, analysis of variance was utilized as the chief statistical method for the analysis of the data obtained. The following conclusions were derived from the data and raw observations:

1. The dietary alterations of fat, carbohydrate, and protein used in this study have a significance (0.0005 level) effect on the physical performance of the male albino rat.
2. This effect is observed in the two types of exercise used, short and long endurance, where marked differences in PER scores can be seen.

The protein, carbohydrate, and control animals in the long endurance program achieved higher PER scores than the protein carbohydrate, control, and fat animals in the short endurance group. Also, the order of PER scores of each diet group varied in each training group. In the short group, the fat diet group achieved the highest PER score and the protein group the lowest; while in the long group, the protein diet group achieved the highest PER score and the fat diet group the lowest.

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CHAPTER I

INTRODUCTION TO THE PROBLEM

For many years, investigators have endeavored to determine the adverse or beneficial effects of dietary alterations on human and animal growth, behavior, pathology, intelligence, and physical activity. The dietary alterations usually have been achieved by increasing or decreasing the quantity of protein, carbohydrate, or fat, and adding or subtracting vitamins and minerals in the diets of experimental subjects. Simultaneously, control subjects have been fed diets containing adequate amounts of all these components. Similar environments and circumstances have been imposed upon both the experimental and control subjects.

The belief that only a "well-balanced" diet prevents nutritional disorders has existed for many years. If one reads the literature, one will find minimum and maximum values for all of the dietary components and must conclude that somewhere intermediate lies the well-balanced diet. This intermediate area is justifiable only because of the fact that each individual varies considerably in terms of metabolic need, hence the necessity for flexibility.

With the growth of athletics in America, the consideration of diet has increased in view of its inherent importance in the training of the individual for physical performance. Recognizing that it is the dietary components which provide the necessary fuel for energy expenditure in athletic performance, athletic coaches and trainers have recommended specific foods and diets as a means of improving athletic performance with very little experimental evidence to justify or refute their choices. Over the past decade, food supplements, including vitamins, minerals, wheat germ, gelatin, and infinitum, have been steadily increasing as integral parts of athletes' diets; although numerous writers have concluded that there is no evidence that athletic performance is improved by supplementing an already nutritionally adequate diet. Many coaches subjectively believe that the dietary needs of the athlete are not the same as those of the average individual and that particular foods enhance the athlete's performance. If this is true, which dietary components are most beneficial and what quantitative alterations should be made as a result?

Because of the lack of experimental evidence concerning dietary alteration and its effect on physical performance, this investigation was undertaken to determine the effects of high protein, high carbohydrate, and high fat diets on the performance of forced but controlled activity in the male albino rat.

Need for the Problem

The problem of dietary alteration and its effects on physical performance has long awaited experimental investigation. Minimum and maximum values for each dietary component have been established, but do these standards hold for subjects engaged in physical activity? Will a rat which is forced to exercise daily over a substantial period of time perform better on a "balanced diet" or a diet which is composed mainly of protein, carbohydrate, or fat?

Scope of the Study

This study involved the dietary alterations of protein, carbohydrate, and fat in the diets of 32 male albino rats and the effects these alterations had on their physical performance. The study covered 14 weeks, beginning when the animals were 23 days old and ending at sacrifice when the animals were 121 to 123 days old.

Definition of Terms

The following terms are defined because of their specific connotations in this study:

High protein - Formula	g/kg
Casein, High Protein	500.0
Dextrose, Hydrate, Technical	300.0
Hydrogenated Vegetable Oil	100.0

Vitamin Fortification Mixture GBI Cat. #40060	10.0
Salt Mix, Bernhart and Tomarelli Cat. #170750	40.0
Non-Nutritive Fiber (cellulose)	50.0

High carbohydrate - Formula

Casein, High Protein	200.0
Dextrose, Hydrate, Technical	600.0
Hydrogenated Vegetable Oil	100.0
Vitamin Fortification Mixture GBI Cat. #40060	10.0
Salt Mix, Bernhart and Tomarelli Cat. #170750	40.0
Non-Nutritive Fiber (cellulose)	50.0

High fat - Formula

Casein, High Protein	200.0
Dextrose, Hydrate, Technical	200.0
Hydrogenated Vegetable Oil	500.0
Vitamin Fortification Mixture GBI Cat. #40060	10.0
Salt Mix, Bernhart and Tomarelli Cat. #170750	40.0
Non-Nutritive Fiber (cellulose)	50.0

Control diet - Standard Wayne Laboratory Blocks

Forced exercise - This standard program was designed using male rats of the Sprague-Dawley strain. All animals were 70 days of age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have Per cent Shock-Free Time and Per cent Expected Revolutions of 75 or higher during the final two weeks. Noted below in Table 1 are the first and last days of training of the eight-week, short-duration, high-intensity and long-duration, low-intensity endurance training programs for adult male rats in controlled-running wheels.

Limitations of the Study

Because of time, expense, and uncontrollable conditions, many limitations and restrictions were imposed on the design of this investigation. The scope and depth of this study were limited because only two exercise programs were included. Thus, all generalizations are limited specifically to the short and long endurance programs outlined elsewhere in this paper. The number of animals was chosen because of housing convenience and time available for programming and running the exercise wheels, rather than determining the exact number necessary for the establishment of statistical significance. The diets were designed so that a maximum amount of a specific compound was utilized. This eliminated the determination of any potential influence that a progressive alteration

TABLE 1

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (sec) or (min)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Bet. Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Work Time TWT (sec)	Total Exp. Revolutions TER
						Short-Duration, High-Intensity							
1	M	1	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
8	F	40	2.0	10s	40	10	6	2.5	0.8	6.0	58:30	600	900
						Long-Duration, Low-Intensity							
1	M	1	3.0	10s	5	40	3	5.0	1.2	1.5	39:45	1200	450
8	F	40	1.0	25.0m	0	1	2	2.5	0.8	2.0	52:30	3000	1500

of component percentages might have had on animal performance. It should also be noted that casein was used as the protein source, dextrose as the carbohydrate source, and hydrogenated vegetable oil as the fat source. It is these specific compounds, rather than all proteins, all carbohydrates, or all fats, that the results are attributed to. Air conditioning and control of humidity were available in the living quarters, but the training area lacked such control. Therefore, it was virtually impossible to determine the amount of environmental stress placed upon the animals and its effect on their physical performance.

It is often argued that animal studies have no place in physical education research since the results cannot be applied directly to humans. However, many experimental designs call for precise control of subjects, observations, and variables over the majority of the subjects' life spans. Certainly, with designs of this nature, animal experimentation is far more feasible than studies which make use of human subjects.

It must be realized, by both those who do animal research and those who read it, that the purpose of animal studies is not to draw inferences which are immediately applicable to human populations, but to provide researchers with insights which other tools and techniques will not allow. It is in this sense that animal studies may be thought of almost as pilot studies, for they provide the investigator with information concerning the possible direction of future research.

CHAPTER II

REVIEW OF THE LITERATURE

The rational for any dietary alteration must be carefully investigated before that alteration is implemented. This survey of pertinent literature was directed at gaining insight in terms of protein, carbohydrate, and fat requirements and alterations in animal and human diets and the influence of such alterations on physical performance.

Animal Studies

The information derived from animal studies is of interest mainly because of its potential application to humans. Unfortunately, rigid control in studies of this nature is extremely difficult using human subjects; therefore, it is hoped that the advantage gained in experimental control through the use of animals is not offset by the limitations in the application of animal data to humans.

Protein Requirements.—Proteins are required by the animal body for the replacement of the tissue proteins which are broken down in the normal metabolic processes, and for the building of new tissues in growth and reproduction.

Proteins digested and absorbed in excess of these requirements are for the most part used for the production of energy—either stored as carbohydrate or fat, or expended in work or heat.

Proteins are made up of amino acids. The amounts and proportions of these amino acids vary tremendously in proteins from different sources. Inasmuch as many amino acids occur as components of body proteins, obviously each of these must be made available, either preformed in the diet or by synthesis in the organism from other materials. The recognition of this fact raises the question as to the nutritional importance of the individual amino acids.

