

EFFECT OF WEIGHT GAIN AND WEIGHT LOSS ON CERTAIN  
BODY AND BLOOD CONSTITUENTS OF RATS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan  
State College of Agriculture and Applied Science  
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C. L. Bedford

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The influence of diets of different composition on certain body and blood constituents and on the rate of weight gain and weight loss of rats was studied.

During the gaining period two diets were fed ad libitum to two groups of rats. One diet, containing corn as the main ingredient, was used in an attempt to produce obesity; the other diet, containing wheat and milk solids as the main ingredients, was used as the control diet.

The corn diet used failed to produce obesity in rats, if obesity is interpreted as excess body weight, but the rats on the corn diet had a significantly higher fat content than those on the control wheat diet. The protein, moisture, and ash content of mature rat carcasses were similar for animals on both diets.

The composition of the diet and age were found to affect the blood cholesterol values. Cholesterol concentration in the blood serum increased with the age of the rats. Blood potassium values were affected by the type of diet, while blood pyruvic acid concentrations were independent of age and diet. A relationship was found between body fat content and the values for blood cholesterol, blood pyruvic acid, and blood potassium.

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ABSTRACT

At the end of the gain-in-weight period the corn-fed rats were paired according to weight, and divided into two groups. One group was fed a diet which was high in carbohydrate, and the other group was fed a diet which was low in carbohydrate; the rats on both diets were restricted to 12 calories per day. No difference was observed between the effects of the high-carbohydrate and the low-carbohydrate restricted diets on the rate of weight loss of rats or on the body and blood constituents that were studied.

The blood cholesterol content of the rats on the restricted diets was lower and the blood potassium content higher than that obtained for the rats on a control wheat diet. The blood pyruvic acid values were similar for all diets.

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## INTRODUCTION

Obesity, which was once considered a desirable phenomenon, has become a nutritional and health problem. Dublin and Lotka (29) stated that "the penalty of overweight is one-fourth to three-fourths excess in mortality." Fisk (36) pointed out that 50 pounds overweight at age forty-five was as dangerous to life as valvular heart disease. In addition to the mortality risk, obese persons were found to be more susceptible to infectious diseases, arterial hypertension, diabetes, gallstones, and were poorer surgical risks than individuals of ideal weight (10).

Sebrell (93) reported that 25 per cent of the adults in the United States were overweight. The hazardous effect of obesity and its widespread distribution has attracted the attention of many workers who have attempted to discover why human beings become obese. At first it was believed that obese persons had lower basal metabolic rates than nonobese individuals, but further studies demonstrated that this was not true (13, 102). As the functions of the endocrine glands became understood, it seemed to many leading authorities in the field that hypofunction of the pituitary, thyroid, or the gonads offered an adequate explanation of obesity. However, further studies (reviewed

by Newburgh, 77) have shown that this is not the case. Evidence from the literature reported by Newburgh (77) showed also that the theories of specific dynamic action of foods, luxusconsumption and hypoglycemia cannot explain the cause of obesity.

The theory that obesity is the result of an intake of energy that exceeds the output was substantiated by Newburgh and Johnston (75) and has been accepted almost universally; yet there are cases of obesity not readily explained by this theory (6, 20, 50). Differences have been reported in the metabolism of carbohydrate and fat of obese subjects from those of normal subjects (112). Lack of knowledge about the causes of this improper balance between intake and output in obese persons has handicapped the treatment of obesity. However, it seemed clear that the treatment must consist of either reducing the food intake or causing the body to burn more calories. Since restriction of caloric intake was found to be more practical than increasing the caloric output of the body, several low-caloric diets have been used for the treatment of obesity. The work of Hagedorn, Holten, and Johansen (43) postulated that obesity arises from an abnormally increased transformation of carbohydrates into fat, due to qualitative anomaly of metabolism. On this basis, Hanssen (44) used a high-fat and low-carbohydrate diet for reducing weight.

There is a conflict between investigators about the advantage of the use of such a diet over the regular low-fat restricted diet. In a study of the physiology of women during weight reduction conducted by the department of Foods and Nutrition at Michigan State College, Cederquist et al. (18) reported that a low-carbohydrate reducing diet, patterned in part after the diet published by Pennington (81), gave more satisfactory results than had been obtained previously with a low-fat reducing diet. Subjects lost more weight without suffering from hunger and there was no flabbiness or looseness of skin; these observations were in agreement with the work of Hanssen (44).

Since the work of both Hanssen (44) and Cederquist et al. (18) was carried out on human subjects who may have had different motives for weight reduction or who may have been under certain emotional stresses, it was felt that a controlled experiment was needed in which these variables could be eliminated. A study, therefore, was planned in which rats were used as the experimental animals. The rate of weight gain and weight loss and the composition of certain body and blood constituents of rats and high- or low-carbohydrate diets were studied.

Since the metabolism of cholesterol has been reported to be allied closely with lipid metabolism, and since pyruvic acid and

potassium have been shown to have roles in fat and carbohydrate metabolism, studies of the blood cholesterol, blood pyruvic acid, and blood potassium during the periods of weight gain and weight loss were made.

## REVIEW OF LITERATURE

It is well known that the chemical composition of an animal body, as well as its weight, is not fixed and constant, but changes throughout life from conception until death.

### Body Weight

The changes in body weight of different animals have been studied by many investigators. Donaldson (28), Ferry (34), and King (60) studied changes in body weights of rats during the growth period. These workers found that after the age of 120 days, the increase in body weight was relatively small. Their data showed that the increase in body weight did not cease entirely when the rat reached maturity, but that it usually continued as long as the animals were in healthy condition. They also reported that differences in body weight between males and females became distinct at the age of 60 days.

King (60) suggested that the weight increase in adult rats is not true growth, but chiefly the accumulation of adipose tissue, and concluded that the body weight of the rat at any given age depended to a considerable extent on the character of the food. This has



been pointed out also by Hatai (47) and Carlson and Hoelzel (17). Others (90, 23) disagree, and reported that as long as the diet is adequate, the increase in weight of the body tends to be of the same composition, despite the differences in the diet.

### Body Composition

During the active growing period, the percentage of body fat generally increases, while the percentage of body water decreases. It has been suggested (72) that if the fat-free composition is considered, a point is reached when the concentrations of water, protein, and ash become more or less stationary, and after which no further appreciable changes take place. Moulton (72) described this as the state of chemical maturity which is reached after 50 days in the rat.

Every mature animal body contains a certain amount of energy stored as fat, the amount of which varies from species to species. Certain individuals among some species were found to store more fat than others. This is believed to be due to an upset in the mechanism which controls the energy intake with the energy output (55). As this excess deposition of fat is desirable for meat animals, many workers have striven to develop such deposition of fat in animals. Murray (73) has reported 50 per cent body fat for sheep and pigs.

There have been no reports in the available literature of other mammals, with the exception of humans (101), that have contained such a high percentage of fat. Beef cattle, selectively bred for their ability to store fat, attained a maximum per cent of 37 (74). Spray (101) reported that the mature female rat at the age of 200 days contained 23.7 per cent fat; this per cent of body fat was higher than that for the male rat of similar age. She suggested this figure as the upper limit for the species since the percentage decreased in the older animals. Values of 13.4 and 12.8 per cent for male rats at 246 and 321 days of age, respectively (101), are similar to the figure 13.4 per cent reported by Chanutin (21) for adult animals. Hatai (47) found only 5.7 per cent fat in rats that were 294 days old. Donaldson (28) and Deuel et al. (24) also reported that female rats contained more fat per 100 grams body weight than male rats. They reported 18.1 per cent for males and 20.1 per cent for females at the age of 84 days.

Smith (96) obtained rats having 50 to 60 per cent fat by injury of the tuber cinereum of the hypothalamus. This method has been used by many investigators to obtain obesity in rats and other animals (87, 49). Obesity obtained by such a method is accompanied by endocrine disturbances. Therefore, this does not represent a

true picture of the obesity which results when energy intake exceeds energy output in the intact animal.

Spray (101) found that the percentage of water in the body depended to a large extent on the percentage of fat. There was a corresponding, though not equal, decrease in water when there was an increase in fat. She found that the average content of water was 56 per cent at the age of 120 days, with an increase to 60 per cent at the age of 370 days. Hatai (47) reported a percentage of 65.3 of water in the body of the mature rat. He concluded that the percentage of water and body weight and age were not related. Deuel et al. (24) reported that the average per cent of water in rats studied in his experiment was 58.2 for males and 56.8 for females.

Spray (101), Donaldson (28), Chanutin (21), and Deuel et al. (24) showed that changes in protein values were reciprocal to those for water. This was demonstrated most clearly in the rat by Spray when the data for water and protein were calculated on a fat-free basis. Spray (101) reported that rat carcasses contained 16.8 per cent protein, a value which was similar to that reported by Hatai (47). Chanutin (21) reported values that were slightly lower, but the curve was a similar one and a constant percentage was reached at an age of approximately 100 days. Deuel et al. (24) found 17.3 per

cent protein in the males and 16.2 per cent for the females. All workers agreed that the per cent of protein remained relatively constant throughout the life of the mature rat.

The ash content of the body of rats studied by Chanutin (21) reached the maximum at the twentieth day of age, after which there was a gradual but marked decrease (21). Hatai (47) reported that the mature rat contained 3.7 per cent ash, which was similar to the results obtained by Chanutin (21). Deuel et al. (24) found that the females had a higher percentage of ash than the males: 3.26 and 2.95 respectively.

The effect of different diets on the weights and body composition of male and female rats has been studied by many investigators (47, 17, 90). Hutchinson (53) reported that the fat content of animals cannot be predicted from their live weights or rates of increase in live weights, because the fat content of the body may be influenced considerably by the ration that is fed. Hutchinson and Baker (54) made a detailed investigation of the effect of various diets in the proportions of protein, fat, and dry matter in the bodies of domestic rabbits. They showed that although the growth curves were similar whether the animals were reared on weeds or on potatoes and hay, the bodies of the potato-fed group contained much more fat than those

of the other group. Wynn and Haldi (113) reported that a diet containing 50 per cent fat induced a decrease in the water and protein content, and a definite increase in the amount of fat in the skin and body of the albino rat; these changes occurred within seven days. Scheer et al. (90), Deuel et al. (24), and Bachmann et al. (4) reported that the fat content of the body of the rat was not closely related to the fat content of the diet.

#### Factors Affecting Weight Loss

The loss of excess weight can be accomplished only by the burning of the excess fat deposits. The only universally accepted method to burn this excess fat and thereby reduce the weight of the obese is to restrict the caloric intake. Evans and Strang (31) reported that the burning of excess fat is in direct proportion to the difference between the energy intake and the energy output. These authors used diets restricted to 600 calories per day but stressed that in limiting the diet there should be an adequate intake of protein, minerals, and vitamins, enough carbohydrate to avoid acidosis, and no fat other than that which could not be separated from the protein ration. Failure of some individuals to lose weight on such a severe regime has been reported in the literature (75). No

satisfactory explanation for such cases has been given. Hagedorn, Holten, and Johansen (43) suggested that obesity arises from abnormally increased transformation of carbohydrate into fat, due to a qualitative anomaly of metabolism. This idea was used as a basis for the treatment of obesity; the percentage of carbohydrate in the diet was reduced to prevent a surplus of carbohydrates being deposited as fat. This treatment receives added justification from the fact that many persons with obesity become diabetic. The low-carbohydrate diets have been subjected to both clinical and experimental investigations. Benedict and Milner (8) compared diets of equal caloric value and demonstrated that diets high in fat caused a more immediate loss in the weight of the subject than diets that were high in carbohydrates. Lyon and Dunlop (65) reported that on short-term experiments, the loss of weight on diets containing 1,000 calories appeared to be inversely proportional to the carbohydrate content of the food. Hanssen (44) showed that his patients lost more weight on a high-fat diet than those on a high-carbohydrate diet. Anderson (2) studied the influence of the composition of submaintenance diets on the loss of weight of obese patients. He concluded that high-fat diets apparently led to a more rapid loss of weight than high-carbohydrate diets. From comparison of analytical figures, however,

he discovered that in addition to the difference in the amounts of carbohydrates and fats, the diets also differed in salt content. The chloride content of the high-carbohydrate diet was found to be 3.99 gram per cent, while that of the high-fat diet was 1.33 gram per cent. He made a comparison of high-fat and high-carbohydrate diets of equal salt content. Results indicated that the excessive water and salt retention on the high-carbohydrate diet was due to the high content of salt rather than the high content of carbohydrate. On isocaloric diets of equally low salt content, whether high in fat or high in carbohydrate, Anderson (2) found that weight losses were identical.

Scheer, Codie, and Deuel (89) found that when the intake of female rats was restricted to 12 calories per day, the animals lost 30 to 40 per cent of their weight in eight weeks. The greatest weight loss occurred in animals fed the diets containing 5 or 10 per cent fat and the lowest loss in rats that were fed 20 to 40 per cent fat.

The question of water balance arises whenever reduction of weight is attempted. Newburgh and Johnston (75) and Newburgh and Lashmet (76) have reported the tendency of obese patients on a low caloric diet to retain water which prevented loss of weight. Bartels and Blum (7) found that a disturbance in water balance occurred during attempts at weight reduction by low caloric diets. Scheer

and co-workers (89) reported that caloric restriction resulted in relatively high water content in the body. Evans and Strang (31) felt that this water retention was due to the spontaneous alteration in the capacity of fat to store water. Newburgh (77) reported that in the living organism, protein is associated with three times its weight of water, and fat with one-tenth its weight. When fat is burned in the body, water is released; and thus the per cent of body water is increased. Evans and Strang (31) studied the nitrogen balance, the oxygen consumption, and the carbon dioxide output of obese patients. They reported that fat was the only component that was burned in the body when the diet was adequate in protein and minerals but restricted in calories. The work of Scheer and co-workers (89) on rats substantiated this observation. They reported that a caloric restriction resulted in a relatively high water content with a slight increase in ash and protein and a great reduction in fat.

#### Blood Constituents

Cholesterol. Cholesterol, one of the animal sterols, is an essential constituent of all cells and fluids of the body (10). Cholesterol contains an alcohol group and exists in the blood in the free state and as esters of fatty acids. Among many other physiological



functions, cholesterol appears to act as a fat-mobilizing agent (109, 22, 41, 10, 84). Some investigators believe that the cholesterol concentration in blood is fairly constant (41, 78, 109, 15, 61), and any change would be a result of a metabolic disturbance in the body. Atherosclerosis, one of the diseases that was found to increase the concentration of blood cholesterol, was found to be common in obese individuals. This suggested that there might be a relationship between cholesterol metabolism and obesity. Other investigators (16, 92, 99, 109, 41, 67) reported varying blood cholesterol concentrations for normal subjects. This variation may be accounted for by differences in the experimental conditions, pathology of the subjects, and to a greater extent by the limitations of the methods used for analysis. Variations in cholesterol values were obtained also for rats. Monnier and co-workers (71) reported a blood cholesterol average of 80 milligrams per 100 milliliters, 70 per cent of which was esterized. Treadwell and Eckstein (107) reported an average of 68 milligrams per 100 milliliters.