Until comparatively recently, only three of these compounds, namely tryptophane, lysine, and histidine, had been shown to be indispensable components of the diet. In 1931, Rose (26) reported investigations using diets in which the proteins were replaced entirely by mixtures of highly purified amino acids. From this work it was apparent that the known amino acids, when incorporated in otherwise adequate diets, were incapable of supporting growth. This led eventually to the isolation and identification, by McCoy, Meyer, and Rose (24) of a new indispensable dietary component, now known as threonine. Feeding experiments with this new amino acid incorporated in an otherwise adequate diet constituted the first successful effort to induce growth in animals maintained upon diets carrying synthetic mixtures of highly purified amino acids in place of proteins. Following this discovery, the importance of the individual amino acids was determined by omitting them from diets one or more at a time. The known essential amino acids for the rat may be summarized as follows:

Lysine
Tryptophane
Histidine
Phenylalanine
Leucine
Isoleucine
Threonine
Methionine
Valine
Arginine

Although a number of proteins are lacking in certain of the essential amino acids (gliadin of wheat is deficient in lysine and zein of corn is devoid of lysine and tryptophane), the feeding of a mixture of proteins of plant and animal origin will usually insure the presence of all the necessary ones. Two proteins, each of which is deficient in one or more of these essential components, may mutally supplement each other to provide an adequate source of nitrogen.

Hogan and Pilcher (18), using rations varying from 7 per cent to 33 per cent protein, found that rats on the higher concentrations made better gains than those on the lower levels. Hogan, Johnson, and Ashworth (19) reported that rats on a high protein ration (26.2 per cent) made greater gains and stored more water and protein, but less fat, than did their pair mates on a low protein food (10 per cent). Forbes, Swift, Black, and Kahlenberg (12) described studies of the effects of four different levels of dietary protein (10, 15, 20, and 25 per cent) when equicaloric amounts of the ration were fed. With increased protein intake there was an increase in total gains, an increase in protein stored and usually a decrease in fat gained per unit of protein stored. Hamilton concluded that the growth-promoting value of a diet is increased as the protein of the ration is increased from 4 to 16 per cent, is unchanged between 16 and 30 per cent, and is decreased when the concentration of protein is above 30 per cent of the food. Diets containing less than 16 per cent protein produce gains having more fat but less protein than is produced on a well-balanced ration. Gains of approximately the same composition are produced by foods containing between 16 and 42 per cent protein.

McCoy (24) studied the growth and body composition of animals receiving different levels of protein in the diet. Vitamin-free, fat-free, salt-low casein was substituted for a part of the dextrin of the basal ration to produce foods containing about 15, 25, and 40 per cent protein.

Animals receiving the high- and medium-protein diets grew more rapidly than those receiving the low-protein ration. In paired feeding experiments the rate of growth and the percentage of nitrogen of the animal parallels the protein intake. The percentage of fat in animals on the 40 per cent diet was consistently lower than in those from either the 25 or 15 per cent rations.

Alteration of protein quantity and quality in the rat without consideration for their physical, mental, and behavioral development can cause irrepairable damage. Delayed physical development and impaired learning ability of rat pups resulted from feeding a marginal diet. Under experimental conditions, two low protein diets which provided 14 and 21 per cent as opposed to 25 per cent resulted in differences in body weights and intelligence (3, 10, 11). When animals were fed a diet containing insufficient quantities of an essential amino acid growth was retarded or abnormal tissue development occurred. Changes in the cellular structure of the pancreas have been reported when rats were fed a low protein diet (27). Changes in the liver, principally in gross appearance and color, slight changes in the pancreas and some atrophic changes in the spleen were reported as a result of a forced-fed diet consisting of a vitamin-sucrose mixture plus corn, rice, wheat, and milo flour as the sole source of protein (27). On the other hand, the activity of many liver enzymes involved in amino acid catabolism increases with an elevated dietary intake of protein. This causes a high rate of deamination which in turn causes higher blood levels of ammonia. Under experimental conditions, diets containing either 25 or 80 per cent casein were fed to control and experimental treatment groups respectively. It was found that the mean blood ammonia level in the rats on the 80 per cent ration was more than three times higher than that of the control group receiving the 25 per cent ration. (33)

<u>Fat Requirements</u>.—In 1929, Burr and Burr (5) reported a deficiency disease of the rat produced by the exclusion of fats from the diet. On such a ration animals grew for a time, but soon developed deficiency symptoms: the skin became scaly; the end of the tail appeared inflamed and swollen, and later became heavily scaled and ridged. Investigators (6) concluded that both linolenic and linoleic acid are effective and are of about equal value in curing rats suffering from a fat deficiency. It has been further suggested that linoleic and linolenic acids are essential for the production of more highly unsaturated fatty acids.

Evidence has accumulated for several years that the type rather than the quantity of fat consumed may be of importance in dietary alteration. On diets containing 20 per cent fat, rats had the best growth rates when saturated fatty acids accounted for 30 per cent of the fat. On the other hand, growth rate may not be an entirely reliable criterion. Greater longevity was associated with lower growth rates when animals were fed diets of either 27 per cent rapeseed oil or butterfat. The rapeseed oil animals had a longer life span than those of the butterfat group, with a mean life span of 669 and 545 days respectively. (31)

A study by Carrol and Bright (8) was designed to determine whether changing the relative proportion of carbohydrate and fat in the diet would influence metabolic responses in rats to different sources of the two nutrients. Four carbohydrate-fat combinations (glucose and fructose each with corn oil (CO) and with hydrogenated coconut oil (HCO)) were combined in high carbohydrate-low fat and low carbohydrate-high fat diets. Protein and caloric values of all diets were equivalent. Eight groups of male weanling rats were each fed one of the experimental diets for two to four weeks. Reducing the carbohydrate-to-fat ratio from 64:5 to 19:25 (by weight) resulted in the following changes in liver functions: 1) marked reduction or complete elimination of responses to the glucose-6-phosphatase and fructose diphosphatase enzyme systems to dietary fructose; 2) significant increase in response of glucose-6-phosphatase to dietary

HCO; 3) decreases in liver glycogen to a different extent with different carbohydrate-fat combinations; 4) striking increases in total lipid in rats fed CO or HCO with glucose; and 5) increases incholesterol in rats fed CO, and in phospholipid in rats fed HCO.

Carbohydrate Requirements.—Many experimenters have studied the influence of individual carbohydrates on growth (16), on the utilization of other dietary components (9), and on body composition (7, 21, 22). There is now a substantial body of evidence that in a number of different ways different kinds of dietary carbohydrate produce different effects in animals. To emphasize this difference an experiment in which the carbohydrates studied formed 80 per cent of the diet can be cited (2). Dextrose, fructose, liquid glucose, and sucrose were the experimental carbohydrates while starch served as the control. The mean body weights of the rats in all treatment groups increased steadily throughout the experiment. At the termination, the rats given dextrose increased significantly less in weight than those given diets containing liquid glucose or sucrose. The mean weights of the heart, kidney, liver, and spleen were calculated as percentages of body weight. The organ weights of the control group were used as normal values. The results are as follows:

Diet	Comparison
Liquid glucose	No difference
Dextrose	Heart weight greater
Sucrose	Heart weight and kidney weight greater
Fructose	Heart, kidney, and liver weights greater

As compared with values found with the control diet, carcass and liver fat were increased by sucrose and fructose diets but not by dextrose and liquid glucose diets. All high carbohydrate diets resulted in lower liver protein than the control diet. The difference was greatest with the fructose and sucrose diets.

Animal Performance

For decades man has wondered if alteration of the quantity of the three dietary components of protein, carbohydrate, and fat would enhance his performance in terms of physical performance. Animal experiments have shed some light on this topic and are utilized because of the difficulty encountered when trying to control variables in studies with humans.

Investigators have found that a diet containing between 14 and 18 per cent protein is best suited for the spontaneous activity of albino rats (28). No change in activity was observed until the protein content reached 50 to 54 per cent. Above that level, a depression of activity occurred (15, 29). Diets containing 66 per cent carbohydrate or 43 per cent fat did not influence the amount of spontaneous activity (23). Studies of spontaneous activity of rats fed diets high in either fat or protein indicated that as much as 56 per cent of the caloric value of food may come from fat without depressing activity.

In the above experiments, activity was defined as the amount of activity which was of a completely voluntary nature. There are many other variables which may have been operating in the above experiments which have not been noted. Very little work has been done on the effects of altering the quantity of each dietary component in regard to forced or controlled exercise.

Human Nutrition and Physical Activity

Focusing attention on the status of American nutrition provides the investigator some insight as to the range and scope of dietary needs inherent to all individuals. A comparison of present day diets in the United States with diets at the beginning of the century shows that the American diet is higher in all nutrients. Fifty of the dietary studies made by Atwater and his associates of the Office of Experimental Stations between 1895 and 1903 were recalculated. The comparison showed that 50 per cent of the dietary studies made by Atwater were below 1958 National Research Council recommended allowances of calcium, riboflavin, vitamin A, ascorbic acid, and thiamine. The percent of calories from fats has increased. The diets of 1895 and 1905 contained 131 grams of fat which was 38 per cent of the total calories, compared to 155 grams or 44 per cent of the total calories of 1955 (30).