Many factors--among them diet, obesity, sex, age, and state of nutrition--have been reported to influence the concentration of blood cholesterol. Rony and Levy (88) found that the blood lipid values of humans were affected by the ingestion of food-fat, and

concluded that some individuals had greater tolerance for fat than others. Bloor (12) reported that a high-fat diet produced an increase in the blood cholesterol in the dog and in the rabbit. The difference was not appreciable in dogs, but was marked in the rabbit. Luden (64) found that diets affected the blood cholesterol in humans. Nhavi and Patwardhan (78) reported that cholesterol values of humans appeared to be independent of the fat intake. Gough (41) found no correlation between the cholesterol intake and its concentration in the blood. Turner and co-workers (109), Bruger and Somach (16), and Schube (92) reported that the blood cholesterol values tended to be constant regardless of the food intake. Steiner and Domanski (104) reported that when cholesterol was mixed with lecithin in the diet, a notable rise in the concentration of blood cholesterol occurred, while the ingestion of cholesterol alone or mixed with fat had no effect. Keys et al. (58) reported that although the cholesterol intake had no effect on the concentration of blood cholesterol, the blood cholesterol was lowered if the diet was free from cholesterol. In rats, Treadwell and Eckstein (107) concluded that diet had no effect on the blood cholesterol.

There are conflicting reports concerning the effect of obesity on blood cholesterol values. Elliott and Nuzum (30) found that

cholesterol values frequently were higher among underweight than among obese persons. Denis (26) reported that plasma cholesterol was elevated in cases of obesity. On the other hand, several studies have been reported in the literature in which the blood cholesterols of obese subjects were found to be normal (52, 15, 82). Blotner (11) found a difference in the blood cholesterol levels between normal and obese individuals when a test meal, high in fat, was ingested.

Higher values of blood cholesterol have been reported for females than males, and lower values for children than adults (61). However, Rafsky and Newman (86), Peters and Man (82), and Keys and his co-workers (58) reported that there was no significant difference between the values for men and those for women. Keys et al. (58) reported higher values for cholesterol in persons over thirty years of age than in younger persons. The increase was found to be approximately 2.2 milligrams of total cholesterol per 100 milliliters of serum per year of age over thirty. Kirk et al. (59), on the other hand, found no significant difference in blood cholesterol concentrations between his subjects who ranged from 21 years of age to 91. In rats, Treadwell and Eckstein (107) found no differences between sexes. Monnier and co-workers (71) reported that the blood cholesterol of mature rats was similar to that of young rats.

Man and Gildea (66) studied malnourished patients and found that the cholesterol content of blood varied with the state of nutrition. The bloods of emaciated patients had lower cholesterol values than those of normal persons; improvement of the nutritional condition resulted in a rise in the cholesterol concentration. This also was observed by Bose and his co-workers (14), Gardner and Landen (38), and Shope (95), who reported low cholesterol values in starved persons. Poindexter and Bruger (83) found that the cholesterol content of the plasma was not altered during periods of weight reduction on restricted diets. However, they observed that in some patients, low caloric diets caused a definite increase in plasma cholesterol for several weeks. Walker and his co-worker (111) observed the same increase in plasma cholesterol in obese individuals who underwent rapid weight reduction by rigid caloric restriction. Poindexter et al. (83) explained this increase in the plasma cholesterol as being the effect of starvation. Amen (1) reported a decrease in the blood cholesterol concentration during weight reduction which was followed by an increase. This was explained as the result of adjustment to the restricted caloric intake.

Potassium. Potassium is quantitatively the most prominent cation of intracellular water. A number of observations, reviewed

by Fenn (33), have indicated the importance of potassium in muscle physiology. A relationship of potassium to carbohydrate metabolism has been indicated by several investigators. Pulver and Verzar (85) reported that the uptake of glucose by the yeast cell is accompanied by a simultaneous uptake of potassium. Other investigators have shown that the anaerobic fermentation of glucose by animal tissues was markedly greater in a medium containing sodium and potassium than in a medium containing sodium alone (33, 63). These results have shown that potassium is able to activate the enzymatic breakdown of glucose, but the question has been left open as to how this activation takes place. Pulver and Verzar (85) assumed that the inward diffusion of potassium was connected with the phosphorylation of glucose as the initial reaction leading to the formation of a polysaccharide. Hastings and Buchanan (46) found that optimum glycogenesis in liver slices required an artificial medium containing potassium, magnesium, and sodium. Fenn (32) observed that the ratio of potassium to glycogen in liver was constant, no matter what concentration of glycogen was produced in the liver. Gardner and co-workers (39) stated that potassium has a dual effect on carbohydrate metabolism. One effect involves the overproduction of adrenal cortical hormones; the second is the effect on tissue glycogenesis.

Factors that affect the potassium concentration in the blood are:

Age: Benetato and Cuirdarui (9) found that the blood serum of rats, eight to nine months old, contained an average of 31.5 milligrams of potassium per 100 milliliters. This seemed to increase with age and reached an average of 39.2 milligrams of potassium per 100 milliliters at the age of two years.

Diet: The dietary intake of potassium was found to have an effect on the amount of potassium in the blood plasma and other tissues (70, 57). A high potassium ration caused an increase of the potassium concentration in rats and dogs. Gardner and co-workers (39) produced potassium-deficient rats by feeding them a diet containing 0.07 millimols of potassium per 100 grams of diet. Dienst (27) reported that sodium displaced potassium in the organism and had a water-retaining action. Potassium drove out sodium and had a flushing action. Whether one or the other ion dominated in its action depended upon the proportion between the two. The absolute amount was unimportant. Keizo (57) reported that on a carbohydrate-poor diet, the potassium-calcium quotient in the blood was higher than on a carbohydrate-rich diet. The rise in the quotient might be caused by a movement of calcium into the tissues and of potassium

from the tissues to the blood. Verzar and Somogyi (110) showed that the intake of glucose, galactose, or fructose caused a sudden increase of blood potassium. The peak of the potassium values was reached only when the sugar concentration in the plasma had begun to decrease.

**Basal Metabolism:** Larson and Brewer (62) found that the metabolic rate affected the potassium content of the blood serum. They suggested that the lowering of serum potassium by anesthetics was a result of lowered metabolic rate.

**Fasting:** Mellinshoff (68) reported that the potassium content of the serum during fasting increased slightly at the beginning of the fasting, then fell and remained for months near the lower limit of normal values. Keizo (57) reported a similar rise of potassium blood values during fasting and suggested that this resulted from a movement of potassium from the tissues to the blood.

Pyruvic acid. The process by which sugar is converted into fatty acids is not well understood. Starling (103) suggested that since carbohydrate metabolism, both in animal and alcoholic fermentation, passes through stages of three carbon compounds, pyruvic acid or lactic acid, it is possible that one or the other of these compounds form the starting point from which fat is synthesized.

Aldehydes possess the property of condensing to form long chain compounds containing secondary alcoholic groups which may lose water with the production of double bonds. Various schemes, based on this property, to explain the formation of fatty acids from carbohydrates have been postulated. Starling (103) suggested that a molecule of acetaldehyde derived from pyruvic acid condenses with a second molecule of pyruvic acid with the production of an unsaturated keto acid. The loss of carbon dioxide yields the unsaturated crotonic aldehyde. The latter, by reduction, would yield butyric aldehyde, which on oxidation would yield butyric acid. Haarmann and Schroeder (42) studied the conversion of fat into sugar. They concluded that the probable intermediate steps are butyric to crotonic to beta hydroxybutyric to acetoacetic to dihydroxybutyric to dihydroxy-crotonic to diketobutyric to methylglyoxal to pyruvic or lactic acid to sugar.

Some of the factors that affect the pyruvic acid concentration in the blood have been studied by various workers. Friedemann and his co-workers (35) found that the ingestion of carbohydrates and proteins raised the concentration of pyruvic acid in the blood of humans. The ingestion of fat did not affect the pyruvic acid content of the blood when large quantities were ingested. Smith (97) also



reported that glucose ingestion caused an increase in the blood pyruvate concentration in humans. Hatz (48) found that the pyruvic acid concentration in the blood of rats was increased after the intravenous or subcutaneous administration of each of fourteen natural amino acids.

Several workers have studied the effect of vitamin deficiencies on the pyruvic acid content of the blood. Bargoni (5) reported that the absence of Vitamin A in the diet caused an increase in the pyruvic acid content of the blood of rats. Friedmann and his co-workers (35) found that thiamine deficiency increased the concentration of pyruvate in the blood of humans. Goldsmith (40) found that a thiamine, riboflavin, or niacin deficiency increased the concentration of pyruvate in the blood of humans.

The effect of various physiological and pathological disorders on the blood pyruvic acid has been studied. Bargoni (5) reported that tumors in rats caused the pyruvic acid content of the blood to increase. Monge (69) found that lactic and pyruvic acid concentrations in human blood significantly increased at high altitudes. This was found to be due to anoxia (35). It was reported by De Michele and Francis (25) that the increase in the blood pyruvic acid concentration of tuberculous patients was not related to the clinical

aspect of the disease, but to the degree of respiratory insufficiency. Palomba and Budroni (80) found that anesthesia increased the pyruvic acid concentration of the blood of humans and that the amount of increase depended upon the type of anesthesia used. They also observed an increase in the pyruvic acid concentration after various operations and that the amount and duration of the increase were proportional to the severity and duration of the operation. Stotz and Bessey (105) believed that this increase may have been caused by excitement and shock. Taylor and McHenry (106) studied the pyruvic acid metabolism of obese and normal subjects and reported that no difference was found. Stotz and Bessey (105) reported that different degrees of fasting caused the blood pyruvic acid concentration in pigeons to decrease.

The effect of age on the concentration of pyruvic acid has been studied by Wortis and his co-workers (114), who found no significant difference between the blood pyruvic acid values of subjects from seven to seventy years of age.

Exercise and excitement were reported to have an effect on the blood pyruvic acid concentration (105). Goldsmith (40) reported that the increase in the concentration of pyruvic acid in the blood of humans was found to be dependent upon the degree of physical activity.

There is little information in the available literature concerning the blood pyruvic acid of rats. Bargoni (5) reported that he found the concentration to be 13 micrograms per milliliter of blood in the normal rats.

## EXPERIMENTAL PROCEDURES

### Experimental Plan

Three-month-old white female rats were used as experimental animals. Upon arrival, one-third of the rats, selected at random, were assigned to Diet A, the composition of which is given in Table 1, and designated as Group I. The remaining two-thirds were assigned to Diet B (Table 1) and designated as Group II.

Period of weight gain. During a period of twenty weeks the two groups were fed the assigned diet and water ad libitum. Individual daily food intake records were obtained. Animals were weighed each week on two consecutive days and the average of the two weights was calculated. After a period of six weeks, two rats from each group were killed for analysis every week for four weeks. For ten weeks thereafter, two rats of each group were killed every two weeks for analysis.

Period of weight loss. During this period, Group I was kept on Diet A, fed and watered ad libitum. These rats served as control animals during the period of weight loss. Rats of Group II were

Table 1. Composition of diets.

Ingredients	Diet			
	A Wheat	B Corn	C Low- Carbo- hydrate	D High- Carbo- hydrate
	(gm)	(gm)	(gm)	(gm)
Whole wheat, ground	66	--	--	--
Whole milk solids	33	--	--	--
Sodium chloride	1	--	--	--
Whole yellow corn, ground	--	72	32	40
Casein	--	15	31	23
Yeast	--	4	4	4
Oil	--	5	29	8
Cod liver oil	--	1	1	1
Wesson salts	--	3	3	3
Sucrose	--	--	--	21
Lettuce	5, twice a week	--	--	--
Ground beef	5, twice a week	--	--	--

paired according to weight, and one of each pair was given Diet C (Table 1); this group of rats was designated as II-a. The other rat of each pair was given Diet D (Table 1); these animals were called Group II-b.

Each rat of Group II-a was given 2.5 grams of Diet C daily, and each rat of Group II-b was given three grams per day of Diet D. Each diet provided 12 calories per day for each rat. All animals were given water ad libitum. Two animals from each group were killed weekly for seven weeks. In the eighth week, five rats from each group were killed for analysis and the experiment was terminated. Examination of Table 18 (Appendix) shows the numbers and weights of the rats in each group.

An autopsy was performed on each animal that died or showed signs of illness during the experiment. Data on these animals were excluded from the experimental results. The number of rats that died from each group and the suspected cause of death is tabulated in Table 23 (Appendix).

Chemical analysis. Before each rat was killed, it was anesthetized and a blood sample was obtained from the heart for cholesterol, pyruvic acid, and potassium content analyses. The rat then

was killed by a blow on the head and determinations were made on the carcass for moisture, fat, ash, and protein content.

Experimental diets. The composition of the experimental diets--Diets A, B, C, and D--is given in Table 1. Diet A, the control diet, which is similar to the one described by Sherman (94), was made up of ground whole wheat, whole milk solids, and sodium chloride. The rats were fed five grams of ground beef and five grams of lettuce twice weekly. The diet furnished 4.2 grams of protein, 2.5 grams of fat, and 13 grams of carbohydrate per 100 calories. Diet B consisted of ground yellow corn, casein, yeast, oil, cod liver oil, and Wesson salts. This diet had been used in earlier experiments in the Department of Foods and Nutrition at Michigan State College, and it had been observed that rats raised on this diet showed large deposits of fat (56). Therefore, this diet was selected as a possible means of producing obese rats. This diet provided six grams of protein, 2.25 grams of fat, and 14 grams of carbohydrate per 100 calories.

Diets C and D were planned to provide ratios of protein, fat, and carbohydrate similar to the diets used in the Department of Foods and Nutrition at Michigan State College in studies with young women (19). Diet C contained 7.0 grams of protein, 6.25 grams of

fat and 4.0 grams of carbohydrate per 100 calories. Diet D contained 7.0 grams of protein, 2.6 grams of fat, and 14.7 grams of carbohydrate per 100 calories.

In preparation of the diets, the dry ingredients were weighed and mixed. The oil was added and the diet was mixed well by hand. Each diet was sifted three times to insure uniform distribution of the ingredients. The diets were prepared once a week. Diet C was refrigerated to prevent rancidity, as it contained a higher percentage of fat than the other diets.

### Experimental Methods

Drawing the blood sample. The animal was anesthetized with ether and about 5 milliliters of blood were withdrawn from the heart using a clean, dry syringe. The blood was transferred immediately to a test tube from which samples were pipetted for analyses.

Preparation of sample for body analysis. The animal was sacrificed after drawing the blood sample, and the intestinal tract was removed, opened, and washed. The body was cut into small pieces with strong shears, then ground three times with the intestinal tract in an electric grinder. If pieces of skin wrapped around the shaft, the grinder was pulled apart and the skin pieces were removed. The



ground body was weighed and an equal weight of distilled water was added to the ground carcass in a blender<sup>1</sup> and blended for 10 minutes. Samples were withdrawn for moisture and ash determinations. The remainder of the ground sample was dried in an evaporating dish as in the moisture determination, then ground and dried to constant weight.

Moisture determination. The official method of the A.O.A.C. (3) for the determination of water in meat was followed with slight modifications as follows: Five grams of the blended sample were weighed into a platinum dish with a cover. This was dried to constant weight at 95° to 100° Centigrade.

Ash determination. The official method of the A.O.A.C. (3) for the determination of ash in meat was used. Approximately 5 grams of the blended sample were heated at 100° Centigrade until the water was expelled. Then the dish was placed in a muffle furnace at approximately 525° Centigrade until the sample was white. The dish was cooled, distilled water added, and the sample dried on a hotplate to constant weight.

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<sup>1</sup> Waring Blendor.

Protein determination. A modified Kjeldahl (3) method was used for the determination of total nitrogen content of the body. One-half to 1 gram of the dried sample was weighed and a mixture of sodium sulfate and copper sulfate was added as a catalyst. Protein was expressed as gram nitrogen x 6.25.

Fat determination. The official method of the A.O.A.C. (3) was followed. Exactly 2 grams of the dried sample were transferred into a prepared Gooch crucible (having a thin layer of asbestos). The sample was covered with extracted cotton and dried for five hours at 105° Centigrade, then cooled and weighed. The sample was extracted for sixteen hours with ether, dried at 105° Centigrade for three hours, and weighed to determine the ether extract.

Blood potassium. The method developed by Overmand and Davis (79) and revised by Hunter (51) was used for the determination of potassium in whole blood. As soon as possible after the collection of the blood sample, 1 milliliter of blood was delivered into a 100 milliliter volumetric flask containing 10 milliliters of a solution of lithium chloride equivalent to 0.01 per cent lithium and approximately 30 milliliters of water. After hemolysis was completed, 4 milliliters of 20 per cent trichloroacetic acid was added. The solution

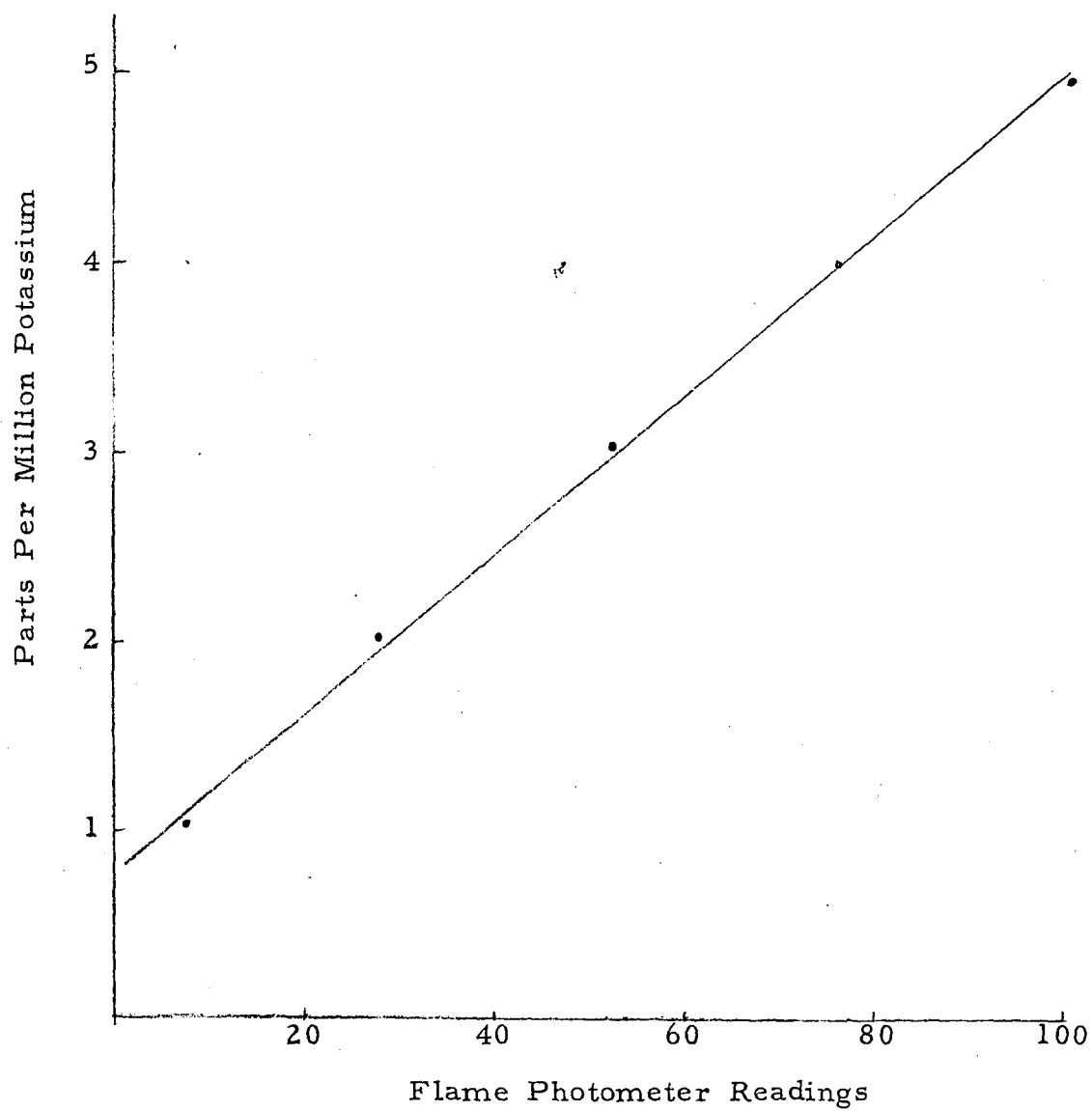
was allowed to stand for ten minutes before diluting to volume. The sample was centrifuged and the clear solution was analyzed for potassium, using a Perkin-Elmer flame photometer. Standard solutions, prepared to contain 0 to 5 milligrams per cent of potassium and 10 milligrams per cent (100 ppm) of lithium were analyzed with each determination. A typical standard curve is shown in Figure 1. Blood values of potassium were expressed as parts per million.

Blood cholesterol.<sup>1</sup> The concentration of total cholesterol in blood serum was determined by the method of Schoenheimer and Sperry (91), with modifications according to Sperry and Brand (100), Sobel and Mayer (98), and Foldes and Wilson (37). Three milliliters of alcohol-acetone (1:1) solution were added to 0.2 milliliter of serum in a 5 milliliter volumetric flask. The solution was brought to boiling and held at this temperature for thirty seconds. The solution was cooled and made to volume. The protein precipitate was separated from the extract by filtration through fat-free filter paper. An aliquot of 1 milliliter of extract was used for the determination of total cholesterol. The cholesterol was precipitated with a 50 per cent

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<sup>1</sup> Acknowledgment is made to Mrs. H. C. Amen and Miss M. Mills for the determination of blood cholesterol and blood pyruvic acid values.

Figure 1. Standard curve for potassium determination.



alcoholic digitonin solution and allowed to stand overnight. The precipitate was separated by centrifuging and then washed with an acetone-ether solution and with anhydrous ether. The ether was removed by evaporation.

The digitonin precipitate was dissolved in glacial acetic acid and a solution of acetic anhydride-sulfuric acid (20:1) was added. The solution was allowed to stand for twenty-seven minutes (no longer than thirty minutes) and the absorption of light was measured in a Beckman spectrophotometer at a wave length of 620 millimicrons. Determinations of serum cholesterol were made in duplicate whenever possible; however, in some instances insufficient blood was obtained to permit duplicate analysis. A series of solutions of 0.01 to 0.10 milligram of pure cholesterol per milliliter of acetic acid was prepared and treated according to the above procedure. The absorption of light was measured and the values were plotted on semi-logarithmic paper against the concentrations of cholesterol. The graph was used as a reference curve for the calculation of cholesterol in blood serum.

Blood pyruvic acid. The micro-method developed by Tsao and Brown (108) was used for the determination of pyruvic acid. After centrifugation of the precipitated blood sample, 0.1 milliliter

of the clear supernatant metaphosphoric acid solution was transferred to a small reaction tube, using a constriction pipette. To each tube, 33 milliliters of 2, 4 dinitrophenylhydrazine reagent were added and the contents were mixed by shaking. To this mixture was added 100 milliliters of xylene. The tubes were stoppered and shaken in a horizontal position for two minutes with a Kahn shaker. After shaking, the tubes were centrifuged at 2,500 revolutions per minute for five minutes. The bottom layer was removed completely by means of a fine capillary pipette and discarded. The hydrazone of pyruvic acid was then extracted from the xylene layer by adding 100 milliliters of 10 per cent sodium carbonate solution and shaking for three minutes. Subsequently, the tubes were centrifuged at 2,500 revolutions per minute for six minutes. With a constriction pipette, 75 milliliters of the lower carbonate layer was transferred to a microcuvette, 2 x 10 x 25 millimeters. Care was taken not to transfer the xylene to the microcuvette. Eighteen milliliters of approximately seven N sodium hydroxide were added and the contents of the cuvette mixed immediately by blowing air gently through the pipette. The optical density at a wave-length of 520 millimicrons was read in the Beckman spectrophotometer with the microattachment properly aligned. The apparatus was set to 100 per cent transmission by means of a

water blank. All readings were made between two and three minutes after the color developed.

Standard solutions containing 1.00, 3.00, and 5.00 milligrams per cent pyruvic acid were prepared from the 100 per cent stock standard solution. The standards and the reagent blank were run simultaneously and in triplicate in exactly the same manner as the blood samples, including the 1:5 dilution with metaphosphoric acid.

## DATA AND DISCUSSION

### Period of Weight Gain

Weight gain. During the period of weight gain of twenty-one weeks, the animals were fed and watered ad libitum. The weekly gain of rats on the corn diet varied from -0.8 to 12.8 grams, averaging 2.66 grams per week; and on the wheat diet, from -0.4 to 11.6 grams, averaging 2.83 grams per week (Table 2). The growth curves of both diets were found to be similar to that reported by King (60) (Figure 2), and the regression coefficients for the two diets did not differ significantly (Fisher t test) (Figure 3). On the corn diet, the total weight gained varied from 19 to 104 grams, averaging 55.9 grams; and on the wheat diet, from 10 to 89 grams, averaging 50.4 grams. There was no significant difference in weight gained between the two diets (Table 3). The final weights of the rats on the corn diet varied from 191 to 263 grams, averaging 220 grams; and on the wheat diet, varied from 170 to 250 grams, averaging 215 grams.

The rats fed the corn diet consumed an average of 253.4 calories per week, while those on wheat diet consumed an average of 261.1 calories per week. These intakes were less than those



Table 2. Average weight, gain in weight, food and caloric consumption of rats during the period of weight gain.

Weeks on Ex- periment	Diets	No. of Rats	Av. Wt. of Rat	Av. Gain in Wt.	Av. Daily Food Consumption	
					Amount	Calories
			(gm)	(gm)	(gm)	
1	Wheat	39	167.8	11.6	68.2	38.9
	Corn	87	176.8	12.8	78.7	38.9
2	Wheat	39	173.2	5.7	66.4	37.9
	Corn	87	182.0	5.2	68.2	36.7
3	Wheat	39	178.6	5.4	68.3	39.0
	Corn	87	186.7	4.7	64.0	34.4
4	Wheat	39	181.2	2.6	68.0	38.9
	Corn	87	191.2	4.5	68.3	36.7
5	Wheat	39	183.8	2.6	65.4	37.4
	Corn	87	194.6	3.4	70.5	37.9
6	Wheat	37	186.6	2.8	64.8	37.0
	Corn	85	198.5	3.9	67.6	36.3
7	Wheat	35	189.5	2.9	65.1	37.1
	Corn	83	200.2	1.7	66.4	35.7
8	Wheat	33	192.7	3.4	65.9	37.7
	Corn	80	203.5	3.3	69.3	37.3
9	Wheat	31	194.8	1.9	64.0	36.7
	Corn	78	205.8	2.3	66.5	36.7
10	Wheat	29	196.9	2.1	63.5	36.3
	Corn	76	209.1	3.3	65.0	34.9

Table 2 (Continued)

Weeks on Ex- periment	Diets	No. of Rats	Av. Wt. of Rat (gm)	Av. Gain in Wt. (gm)	Av. Daily Food Consumption	
					Amount (gm)	Calories
11	Wheat	27	200.4	3.5	65.1	37.1
	Corn	74	208.9	-0.2	59.5	32.0
12	Wheat	25	204.1	3.7	64.8	37.0
	Corn	72	210.0	2.0	66.5	35.6
13	Wheat	25	206.7	2.6	66.2	37.9
	Corn	72	211.9	1.0	66.6	35.7
14	Wheat	24	207.1	0.4	62.4	35.7
	Corn	70	213.0	1.1	68.2	36.6
15	Wheat	24	208.6	1.5	63.3	36.3
	Corn	70	214.9	1.9	74.2	39.9
16	Wheat	22	210.1	1.5	65.2	37.3
	Corn	68	214.9	0.0	63.5	34.1
17	Wheat	22	213.2	3.1	64.8	37.0
	Corn	68	219.3	4.4	78.5	42.1
18	Wheat	20	212.8	-0.4	63.8	36.5
	Corn	66	220.2	0.9	68.3	36.7
19	Wheat	20	211.9	-0.9	62.9	36.0
	Corn	66	222.0	1.8	74.4	40.0
20	Wheat	19	214.4	2.5	66.7	38.1
	Corn	64	220.7	-1.3	58.6	31.4
21	Wheat	19	215.3	0.9	65.2	37.3
	Corn	64	219.9	-0.8	59.3	31.9

Figure 2. Graph showing rate of growth of rats fed corn and wheat diets compared with rate of growth reported by King (60).