The average American consumes approximately 40 per cent fat, 40 per cent carbohydrate, and 20 per cent protein in addition to an assortment of vitamins and minerals in his daily diet. Protein in the diet is necessary to afford sources of nitrogen and amino acids to be utilized in the synthesis of body protein and other nitrogen containing substances. Protein is involved in a variety of important metabolic functions. Protein in excess of requirements serves only as a source of energy since it can not be stored as a protein and presents the kidney with excess organic acids to filter and excrete. Fat is an important human dietary component, because of its high fuel value, because of the essential fatty acids in natural fats, and because fats are carriers of the fat soluble vitamins. The optimal amount of fat in the diet cannot be stated with exactness but the National Research Council, the British Medical Association, and the American Medical Association's Council on Foods and Nutrition recommend that the average diet should contain 20 to 25 per cent of its calories as fat and that the diet of the active person, child, and adolescent should contain 30 to 35 per cent (25). The value of carbohydrates lies solely in their capacity to provide fuel for energy expenditure. Of the three components, carbohydrates are the most flexible when alteration of dietary quantity is in question.

Over the years, coaches have been attempting to control the diets of their athletes in order to insure maximum performance during competition. These coaches have been under the belief that the inclusion or exclusion of certain foods in the diet impairs or aids performance of the athlete. Unfortunately they have had little scientific basis for their endeavors and as a result they have relied upon past traditions, unfounded beliefs, and the clever Madison Avenue advertisements of big business.

It has been generally assumed that carbohydrate is the main and primary energy source during exercise. Experiments with high carbohydrate diets have shown 25 per cent (32), 11 per cent (14), and 8.3 per cent (4) increases in muscular efficiency. It was also found that a subject could continue strenuous work three times as long on a high carbohydrate diet as on a high fat diet (20). Fat has been considered only as reserve fuel, used mainly during recovery. However, in recent years evidence has accumulated which seems to suggest that the plasma free fatty acids are an important fuel for prolonged muscular work and that carbohydrate metabolism starts to play a major role only in heavy exercise when the oxygen supply of the muscle becomes insufficient (13). Also, the oxygen uptake of the skeletal muscle in man is so high that it cannot be explained by the oxidation of glucose alone (1). Another study (17) shows that on the basis of respiratory quotient values, fat appeared to be the principle energy source in men exercising after an overnight fast.

In light of the contradictory results from the above studies, it seems of considerable practical importance to study the effects of high carbohydrate fat, and protein diets on physical performance.

CHAPTER III

METHODS OF RESEARCH

This investigation was undertaken to determine the effects of high protein, high carbohydrate, and high fat on the performance of forced but controlled activity in the male albino rat.

Experimental Design

Experimental Animals

Weaned (23-days-old) male rats of the Sprague-Dawley Strain were utilized as experimental animals in this investigation.

Adjustment Period

To permit adjustment to the laboratory environment and the experimental diets, all animals were housed for six weeks in standard, individual cages for small animals. These cages shall be referred to as "sedentary cages" hereinafter, and are 24cm. long by 18cm. wide by 18cm. tall. For two weeks prior to the commencement of the training phase of the study, the animals were permitted voluntary activity in exercise wheels, of the type described by Richter and

Wang (23), which were attached to their individual sedentary cages. This was done so that the animals were able to learn how to run and to become acquainted with an exercise wheel. At the end of the voluntary activity period, the animals were returned to a sedentary existance, except for their exercise bout each day, for the remainder of the study.

Treatment

1. At twenty-three days of age, each animal was randomly assigned to one of the following four diet groups:

(a) High protein - received casein, 500.0 g/kg or 50 per cent, dextrose, 300.0 g/kg or 30 per cent, hydrogenated vegetable oil, 100.0 g/kg or 10 per cent; and vitamin, salt mix; and non-nutritive fiber in a combination of 10 per cent.

(b) High carbohydrate - received casein, 200.0 g/kg or 20 per cent, dextrose, 600.0 g/kg or 60 per cent, hydrogenate vegetable oil, 100.0 g/kg or 10 per cent; and vitamin, salt mix; and non-nutritive fiber in a combination of 10 per cent.

(c) High fat - received casein, 200.0 g/kg or 20 per cent, dextrose, 200.0 g/kg or 20 per cent, hydrogenated vegetable oil, 500.0 g/kg or 50 per cent; and vitamin, salt mix; and non-nutritive fiber in a combination of 10 per cent.

(d) Control diet - received standard Wayne Laboratory Blocks.

2. At sixty-five days of age, each animal was assigned to one of two activity treatments. The groups were watched according to activity records compiled over the two weeks that the animals had access to their individual exercise wheels, according to body weights, and according to diet treatments.

(a) A Short duration running group was subjected to a short-duration, high-intensity program of interval running in controlled exercise wheels for small animals. By the end of the eighth week of training these animals were completing six, ten-minute bouts, with two and a half minutes of rest between bouts. Each bout consisted of ten repetitions of ten seconds of work alternated with forty seconds of rest. This group ran at six feet per second during the work intervals.

(b) A Long duration running group was subjected to a long-duration, low-intensity program of continuous running in the controlled exercise wheel for small animals. By the end of the eighth week of training, these animals were completing two, twenty-five-minute bouts, with two and a half minutes of rest between bouts. This group ran at two feet per second during the work interval.

The controlled exercise wheel, designed, constructed, and tested at Michigan State University, was utilized in this study. With this apparatus, the speed and duration of running can be controlled separately and in combination. Either interval training or continuous regimens of exercise can be conducted depending on the objectives of the investigator.

Treatment Periods

The diet treatments were administered once a day, seven days a week, in the afternoon over a 14-week period. The various experimental exercise treatments were administered once a day, between 6:00 A.M. and 11:00 A.M., five days a week (Monday through Friday). These treatments were continued over a eight-week or forty-day period.

Number of Animals

Thirty-two Sprague-Dawley Strain male rats were utilized as the experimental animals in this investigation.

Noted below, in Table 2, is the combination diet group-training group design and the distribution of animals in each of the listed categories.

TABLE 2

Diet Group		Training Group	
		Short Endurance	Long Endurance
Protein	8	4	4
Fat	8	4	4
Carbohydrate	8	4	4
Control	8	4	4

Animal Care

Throughout the experiment, all of the animals received water and were fed their specific diets *ad libitum*. Each animal was handled, fed, and watered daily.

Treatment of the Data

The following data were collected during the study and analyzed:

1. Daily records were kept of the body weights of all animals:

   (a) Body weight before exercise

   (b) Body weight after exercise

2. Daily records were kept of the environmental

conditions in the room where the animals were exercised:

(a) Air temperature

(b) Percent humidity

(c) Barometric pressure

3. Daily records were kept of the controlled exercise wheel settings used with the short and long running groups:

(a) Acceleration time

(b) Work time

(c) Rest time

(d) Repetitions per bout

(e) Number of bouts

(f) Time between bouts

(g) Amount of shock available

(h) Speed

4. Daily records were kept of the training results for each of the two exercise groups:

(a) Total expected revolutions

(b) Total revolutions run

(c) Percent expected revolutions

(d) Total work time

(e) Cumulative duration shock

(f) Percent shock free time

5. Analysis of variance, correlations, and means were used in the statistical analysis of the compiled data

from this investigation.

6. These animals were also utilized in two other studies involving blood pressures.

CHAPTER IV

ANALYSIS OF DATA

This study involved the dietary alterations of protein, carbohydrate, and fat in the diets of 32 male albino rats and the effects those alterations had on their physical performance. The study covered 14 weeks, beginning when the animals were 23 days old and ending at sacrifice when the animals were 121 to 123 days old. Over this period of time data was accumulated dealing with the aspects of the daily life of the experimental animals, the environmental conditions, and the training results recorded for each animal.

Because of its inherent value, analysis of variance was utilized as the chief statistical method for the analysis of data obtained in this study.

Tables 3 through 6, and Graphs 1 through 8 provide the analysis of variance, mean, standard deviation, and visual interpretations of the data compiled from this study. Animal Number, Training Group, Diet Group, and Training Group plus Diet Group are the category variables; whereas Body Weight Before Training, Body Weight After Training, Body Weight Loss, Percent Body Weight Loss, PER, and PSF are dependent variables.

The following points should be noted:

(1) Diet Group and Training have been defined in Chapter 1, therefore, reiteration is not necessary.

(2) Animals Number 5, 13, and 18 deceased for reasons of unknown origin. The data obtained from these animals was not used in order to facilitate easy statistical programming and interpretation.