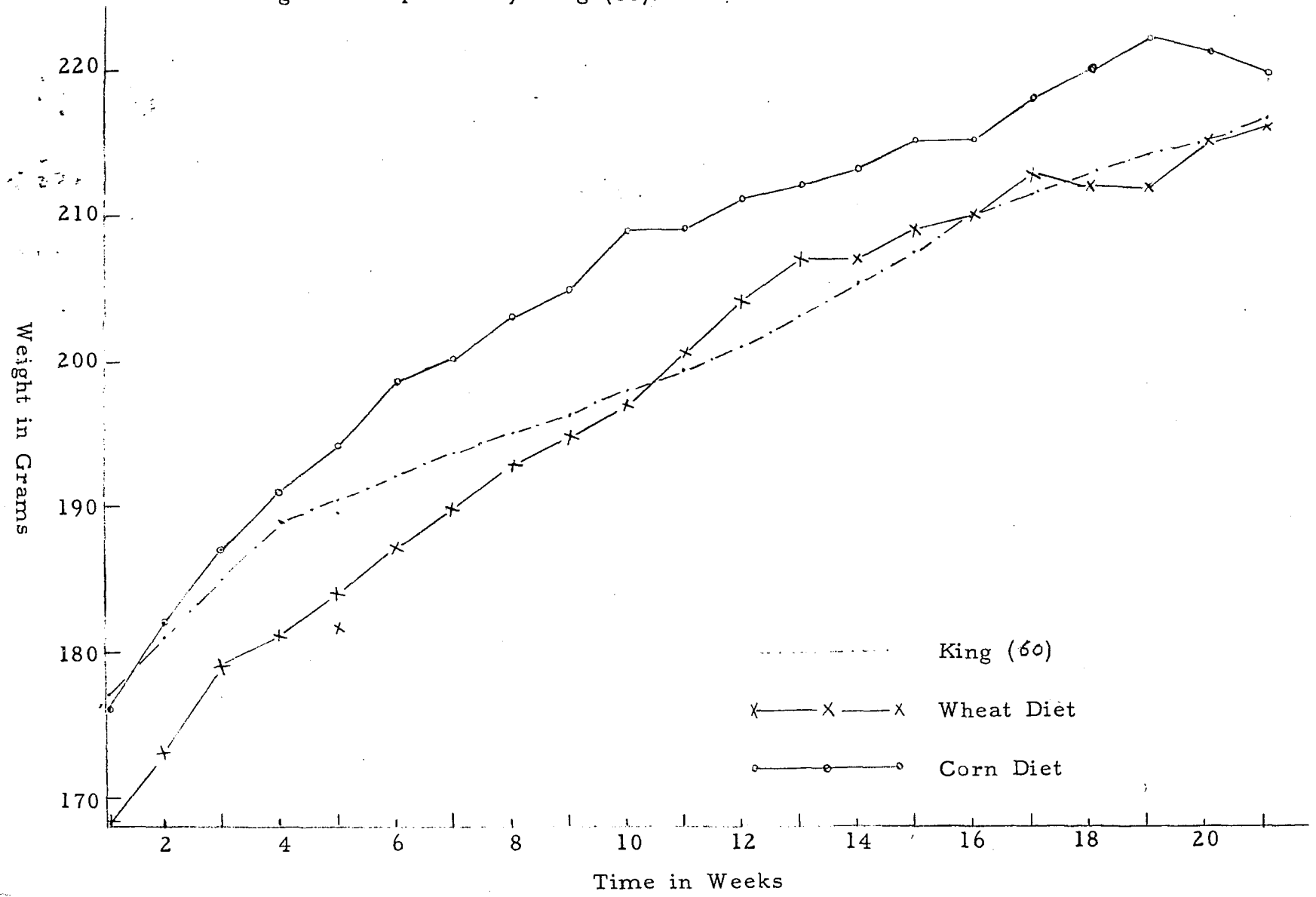


Figure 3. Regression of weight gain on time of diet during period of weight gain.

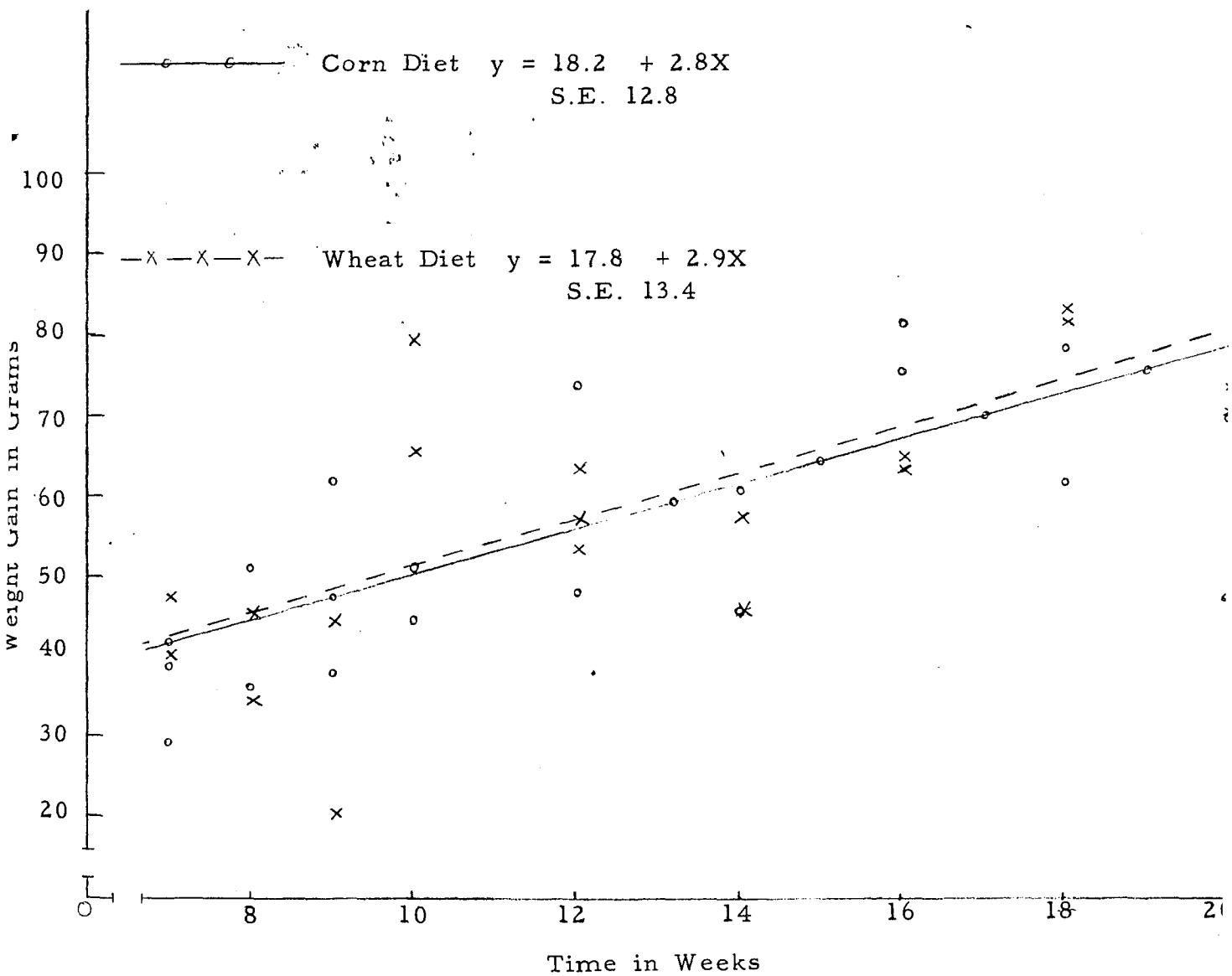


Table 3. Average weight gain of rats on corn and wheat diet during period of weight gain.

Gm Gain in Weight		Analysis of Variable			
Wheat Diet	Corn Diet	No. of Rats	Sample F. Value	Predicted F. Value	
				5%	1%
50.40	55.90	130	0.11	3.07	4.78

calculated from the predicting equation published by Harte, Travers, and Sarich (45). According to this equation, the corn-fed rats with an average weight of 206 grams would voluntarily consume an average of 345 calories per week (12.5 calories per square decimeter of body surface per day) to obtain a normal growth and the wheat-fed rats, with an average weight of 200 grams, would consume an average of 339.5 calories per week.

These data suggest that the corn and wheat diets used in this experiment supported normal growth without the development of obesity, which had been observed previously in animals fed the corn diet (56). However, some rats on both diets reached weights which were about 15 per cent greater than the group average and therefore might be considered to be obese if obesity is interpreted as excess body weight.

Body composition. The moisture content of the carcasses of rats fed the wheat diet averaged 62 per cent, and those fed the corn diet averaged 58.9 per cent (Table 4). This difference was found to be significant (Table 5). However, when the moisture content was calculated on a fat-free basis, the per cent of water in the rats fed the corn and wheat diets averaged 70.6 and 70.4, respectively, and were found not to differ significantly. The

Table 4. The average body composition of rats during the period of weight gain.

Body Constit- uents	7th Week		8th Week		9th Week		10th Week		12th	
	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	
	<u>Fresh Basis</u>									
Protein (%)	16.8	17.4	18.7	17.4	17.6	17.1	18.0	17.0	18.1	
Water (%)	64.2	59.3	60.9	60.0	61.6	58.7	62.3	60.4	61.7	
Fat (%)	13.2	17.0	14.7	16.8	13.5	16.5	12.2	15.5	12.2	
Ash (%)	4.2	3.6	3.6	3.5	3.9	3.4	3.9	3.9	3.9	
	<u>Fat-Free Basis</u>									
Protein (%)	18.3	20.9	21.5	20.9	21.1	20.4	21.4	20.7	20.7	
Water (%)	70.0	71.3	70.1	72.1	70.4	72.1	70.7	70.8	70.2	
Ash (%)	4.8	4.3	4.1	4.2	4.5	4.4	4.5	4.7	4.4	

Table 4 (Continued)

Week	14th Week		16th Week		18th Week		20th Week		Average for the Period	
Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet
<u>Fresh Basis</u>										
17.6	18.1	17.5	17.8	16.5	17.5	17.6	18.0	18.7	17.8± 2.7	17.4± 1.3
57.6	63.1	59.3	61.5	57.8	59.8	56.5	63.9	60.7	62.0± 3.24	58.9± 2.73
18.4	11.3	15.9	12.3	22.9	15.7	18.6	9.5	13.3	12.8± 1.7	17.2± 3.8
4.0	4.0	4.0	4.0	3.6	3.8	3.7	4.0	4.0	3.9± 0.43	3.7± 0.11
<u>Fat-Free Basis</u>										
20.8	21.4	21.0	20.3	20.1	20.6	21.7	20.8	21.5	20.7	20.9
70.2	71.0	69.5	70.2	70.2	70.4	69.2	70.6	70.0	70.4	70.6
4.9	4.5	4.4	4.6	4.5	4.5	4.6	4.5	4.6	4.5	4.5



Table 5. The average body composition of corn and wheat fed rats (period of weight gain) and analysis of variance.

Average Body Composition			Analysis of Variance			
			No. of Rats	Sampled F Value	Predicted F Value	
Wheat Diet	Corn Diet				5%	1%
<u>Fresh Basis</u>						
Protein (%)	17.80	17.40	36	3.10	4.13	7.44
Water (%)	62.00	58.90	36	10.00**	4.13	7.44
Ash (%)	3.90	3.70	36	2.90	4.13	7.44
Fat (%)	12.80	17.20	36	7.44**	4.13	7.44
<u>Fat-Free Basis</u>						
Protein (%)	20.66	20.93	36	0.64	4.13	7.44
Water (%)	70.39	70.60	36	0.25	4.13	7.44
Ash (%)	4.48	4.50	36	0.06	4.13	7.44

\* From Snedecor, G. W. Statistical Methods, Table 10.3.

\*\* Highly significant.

rats fed the corn diet showed a tendency to lose moisture, while those on the wheat diet showed a tendency to gain water as they grew older. There was, however, no correlation between time on the diet and moisture content (Table 6), and the slopes of the corn and wheat diet curves were not significantly different (Figure 4). Data obtained showed that there was a correlation between fat content and the moisture content of the body in wheat-fed rats, but not in the corn-fed rats. The amount of water decreased as the amount of fat increased (Figure 5).

The average protein content of the rats was 17.4 and 17.8 per cent, respectively, for the corn and wheat diets on the fresh weight basis, and 20.9 and 20.7 per cent, respectively, on the fat-free basis (Table 4). The differences were not significant (Table 5) and no correlation was observed between the time on the diet and the protein content of the rats for either diet (Table 6); the slopes of the corn and the wheat diet curves were not significantly different (Figure 6).

The rats on the corn diet had an average ash content of 3.7 per cent and those on the wheat diet, 3.9 per cent (Table 4). The ash contents were not significantly different (Table 5), and as was the case for the protein contents, no correlation was found between

Table 6. Correlation coefficient of body weight, body constituents, and time on diet of rats during the period of weight gain.

Variables	Diet	No. of Observations	Degrees of Freedom	Sample Value of r	Predicted r Value	
					5%	1%
Time on diet and gain in weight	Wheat	18	16	0.72**	0.47	0.59
	Corn	18	16	0.73**	0.47	0.59
Time on diet and body content of fat	Wheat	18	16	-0.34	0.47	0.59
	Corn	18	16	0.01	0.47	0.59
Body content of fat and body content of water	Wheat	18	16	-0.90**	0.47	0.59
	Corn	18	16	-0.03	0.47	0.59
Time on diet and protein content	Wheat	18	16	0.03	0.47	0.59
	Corn	18	16	0.56*	0.47	0.59
Time on diet and water content of body	Wheat	18	16	0.07	0.47	0.59
	Corn	18	16	0.06	0.47	0.59
Time on diet and ash content of body	Wheat	18	16	0.02	0.47	0.59
	Corn	18	16	0.35	0.47	0.59
Body weight and content of fat	Wheat	18	16	0.18	0.47	0.59
	Corn	18	16	0.64**	0.47	0.59

\* Significant.

\*\* Highly significant.

Figure 4. Regression of moisture content on time during the period of weight gain.

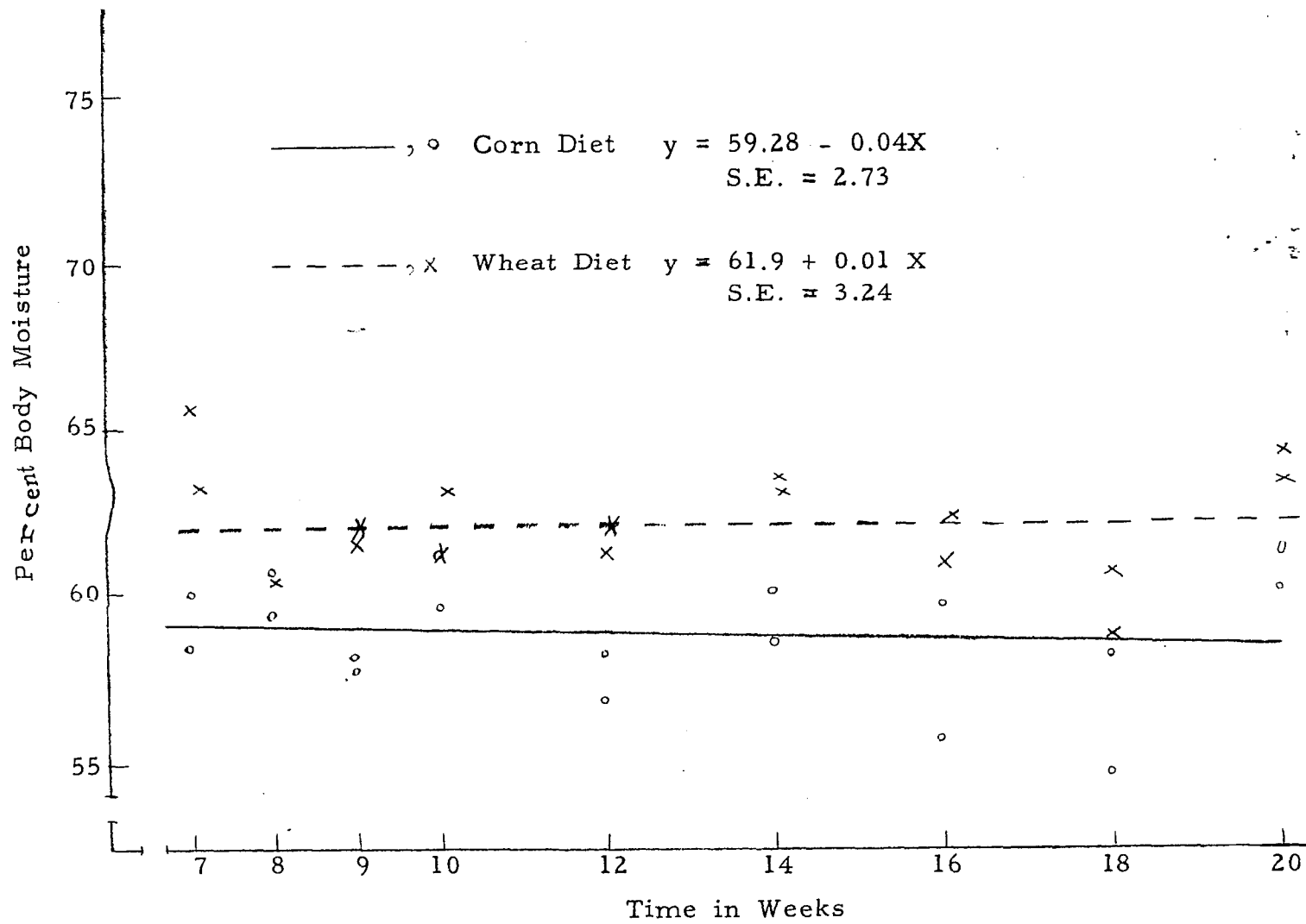


Figure 5. Regression of body moisture content on body fat during period of weight gain.

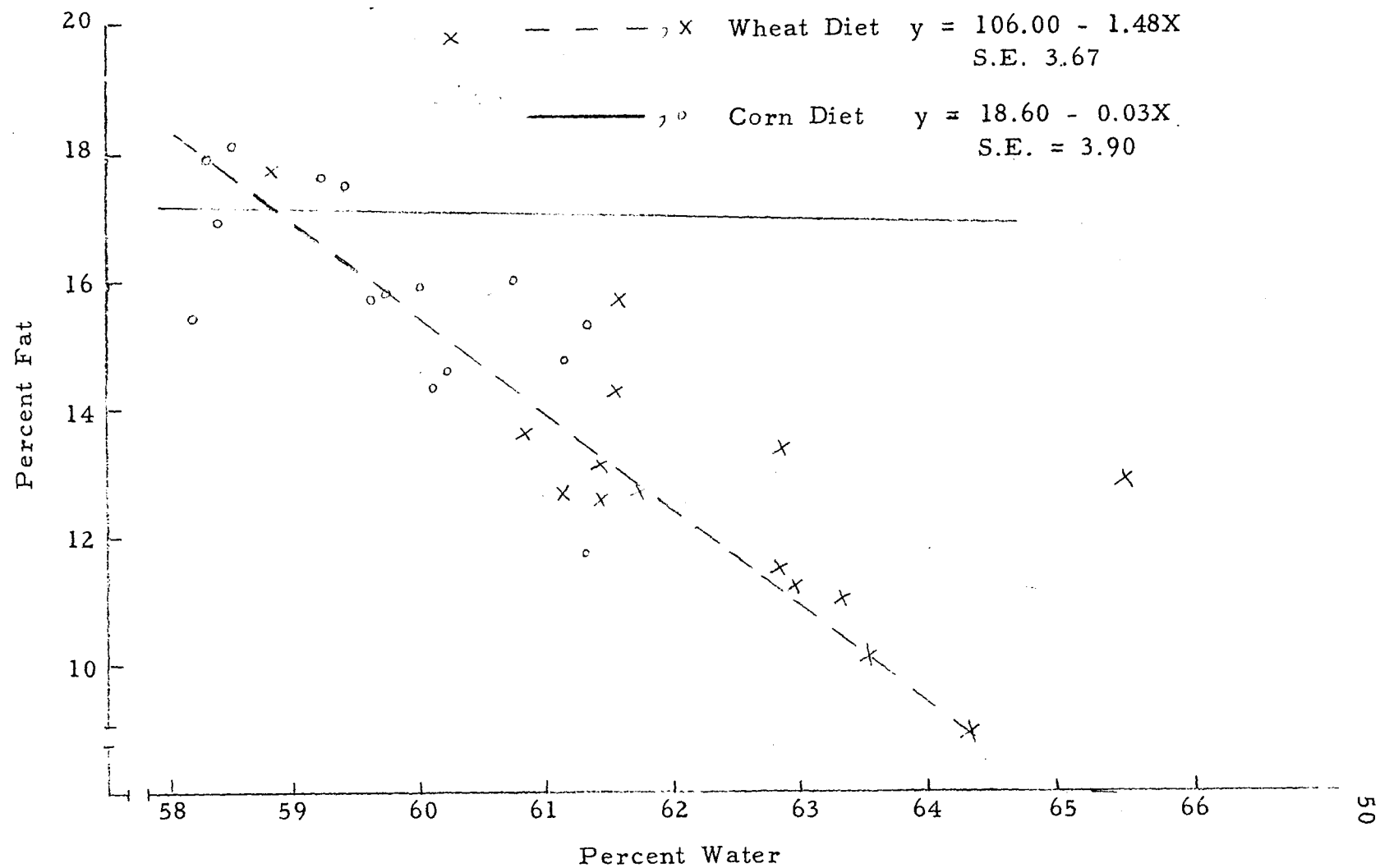
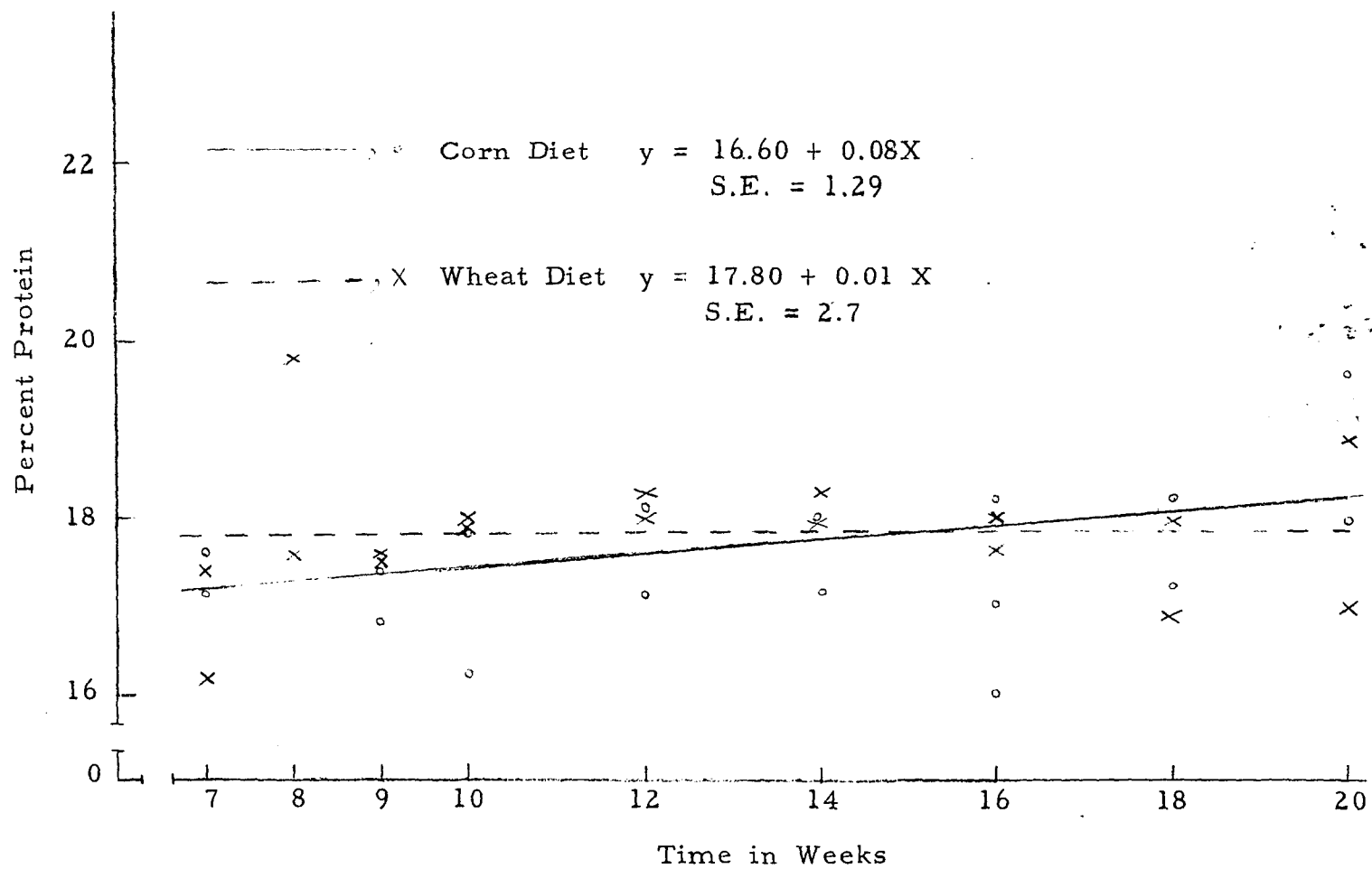


Figure 6. Regression of protein content on time during period of weight gain.

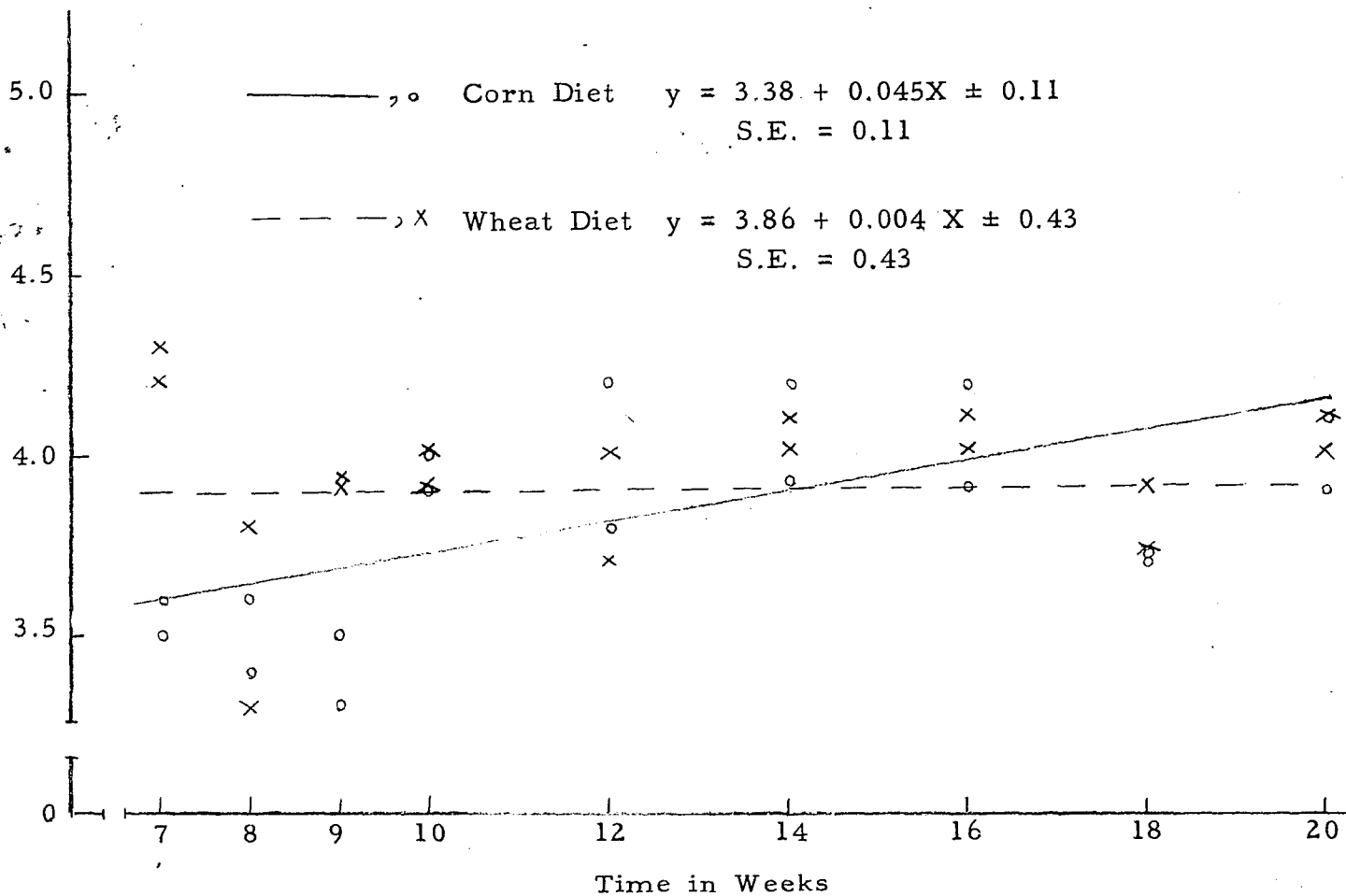


time on the diet and ash content (Table 6), and also no difference in the slopes of the two diet curves was found (Figure 7). On the fat-free basis, the average ash content was 4.5 per cent for both diets (Table 5).