(3) The terms PER and PSF should be defined in order to facilitate understanding of the data presented here. PER can be defined as the per-cent of expected revolutions. The planning of the exercise programs was such that an animal was expected to run a set number of revolutions in the exercise wheel in a given amount of time and at a particular rate. For example, if an animal was expected to run 400 revolutions and then ran 450 or 350, its PER would be 112.5 per cent or 87.5 per cent respectively. PSF is the percentage of shock free time. The programs were based on a time principal, so arranged as to allow the animals a predetermined

amount of acceleration and performance time. If they failed to maintain the required pace, a stimulatory shocking device was utilized to facilitate their performance. The percent shock free time during each exercise period was calculated by dividing the sustained shock time by the program time and subtracting from 100. For example, if cumulative shock time was 50 seconds and program time was 1,200 seconds, the PSF would be 95.8 per cent.

Each table and graph has been designed for easy reading and interpretation. Special attention should be devoted to the dependent variables, Body Weight, PER, and PSF; also, the category variable Training Group and Diet Group (Table 6) and Graphs 1 through 8, since they are of extreme importance in this study of animal performance.

Table 3 provides a summary of individual animal scores over the 40-day training period. It was earlier stated that animals which had PER values of 75 or better may be considered capable performers. Table 3 indicates that only Animal Number 19 failed to achieve the 75 per cent criteria. The data for this animal was included in the statistical analysis because of the already small sample size.

TABLE 3
Analysis of variance, means, and standard deviations: where the category variable is animal number

CATEGORY: ANIMAL NUMBER	BODY WEIGHT BEFORE MEAN	S.D.*	BODY WEIGHT AFTER MEAN	S.D.	BODY WEIGHT LOSS MEAN	S.D.
1	246.6	7.66	245.9	17.0	0.7	16.1
2	256.5	24.25	253.1	23.4	3.3	2.2
3	312.3	30.38	303.5	34.4	8.8	22.3
4	309.6	29.60	301.5	46.5	8.0	39.1
6	326.0	32.21	318.2	34.0	7.7	15.4
7	344.1	34.01	336.4	34.4	7.7	16.0
8	316.2	29.47	312.5	28.5	3.7	2.5
9	287.2	23.79	285.3	29.5	1.9	16.1
10	254.2	23.10	260.1	21.9	4.1	2.5
11	296.2	24.07	292.7	23.9	3.5	1.9
12	277.7	25.58	274.7	25.1	3.0	1.8
14	322.1	21.67	316.5	22.5	5.6	2.5
15	327.5	22.15	323.0	21.5	4.5	2.3
16	348.0	22.30	337.7	34.1	10.3	22.8
17	277.7	20.28	276.9	23.4	0.7	15.6
19	250.1	36.87	246.8	35.7	3.2	2.2
20	238.9	23.21	236.6	22.9	2.3	1.2
21	312.7	28.37	309.2	28.1	3.5	2.0
22	308.1	25.93	303.9	24.9	4.1	2.8
23	312.7	34.01	307.4	33.5	5.3	2.2
24	252.5	37.80	247.8	39.1	4.6	22.7
25	285.9	21.16	285.5	27.1	0.4	15.3
26	270.1	25.45	266.8	25.0	3.3	1.9
27	292.7	25.59	288.8	25.7	3.9	1.7
28	227.5	8.66	225.9	11.5	1.6	6.2
29	329.2	28.69	318.4	32.8	10.8	22.1
30	305.7	29.97	301.0	29.0	4.7	2.2
31	300.7	23.99	301.0	31.1	-0.2	22.1
32	345.5	23.85	340.2	22.7	5.3	3.1

*Where S. D. will represent Standard Deviation

The overall Mean was 294.6 and the overall Standard Deviation was 42.0 for Body Weight Before.

The overall Mean was 290.2 and the overall Standard Deviation was 42.1 for Body Weight After.

The overall Mean was 4.39 and the overall Standard Deviation was 14.0 for Body Weight Loss.

TABLE 3 (Continued)

CATEGORY: ANIMAL NUMBER	PERCENT BODY WEIGHT LOSS MEAN	S.D	PERCENT EXPECTED REVOLUTIONS MEAN	S.D.	PERCENT SHOCK FREE TIME MEAN	S.D.
1	0.2	6.6	95.4	29.0	79.8	20.8
2	1.2	0.7	86.9	8.6	84.2	8.4
3	2.7	6.7	82.9	8.2	78.1	9.0
4	2.4	13.6	85.2	20.5	74.8	21.1
6	2.3	4.5	75.1	21.0	68.9	16.0
7	2.1	4.1	80.6	28.0	74.2	15.9
8	1.1	0.7	112.9	19.9	88.7	9.2
9	0.7	5.5	79.0	33.3	57.4	27.6
10	1.5	0.8	94.9	24.2	87.3	11.1
11	1.1	0.6	115.1	35.5	90.1	10.2
12	1.0	0.6	86.6	15.8	82.5	9.6
14	1.7	0.8	103.7	36.2	80.9	18.3
15	1.3	0.6	81.6	18.3	77.9	11.3
16	3.0	6.8	91.7	25.7	80.6	17.4
17	0.2	5.8	111.5	47.3	83.3	13.0
19	1.2	0.7	58.4	4.4	66.7	12.4
20	0.9	0.5	83.1	27.1	70.9	24.8
21	1.1	0.6	98.7	27.8	79.1	15.4
22	1.3	0.8	109.9	17.6	88.9	10.3
23	1.7	0.6	124.3	45.0	90.5	7.7
24	1.6	6.9	82.7	28.4	76.8	14.7
25	0.1	5.1	97.8	20.8	83.5	14.2
26	1.2	0.6	108.2	38.5	87.4	9.9
27	1.3	0.6	73.4	29.7	75.8	14.3
28	0.7	2.6	96.0	27.7	86.4	13.3
29	3.2	6.2	78.4	14.9	75.1	13.8
30	1.5	0.6	86.2	18.9	76.5	11.4
31	-0.1	7.7	90.8	30.1	82.0	13.6
32	1.5	0.8	105.1	36.8	83.0	15.7

The overall Mean was 1.4 and the overall Standard Deviation was 4.6 for Percent Body Weight Loss.

The overall Mean was 92.3 and the overall Standard Deviation was 30.8 for Percent Expected Revolutions.

The overall Mean was 79.7 and the overall Standard Deviation was 16.5 for Percent Shock Free Time.

Table 4 utilizes the training groups, short and long, as its category variable. This provides information concerning the differences between the two training groups as a whole. It is of extreme importance to note that a comparison of short against long is of little value because performance is relative to the objectives of each particular program. This table is useful chiefly in analyzing the performance within each group.

Table 5 shows the effect of diet as a category variable, but omits the independent effect of training group per se. An interesting point can be made concerning the differences in body weight between diet groups. Body weight was not controlled in terms of the amount of food consumed each day. The animals were permitted complete freedom of food intake. Large body weight differences between diet groups could be observed as early as the second week. These differences became greater in the performance period of the study and continued to increase up to sacrifice during the fifteenth week. The 0.0005 level of significance indicates the importance of diet to animal weight. However, several alternative reasons exist for the marked differences other than dietary composition itself. For example, the texture and taste of the various foods may have been either desirable or disagreeable. As a result, one group of animals may have eaten considerably more than

TABLE 4

Analysis of variance, means and standard deviations: where the category variable is training group

CATEGORY: TRAINING GROUP	BODY WEIGHT BEFORE		BODY WEIGHT AFTER		BODY WEIGHT LOSS	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
SHORT ENDURANCE	298.1	41.3	293.5	42.3	4.6	17.9
LONG ENDURANCE	290.4	42.4	286.5	41.6	4.1	6.9
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.002		0.004		0.565	

The overall Mean and Standard Deviation was the same as shown in Table 3 for all DEPENDENT VARIABLES

CATEGORY: TRAINING GROUP	PERCENT BODY WEIGHT LOSS		PERCENT EXPECTED REVOLUTIONS		PERCENT SHOCK FREE TIME	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
SHORT ENDURANCE	1.4	5.9	91.8	33.7	77.8	18.7
LOND ENDURANCE	1.3	2.1	92.9	26.7	82.0	12.9
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.781		0.554		0.0005	

TABLE 5

Analysis of variance, means and standard deviations: where the category variable is diet group

CATEGORY: DIET GROUP	BODY WEIGHT BEFORE MEAN	S.D.	BODY WEIGHT AFTER MEAN	S.D.	BODY WEIGHT LOSS MEAN	S.D.
Control	325.2	30.8	319.1	32.7	6.1	16.1
Carbohydrate	275.2	30.6	272.5	31.6	2.7	8.3
Fat	270.2	38.1	266.3	39.6	3.9	19.1
Protein	312.4	39.4	307.2	39.2	5.1	11.3
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.0005		0.0005		0.023	

The overall Means and Standard Deviations were the same as shown in Table 3 for <u>all</u> DEPENDENT VARIABLES listed.