Spray (101) found that the water, protein, and ash contents of mature rats were stable when calculated on a fat-free basis. The data obtained in this study are in good agreement with her results (Table 5).

Although no significant differences were found between the weights of the rats fed the corn and wheat diets (Table 3), the per cent of body fat differed significantly for the two groups (Table 5). The rats on the corn diet had an average of 17.2 per cent fat and those on the wheat diet, 12.8 per cent (Table 4). The larger deposition of fat occurred in the rats on the corn diet even though the corn diet had a lower fat content than the wheat diet (8.6 per cent as compared to 10.1 per cent) and the caloric intake on the corn diet was less. These results are not in agreement with those of Wynn and Haldi (113), who reported that the amount of fat in the diet had a definite effect on the amount of fat in the body but appeared to agree with those of Scheer et al. (90) and others (23) who stated that the fat content of the body was not related to the fat content of the

Figure 7. Regression of ash on time during period of weight gain.





diet. The content of body fat of the rats fed on the wheat diet tended to decrease as the rats grew older (Figure 8). This is in agreement with the results reported by Spray (101). No correlation was found between body weight and the content of body fat (Figure 9). The data for rats fed the corn diet showed no correlation between their age and body fat content (Table 6), but there was a correlation between the body weight and the fat content of the body (Figure 9, Table 6).

If obesity is defined as the per cent of fat in the body rather than as live weight, the corn-fed rats might be considered obese since the carcasses of the rats fed the corn diet contained significantly more fat than those rats fed the wheat diet, and also more than that reported by several investigators (21, 47, 60) as the average for rats exhibiting normal growth.

Blood constituents. The blood cholesterol of rats fed the control wheat diet varied from 40.8 to 83.3 milligrams per 100 milliliters with an average of 69.8 milligrams per 100 milliliters of serum, and was higher than the values for the rats fed the corn diet which varied from 34.5 to 90.0 milligrams with an average of 62.6 milligrams per 100 milliliters of serum (Table 7). This difference was found to be significant (Table 8). The amount of cholesterol in

Figure 8. Regression of body fat content on time during period of weight gain.

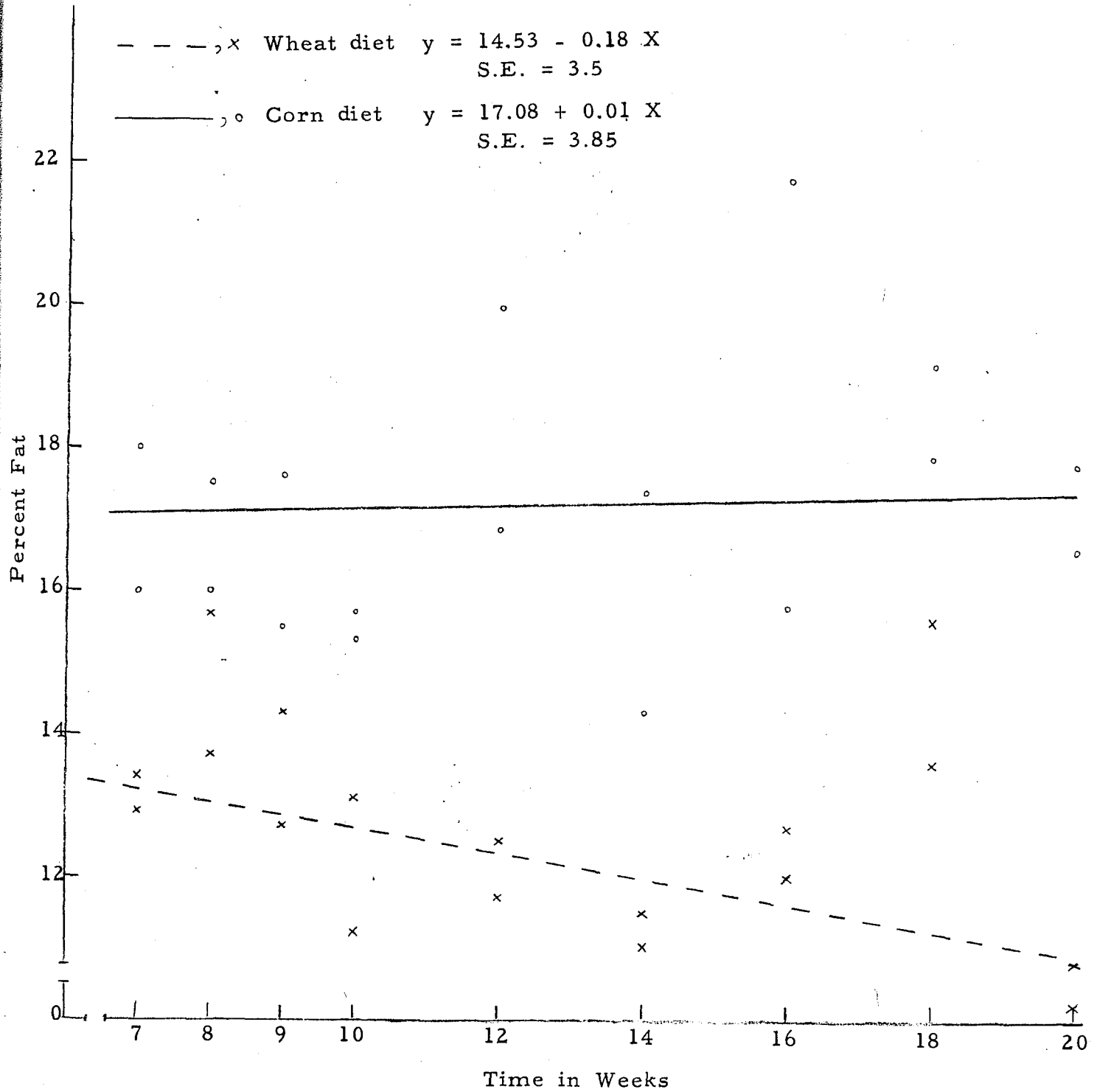


Figure 9. Regression of body weight on fat content during period of weight gain.

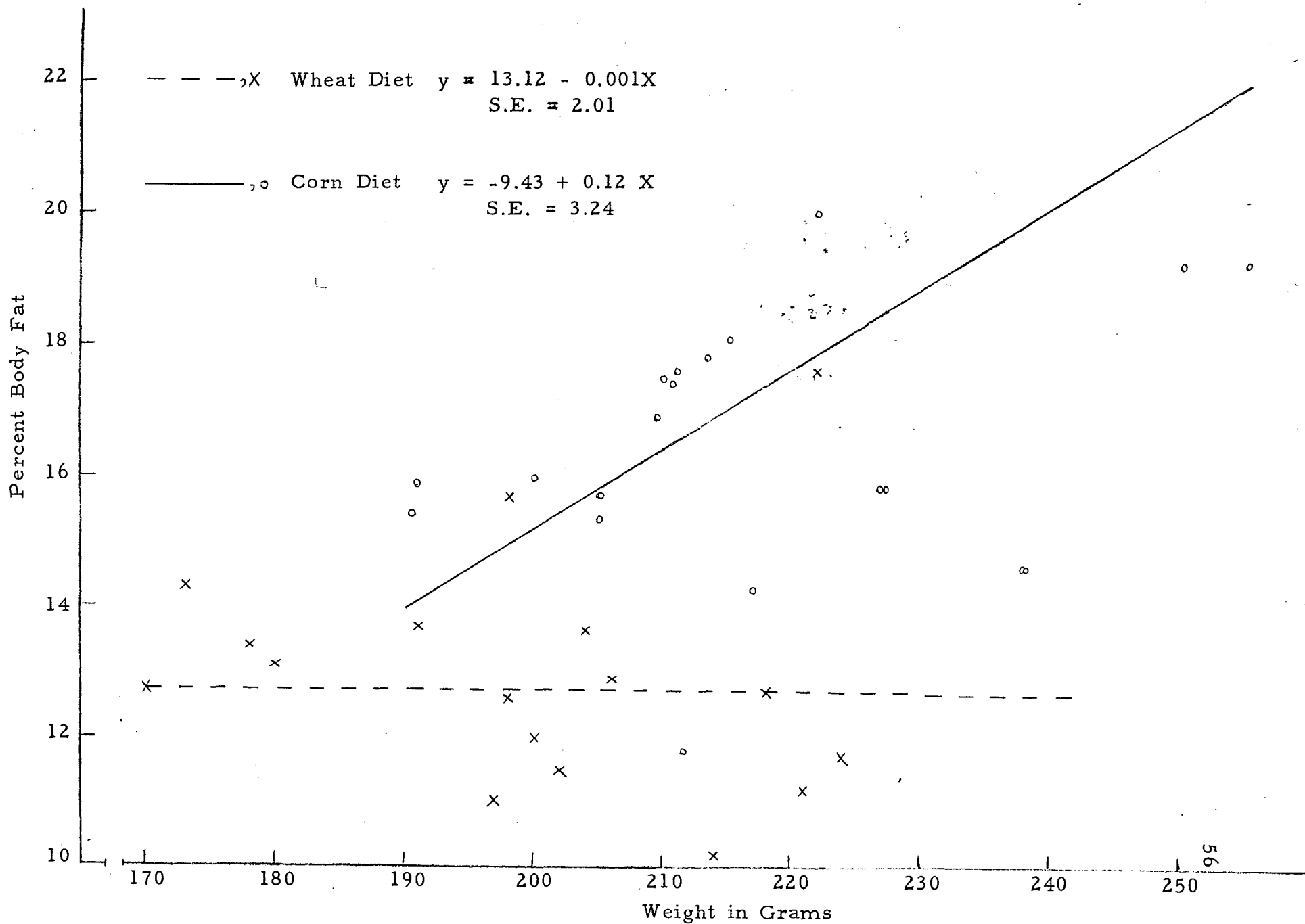


Table 7. Blood cholesterole, pyruvic acid, and potassium of rats during the period of weight gain

Blood	7th Week		8th Week		9th Week		10th Week		12th
	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet
Cholesterol (mg/100 ml)	58.41	60.30	83.29	67.51	40.78	34.50	78.12	46.51	76.30
Pyruvic Acid (mg/100 ml)	2.60	1.59	1.46	1.96	1.95	3.93	2.38	2.14	--
Potassium (ppm)	150	201	210	185	186	163	156	184	158

Table 7 (Continued)

Week	14th Week		16th Week		18th Week		20th Week		Average for the Period	
Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet	Wheat Diet	Corn Diet
77.12	78.43	90.00	76.50	72.34	67.81	77.72	82.68	57.67	69.80	62.60
--	2.37	2.00	2.71	2.83	2.47	2.46	2.06	1.55	2.22	2.30
186	202	311	183	198	183	185	160	184	174.6	189.9

Table 8. The average blood constituents of rats during the period of weight gain.

Blood Constituents	Average Blood Content		Analysis of Variance			
	Wheat Diet	Corn Diet	No. of Rats	Sampled F. Value	Predicted F Value	
					5%	1%
Cholesterol (mg/ 100 ml)	69.80	62.60	34	6.45*	4.13	7.44
Pyruvic acid (mg/ml)	2.22	2.30	30	1.00	4.20	7.64
Potassium (ppm)	174.60	189.90	34	4.70*	4.15	7.50

\* Significant.

the blood serum tended to increase with the time the rats were on the experiment during the weight-gain period (Figure 10). This may represent the normal increase of serum cholesterol with age, since the data obtained by Keys and co-workers (58) indicated that there was an average increase of 2.2 milligrams of total cholesterol per 100 milliliters of serum per year for humans more than thirty years of age.

For rats on the wheat diet, the blood cholesterol concentration decreased as the body fat content of rats increased, while for the rats on the corn diet no relation was found between the blood cholesterol concentration and body fat content (Figure 11). As the rats fed the corn diet were found to have a lower average blood cholesterol concentration than those of the rats fed the control wheat diet, and since the rats on the control wheat diet which had a higher content of body fat than the average were found also to have lower cholesterol values than the average, it might be concluded that the deposition of fat was associated with low cholesterol values. This is in agreement with the results of Elliott and Nuzum (30), who reported that obese persons have lower cholesterol values than underweight persons.

Figure 10. Regression of cholesterol on time during period of weight gain.

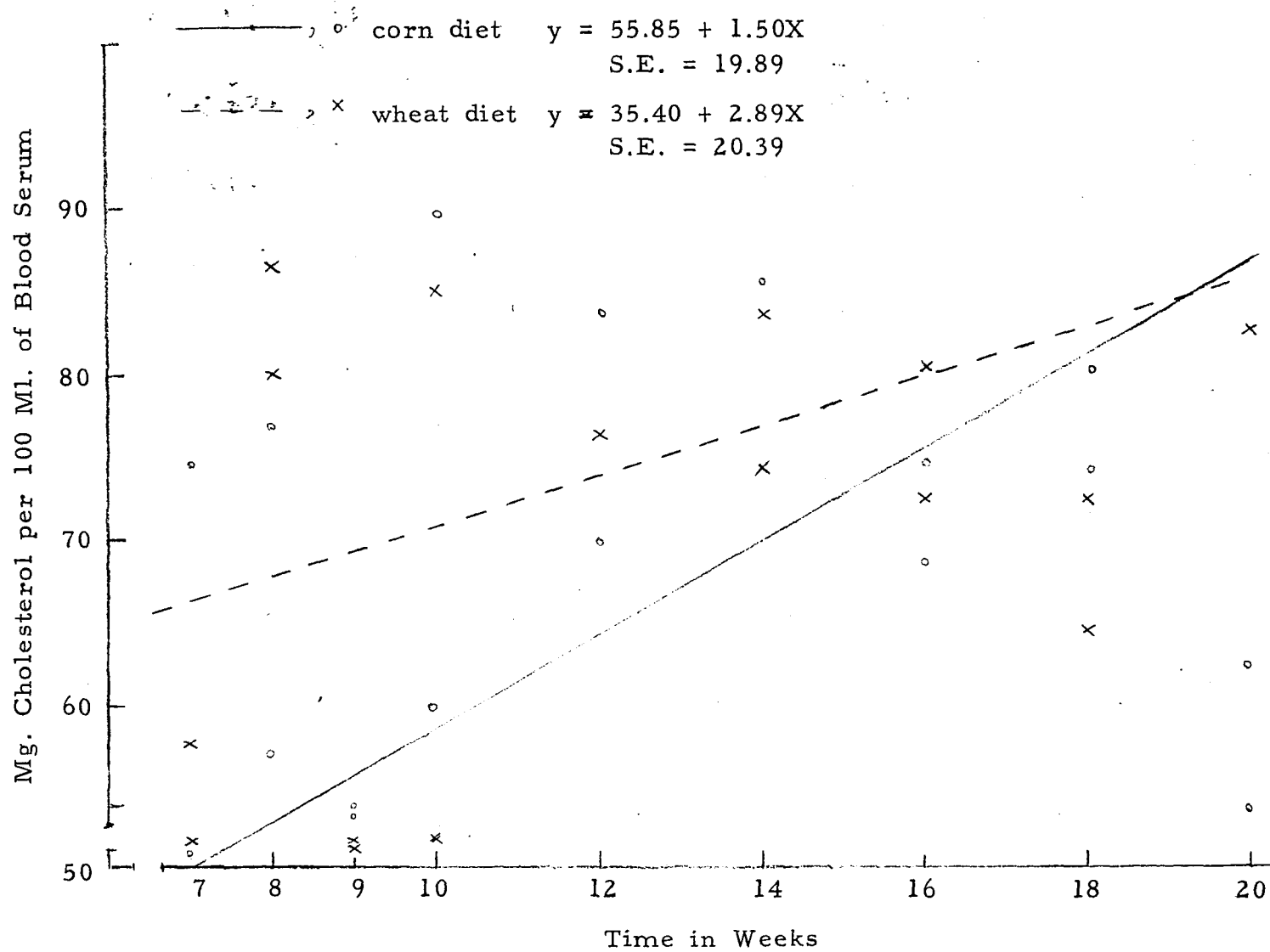
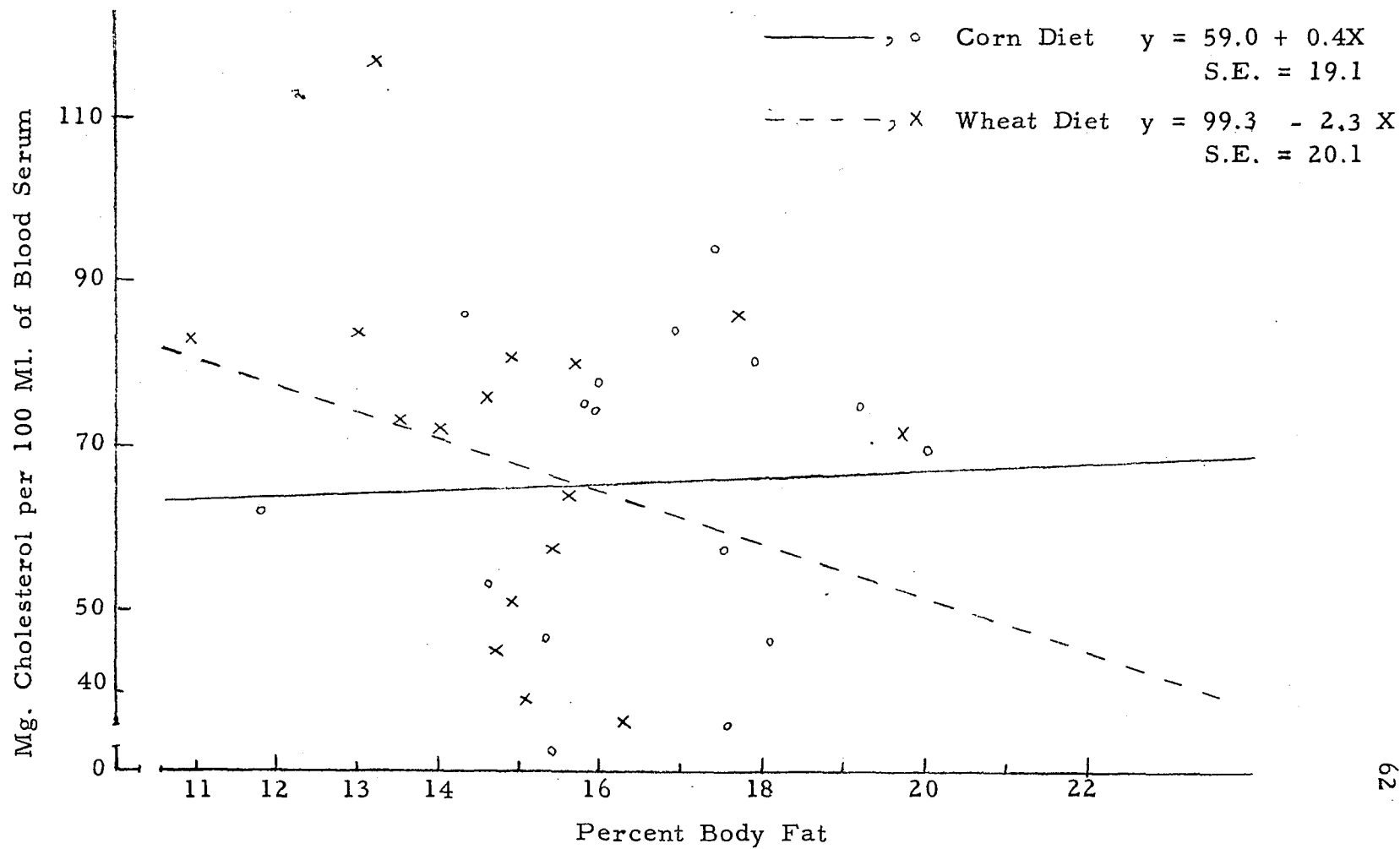




Figure 11. Regression of cholesterol on body fat.



The potassium content of the blood of the rats on the corn diet ranged from 163 to 211 parts per million, with an average of 189.9 parts per million; for rats on the wheat diet the blood potassium values ranged from 150 to 210 parts per million, with an average of 174.6 parts per million during the gaining period (Table 7). The difference in potassium content of the blood of the rats on the two diets was found to be significant (Table 8).

There was no correlation between the time of weight gain and potassium content of the blood (Table 9), but there was a tendency for the blood potassium of rats to decrease the longer the rats were on the weight-gaining diet (Figure 12). These results are not in agreement with those of Benetato and Curdain (9), who found that blood potassium of rats seemed to increase with age.

The blood potassium content of the rats tended to increase with an increase in body fat content on both wheat and corn diets (Figure 13). The rate of increase was most pronounced in the rats fed the wheat diet. The slower rate of increase in blood potassium on the corn diet may be explained by the fact that the corn-fed rats attained a higher blood potassium content near the start of the gaining period which seemed to be near the maximum.

Figure 12. Regression of potassium on time during period of weight gain.

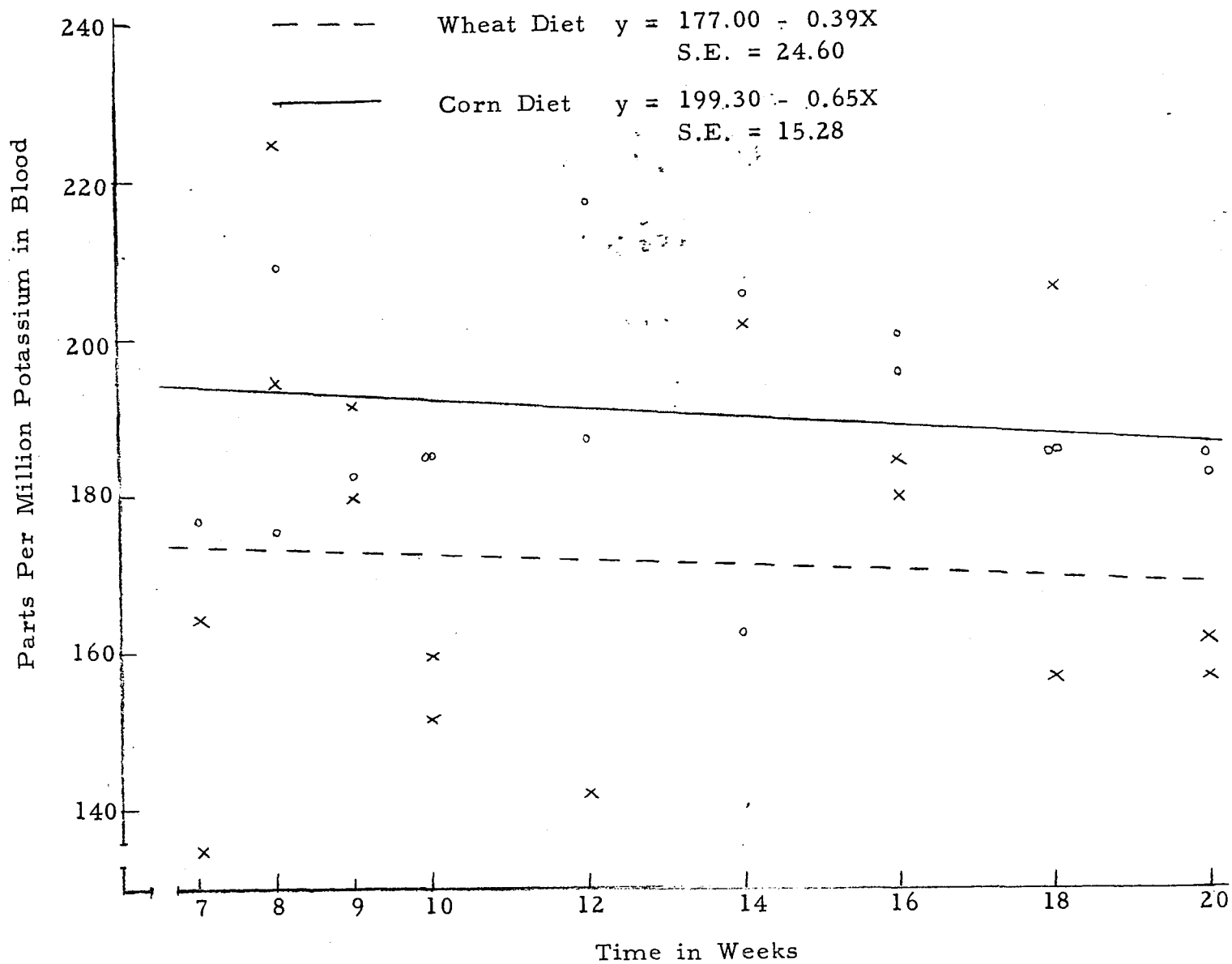
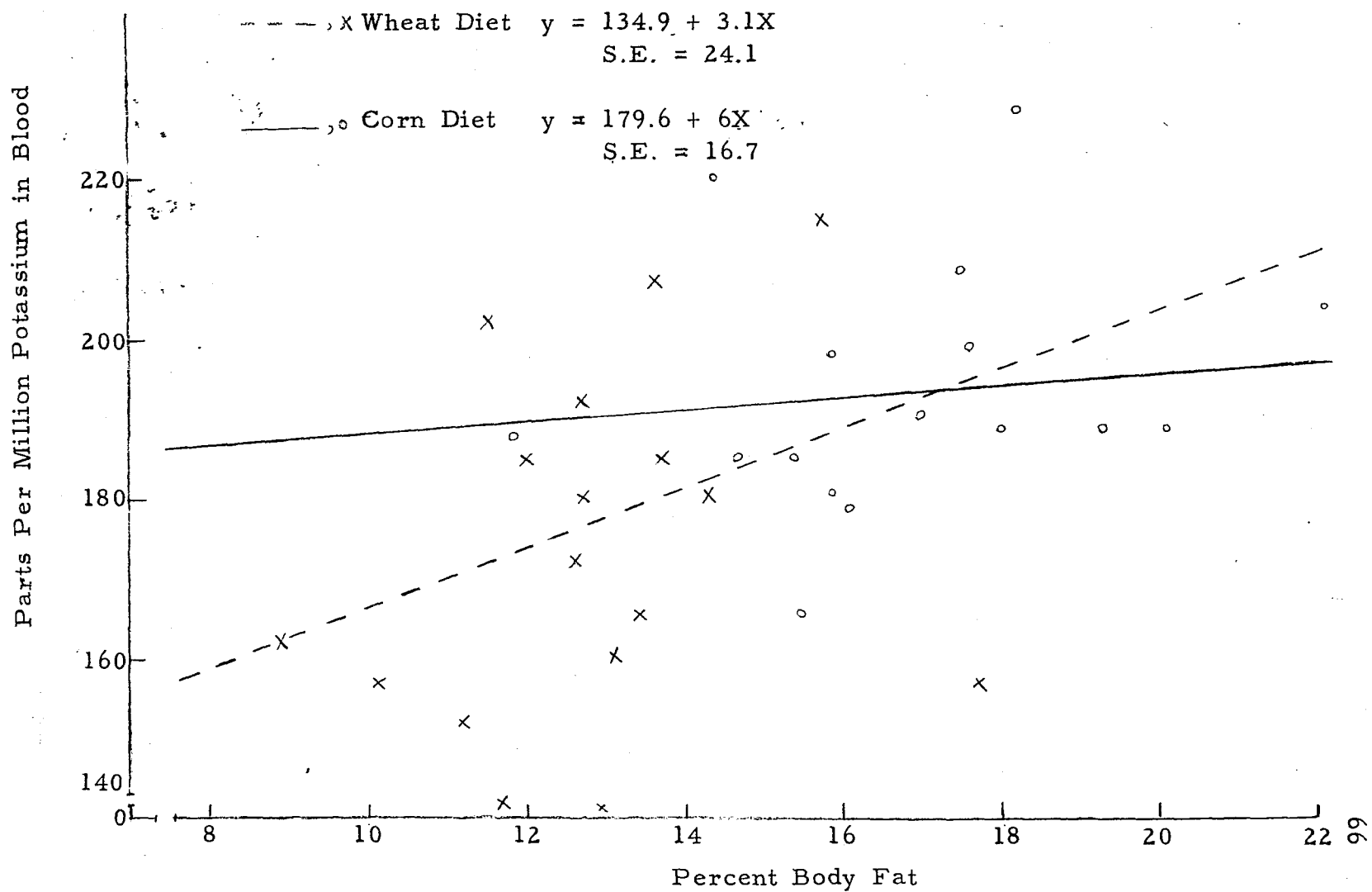


Figure 13. Regression of potassium on fat content during period of weight gain.