CATEGORY: DIET GROUP	PERCENT BODY WEIGHT LOSS MEAN	S.D.	PERCENT EXPECTED REVOLUTIONS MEAN	S.D.	PERCENT SHOCK FREE TIME MEAN	S.D.
Control	1.8	5.0	92.6	29.5	79.6	15.3
Carbohydrate	1.0	2.8	93.9	31.6	81.3	17.7
Fat	1.3	6.6	86.1	28.8	76.8	17.6
Protein	1.6	3.3	95.7	31.8	80.7	14.7
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.149		0.001		0.006	

another group.

Table 5 would indicate that a high fat diet is not conducive to optimal physical activity.

Table 6 and Graphs 1 through 8 represent the most valuable data obtained from this study. The importance of classifying the animals by both Diet Group and Training Group is shown by the fact that mean differences in all variables except Body Weight Loss and Percent Body Weight Loss reached the 0.0005 level of significance.

Table 6 compares the category variable, diet-training group, against the previously noted dependent variables. Each graph gives the viewer a visual impression of the daily PER and Body Weight of a particular diet-training group. Surprisingly within the short endurance group, the animals on the fat ration achieved the highest PER of 93.8 per
Carbohydrate, 91.3 per cent, control, 91
tein, 90.7 per cent, diet animals
the long endurance group, one finds that the animals on the protein ration achieved the highest PER of 100.6 per cent, followed by carbohydrate, 96.5 per cent, control, 95.6 per cent, and fat, 75.8 per cent. It is interesting to note that carbohydrate and control diet animals were second and third in performance both in short and long endurance. But, there was a complete reversal of position for fat and protein diet animals. Animals on the fat diet were last in the short

TABLE 6

Analysis of variance, means and standard deviations where the category variable is diet-training group

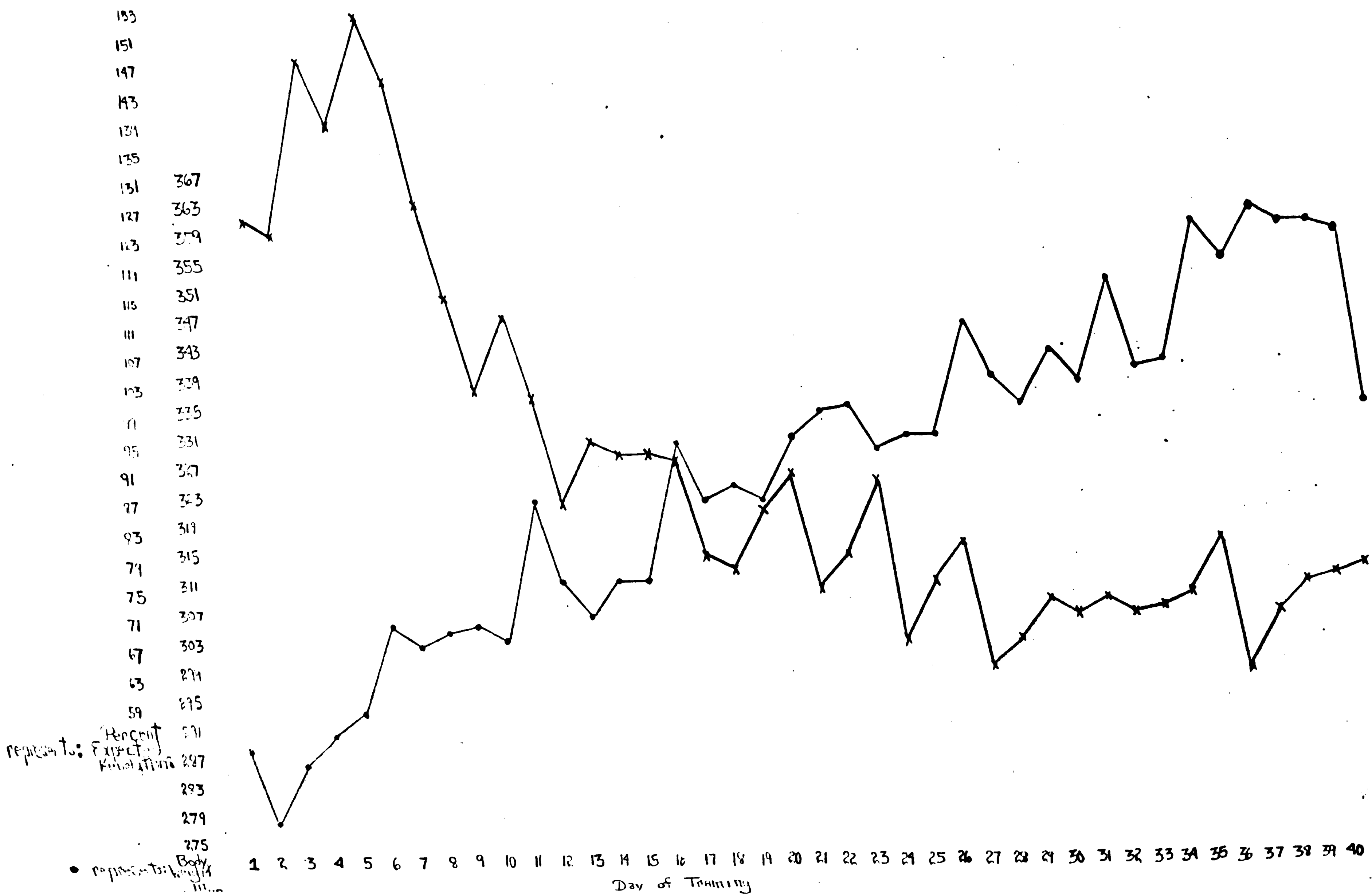
CATEGORY DIET-TRAINING GROUP	BODY WEIGHT BEFORE		BODY WEIGHT AFTER		BODY WEIGHT LOSS	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
SHORT/CONTROL	325.2	29.4	318.5	32.9	6.6	19.7
SHORT/CARBOHYDRATE	290.5	23.8	288.1	26.6	2.4	11.1
SHORT/FAT	268.2	35.3	265.2	39.1	2.9	22.5
SHORT/PROTEIN	308.7	48.6	302.3	48.4	6.3	15.8
LONG/CONTROL	325.4	33.5	320.3	32.6	5.0	2.7
LONG/CARBOHYDRATE	259.8	29.0	256.8	28.4	3.0	3.7
LONG/FAT	272.9	41.5	267.8	40.4	5.1	13.1
LONG/PROTEIN	316.1	27.2	312.1	26.5	4.0	2.4
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.0005		0.0005		0.046	

CATEGORY TRAINING GROUP AND DIET GROUP	PERCENT BODY WEIGHT LOSS		PERCENT EXPECTED REVOLUTIONS		PERCENT SHOCK FREE TIME	
	MEAN	S.D.	MEAN'	S.D.	MEAN	S.D.
SHORT/CONTROL	1.9	6.1	91.2	29.0	79.6	16.0
SHORT/CARBOHYDRATE	0.8	3.7	91.3	34.3	76.7	21.5
SHORT/FAT	0.9	8.1	93.8	34.1	77.2	20.7
SHORT/PROTEIN	1.9	4.6	90.7	37.2	77.6	16.0
LONG/CONTROL	1.5	0.7	95.6	30.5	79.7	14.0
LONG/CARBOHYDRATE	1.1	1.4	96.5	28.6	85.9	11.2
LONG/FAT	1.7	3.9	75.8	14.5	76.3	12.4
LONG/PROTEIN	1.2	0.7	100.6	24.4	83.6	12.8
APPROXIMATE SIGNIFICANCE PROBABILITY:	0.184		0.0005		0.0005	

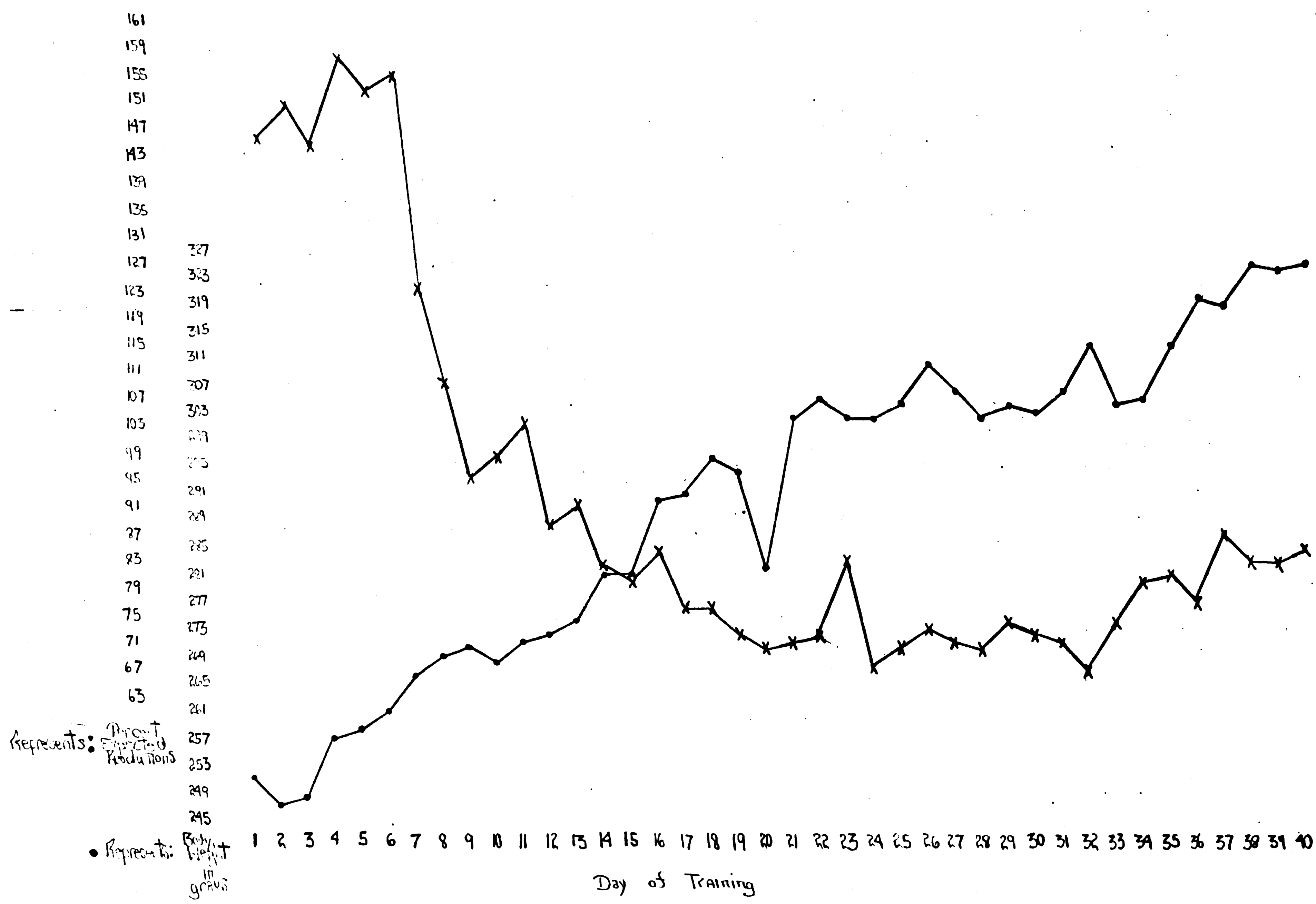
endurance program and last in the long endurance; while animals on the protein diet were last in the short endurance program and first in the long endurance. The original hypothesis that there might be a difference in performance as influenced by various diets has been substantiated at the 0.0005 level of significance. From the data accumulated in this particular study, it may be speculated that the short and long exercise programs involve different metabolic requirements, possibly different metabolic pathways, and more than likely different energy expenditures.

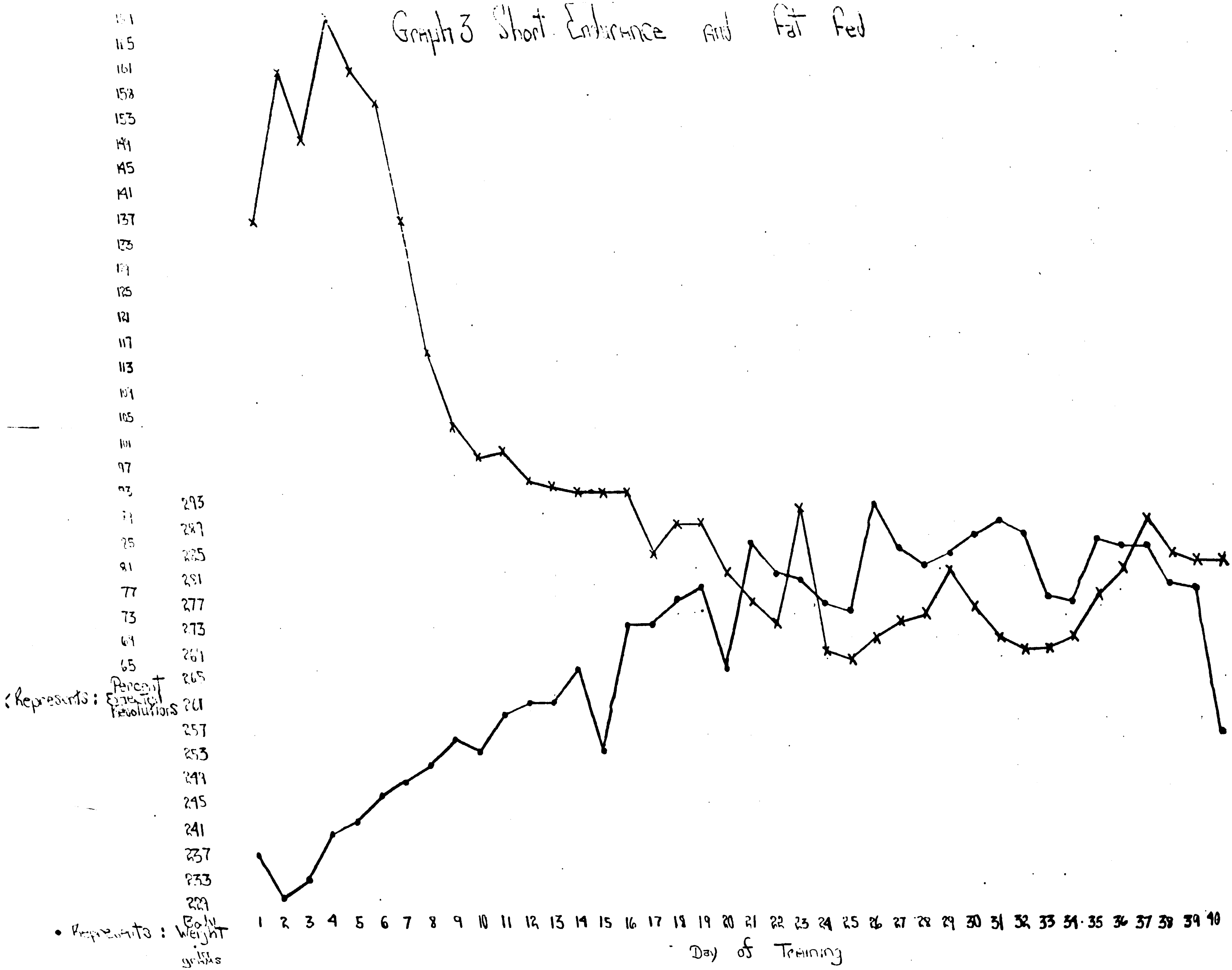
Except for the fat-fed, long-endurance animals, the PER of each of the other groups follow similar patterns as illustrated in Graphs 1 through 8. An interesting, but far from substantiated, observation is that as body weight increases over time, PER decreases. There are many possible explanations for this occurrence. For instance, body weight may influence animal performance after a given point, or the program difficulty increasing as a function of time may be the sole cause of the PER decrease.

Graph 1 Short Endurance And Block Fed



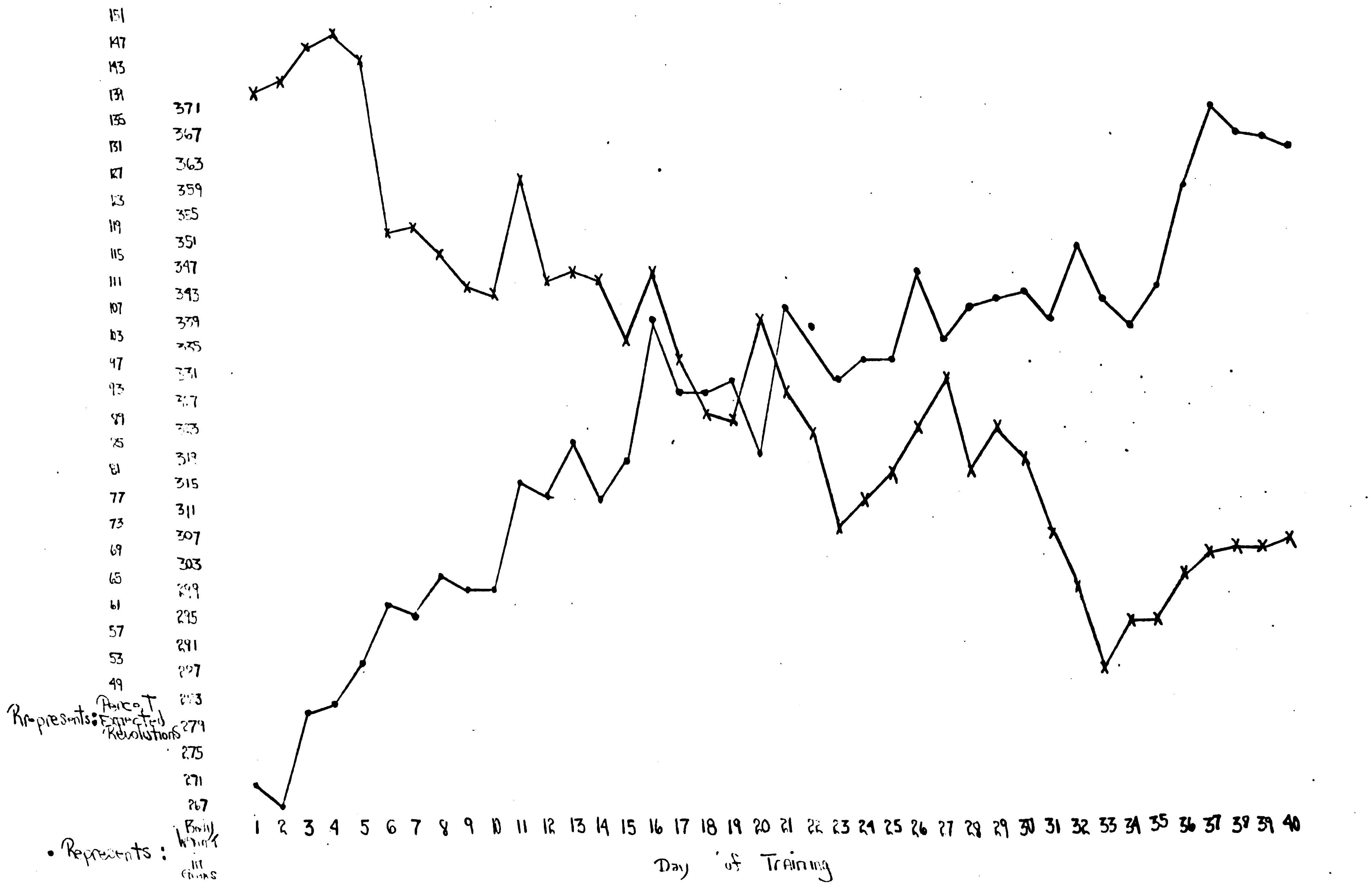
Graph 2 Short Endurance and Carbohydrate Fed



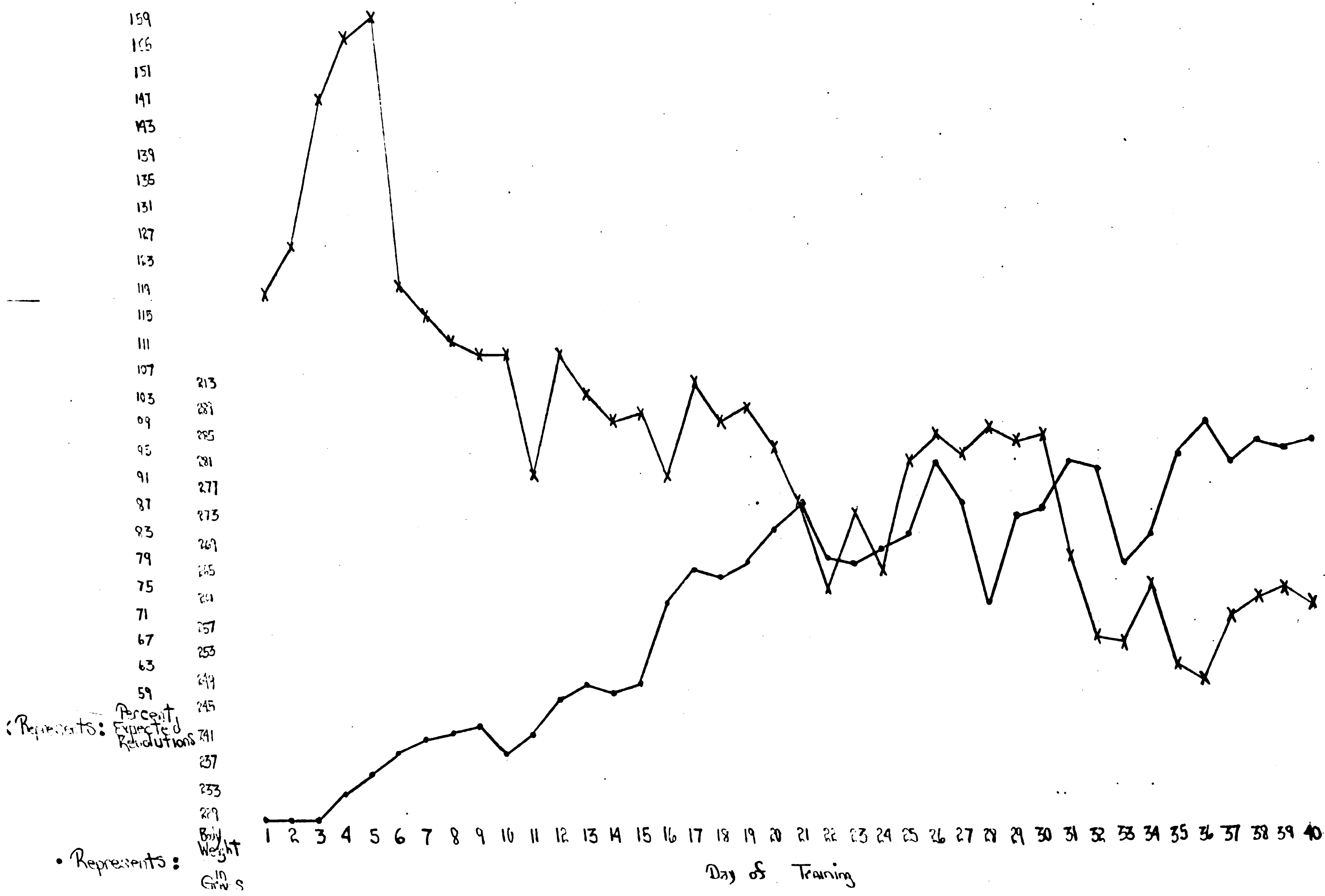

Graph 3 Short Endurance and Fat Fed
x Represents: Percent Essential Revolutions
• Represents: Body Weight grams
Day of Training
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Graph 4 Slot Endurance and Protein Fed

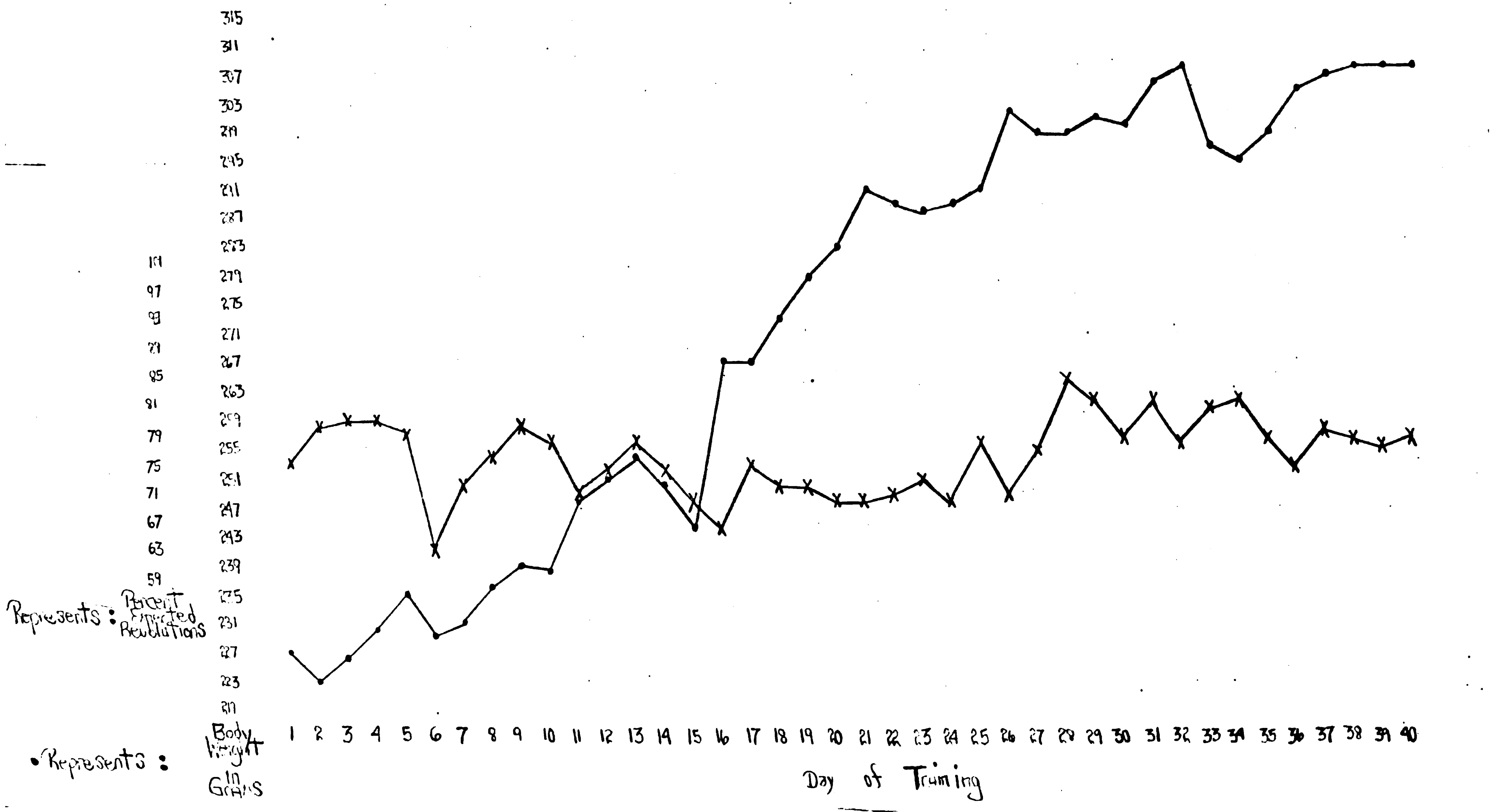
Graph 5 Long Endurance and Block Fed



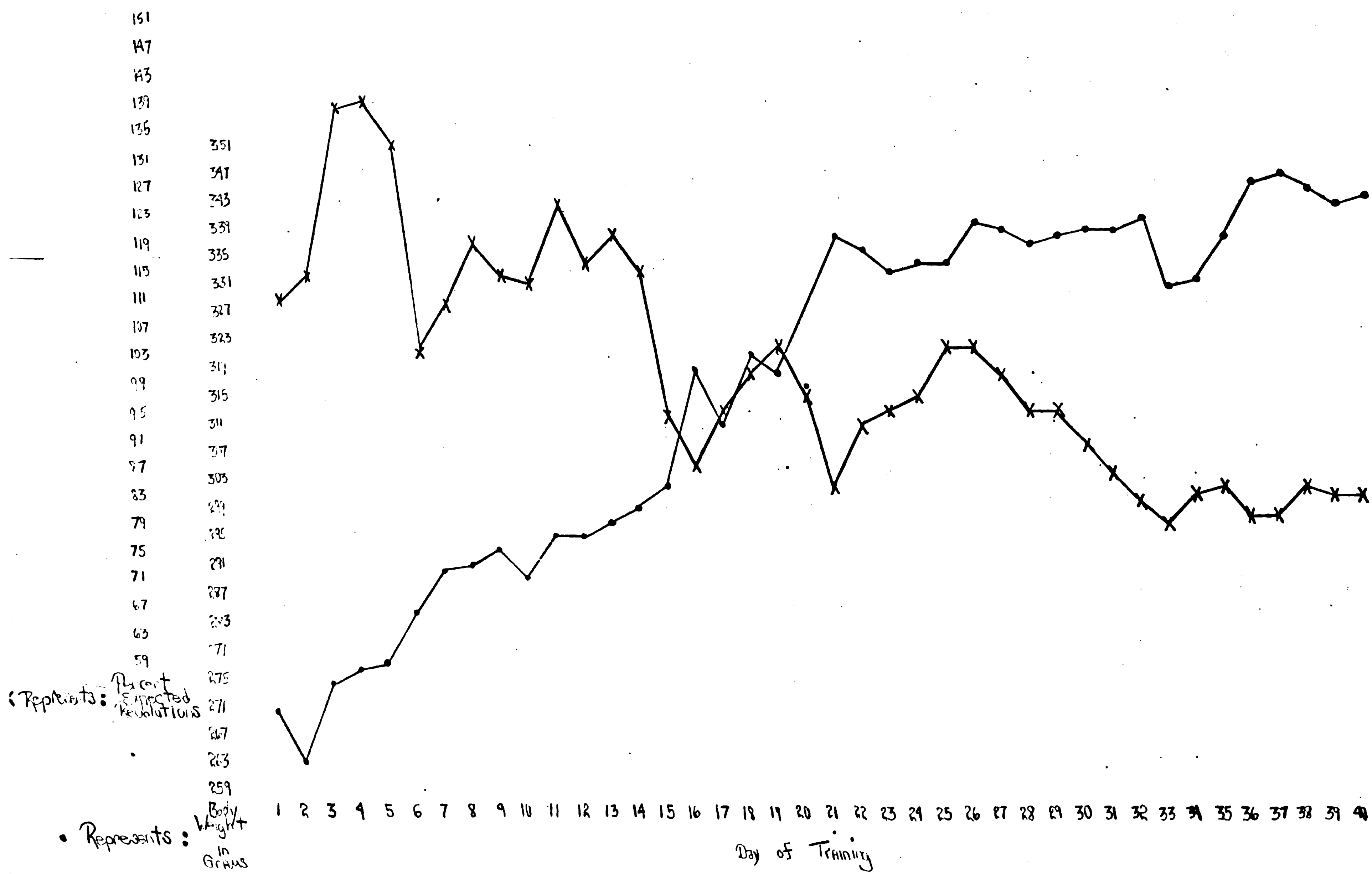
Graph 6 Long Endurance and Carbohydrate Fed



Graph 7. Long Endurance and Fat Fed



Graph 8 Long Endurance and Protein Fed



CHAPTER V

SUMMARY AND CONCLUSIONS

In lieu of the lack of experimental evidence concerning dietary alteration and its effect on physical performance this investigation was undertaken to determine the effects of high protein, high carbohydrate, and high fat diets on the performance of forced but controlled activity in the male albine rat.

Thirty-two weaned, (23-days-old) male, albino rats of the Sprague-Dawley Strain were used as experimental animals in this study. Following an adjustment period of six weeks, each animal was assigned to either the short duration or long duration activity treatment. The following conclusions have been derived from the analysis of the data and raw observations obtained from this experimental investigation.

1. The dietary alterations of fat, carbohydrate, and protein used in this study have a significant (0.0005 level) effect on the physical performance of the male albino rat.

2. This effect is observed in the two types of exercise used, short and long endurance, where marked

differences in PER scores can be seen. The protein, carbohydrate, and control animals in the long endurance program achieved higher PER scores than the protein, carbohydrate, control, and fat animals in the short endurance group. Also, the order of PER scores of each diet group varied in each training group. In the short group, the fat diet group achieved the highest PER score and the protein group the lowest; while in the long group, the protein diet group achieved the highest PER score and the fat diet group the lowest.

3. All animals except number 19 achieved a 75 per cent or better PER score, which was the criterion each animal had to meet to be considered a capable performer.

4. Graphs 1 through 8 indicate that PER scores and Body Weight varied from day to day over the 40-day training period. Except for Graph 7, long endurance and fat fed animals, PER scores and daily Body Weight scores followed very similar patterns. It may be concluded that the difference in daily PER scores may be a function of program difficulty, a reflection of the dietary influence on the physical performance, or a reflection of how well the animal was able to run. More than likely it is a combination of all three possibilities.

5. There is substantial evidence from the tables and graphs that body weight may influence PER scores; however, no definite statement can be made.

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