The pyruvic acid content of the blood varied from 1.46 to 2.71 milligrams per 100 milliliters of blood, averaging 2.2 milligrams during the period of weight gain on the corn and wheat diets (Table 7). No significant differences were found between the two diets (Table 8) and no difference between the slopes of the two curves was found (Figure 14). There was no correlation between the time on the diets and the pyruvic acid content of the blood (Table 9).

The blood pyruvic acid content increased with an increase in the body fat content (Figure 15). This was most pronounced in the blood of the rats fed the corn diet. Although the correlation coefficients between the increase in blood pyruvic acid and increase in body fat contents were not significant (Table 9), the results obtained with the rats fed the corn diet suggested that the deposition of fat might be associated with a change in carbohydrate metabolism, as pyruvic acid concentration increased in rats that deposited fat.

#### Period of Weight Loss

Control animals. During the period of weight loss the control rats which were fed the wheat diet ad libitum gained from -2.0 grams to 4.3 grams per rat per week, with an average weekly gain of 0.65 grams per rat (Table 10).

Figure 14. Regression of pyruvic acid on time during period of weight gain.

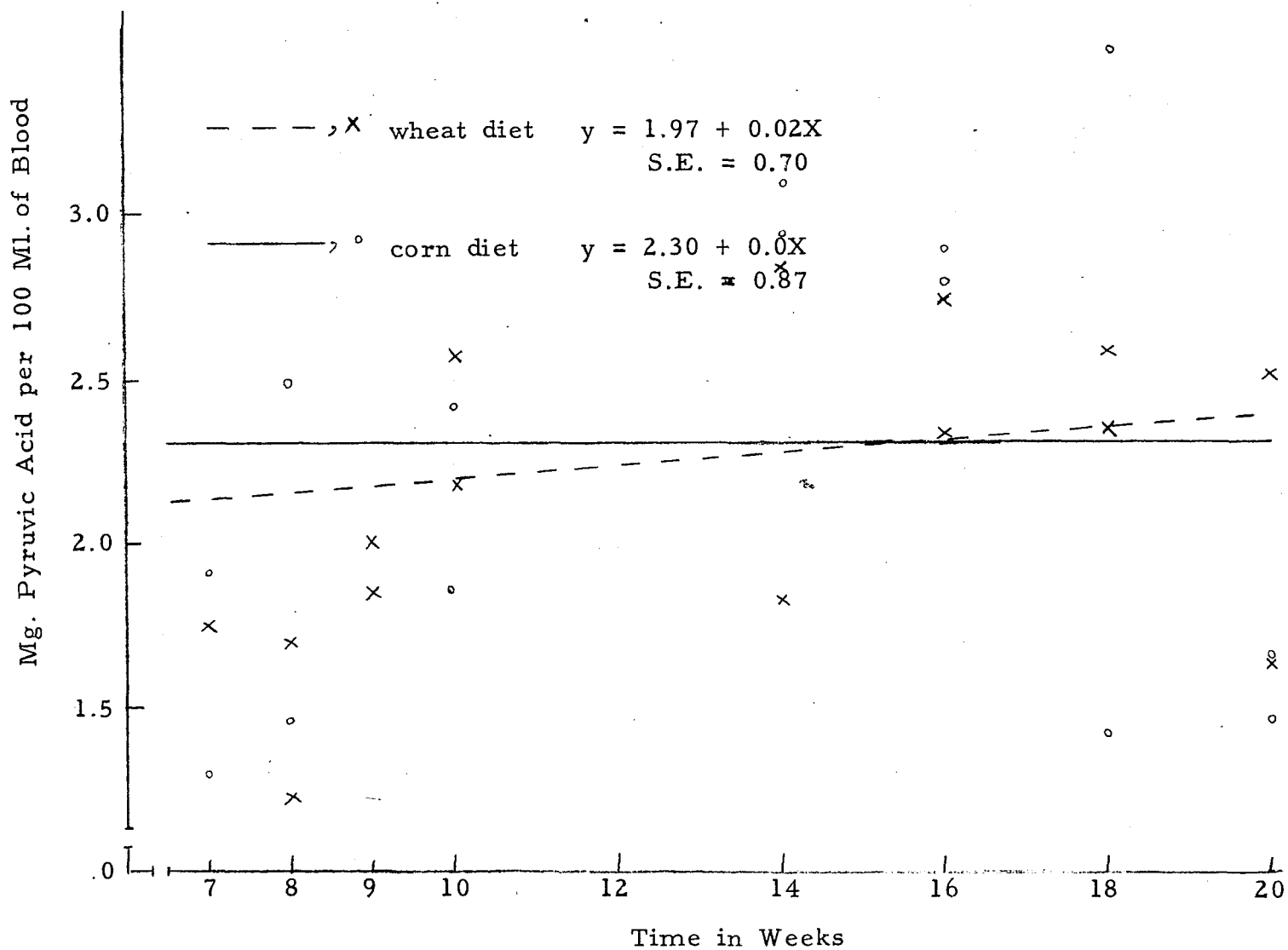


Figure 15. Regression of body fat on pyruvic acid during period of weight gain.

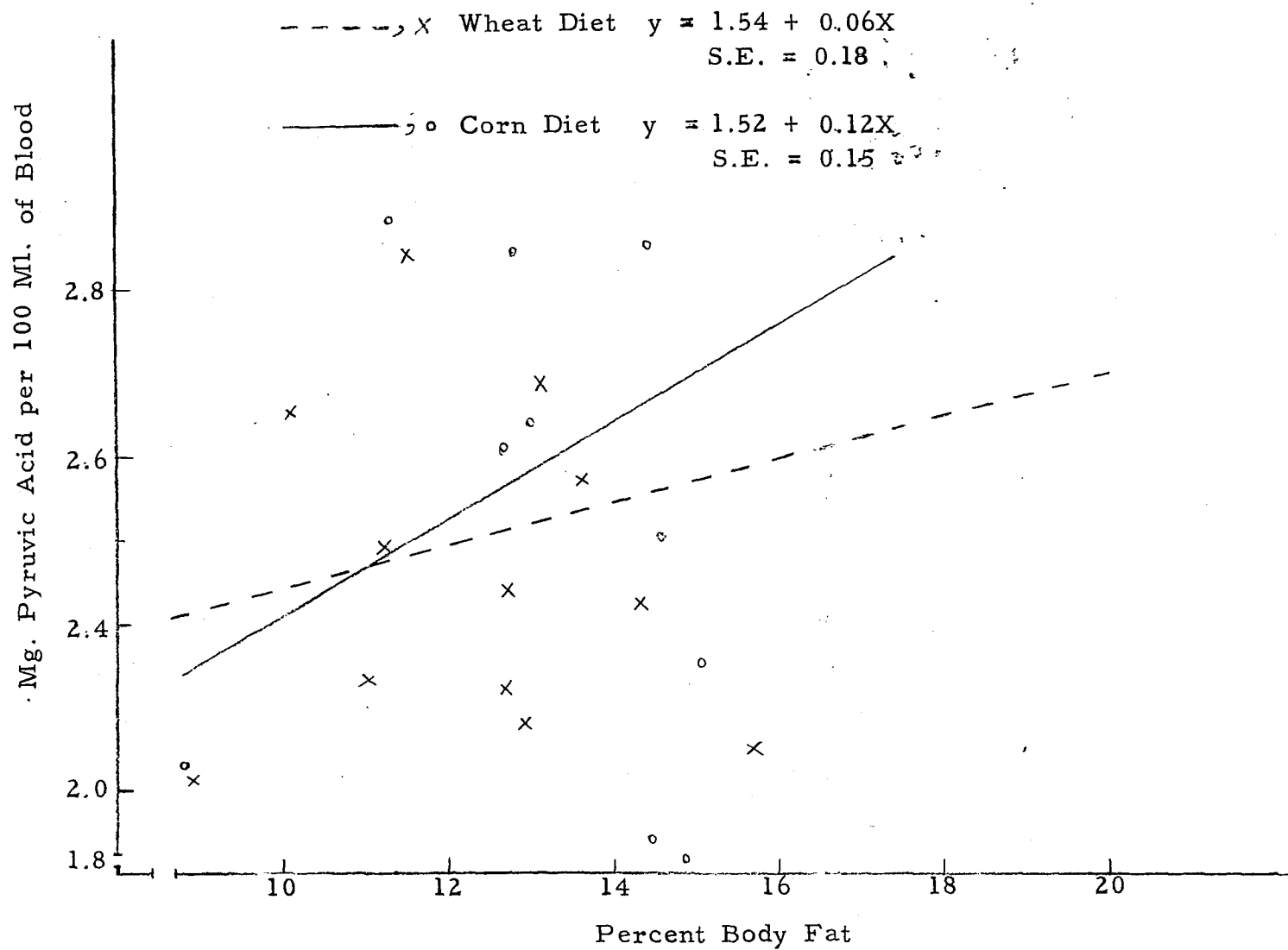


Table 10. Average changes in weight of rats during the period of weight loss.

Changes in Weight	Week		
	1	2	3
	(gm)	(gm)	(gm)
Low carbohydrate diet	-20.13	-7.00	-6.70
High carbohydrate diet	-20.73	-7.80	-5.00
Wheat diet	-2.00	+4.32	+1.00



Table 9. Correlation coefficient of blood constituents and time on diet of rats during the period of weight gain.

	Diet	No. of Rats	Degrees of Freedom	Sample Value of r	Predicted Value of r	
					5%	1%
Time on diet and blood cholesterol	Wheat	16	14	0.257	0.497	0.623
	Corn	16	14	0.519*	0.497	0.623
Cholesterol and body fat	Wheat	16	14	-0.265	0.497	0.623
	Corn	16	14	0.080	0.497	0.623
Time on diet and pyruvic acid in blood	Wheat	16	14	0.137	0.497	0.623
	Corn	16	14	0.007	0.497	0.623
Time on diet and potassium in blood	Wheat	16	14	-0.060	0.497	0.623
	Corn	16	14	-0.155	0.497	0.623
Body content of fat and potassium in blood	Wheat	16	14	0.248	0.497	0.623
	Corn	16	14	0.328	0.497	0.623
Body content of fat and blood pyruvic acid	Wheat	15	13	0.018	0.514	0.641
	Corn	15	13	0.321	0.514	0.641

\* Significant.

Table 10 (Continued)

Week					Average, Total Period
4	5	6	7	8	
(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
-9.61	-8.02	-9.87	-15.25	-10.61	-87.20
-5.40	-10.00	-11.30	-11.90	-10.18	-82.21
-0.53	+2.32	-0.61	+1.33	-0.62	+5.10

The body composition of the rats fed the control wheat diet remained relatively constant during the period of weight loss, especially when the values were calculated on a fat-free basis (Table 12). The average body content of fat, water, protein, and ash, calculated on a fresh weight basis was  $16.4 \pm 1.7$ ,  $59.3 \pm 3.2$ ,  $17.6 \pm 1.3$ , and  $3.8 \pm 0.4$  per cent, respectively (Table 13). The constancy of the various components which were analyzed during the period indicated that age per se of the animals had no apparent influence on the factors studied. Therefore, it might be expected that any changes observed while the animals were fed the restricted diets could be associated with the diet of the animals and was not an effect produced by aging.

Weight loss. There is no agreement among investigators on the relative effects of high-fat, low-carbohydrate diets and low-fat, high-carbohydrate diets on the rate of weight loss of individuals. In this study, rats that were restricted to an intake of 12 calories per day lost an average of 82.21 grams over a period of eight weeks when a low-carbohydrate diet (Diet C) was fed, and 87.19 grams when a high-carbohydrate diet (Diet D) was fed. These losses represent an average of 35.3 and 37.4 per cent, respectively, of the original weights of the rats (Table 10). The differences between the total losses were not significant (Table 11), and the regression coefficients

Table 11. Changes in weight of rats during period of weight loss.

Grams Change in Weight			Analysis of Variance				
			Predicted Value of F		L.S.D.		
Wheat Diet	Low Carbo-hydrate	High Carbo-hydrate			5%	1%	
			5%	1%			
+5.10	-82.21	-87.19	68.30**	3.47 5.78	12.17	16.35	

Table 12. The average body composition of rats during the period of weight loss.

Body Con- stit- uent	Week											
	1			2			3			4		
	Diet			Diet			Diet			Diet		
	A	C	D	A	C	D	A	C	D	A	C	D
	<u>Fresh Weight</u>											
Fat (%)	17.8	18.3	16.0	15.3	12.2	17.0	17.5	10.6	6.9	17.5	12.0	9.5
Water (%)	58.2	58.8	58.3	60.5	60.7	55.3	56.5	62.5	65.4	58.8	60.7	61.3
Protein (%)	17.5	16.6	18.7	17.4	18.7	17.4	18.8	19.5	20.0	17.3	20.6	21.1
Ash (%)	3.4	3.8	4.2	3.8	4.2	4.1	4.1	4.5	5.0	3.9	4.1	4.2
	<u>Fat-Free Weight</u>											
Water (%)	70.9	72.1	69.6	71.4	69.1	66.6	68.5	70.0	70.3	70.4	69.0	67.6
Protein (%)	21.2	20.3	22.2	21.2	21.2	21.1	22.8	21.8	21.5	20.8	23.4	23.4
Ash (%)	4.1	4.6	5.0	4.4	4.8	5.0	5.0	5.0	5.4	4.7	4.7	4.7

Table 12 (Continued)

Week											
5			6			7			8		
Diet			Diet			Diet			Diet		
A	C	D	A	C	D	A	C	D	A	C	D
<u>Fresh Weight</u>											
18.5	12.5	6.1	14.2	3.5	6.1	13.2	3.6	8.2	17.6	2.8	4.0
58.0	59.2	64.7	61.0	66.0	71.9	62.8	66.4	62.1	58.9	68.7	67.6
16.8	21.1	21.4	18.1	21.7	16.2	17.0	22.4	23.1	17.7	21.3	21.8
3.3	4.1	5.1	4.2	5.5	3.9	4.2	5.4	4.2	--	--	--
<u>Fat-Free Weight</u>											
71.2	67.6	69.0	71.1	68.3	70.3	72.3	69.0	67.7	70.7	71.0	70.5
20.6	24.2	22.8	21.1	22.5	22.0	14.7	23.3	25.1	21.2	21.9	22.6
4.1	4.8	5.4	4.9	5.8	5.3	4.8	5.6	4.9	--	--	--

Table 13. Average body constituents of rats during the period of weight loss.

Body Constit- uents	Diet of Rats			No. of Rats	Sample F Value	Predicted F Value		L.S.D.	
	Wheat	Low	High			5%	1%	5%	1%
		Carbo- hydrate Diet C	Carbo- hydrate Diet D						
<u>Fresh Basis</u>									
Fat (%)	16.40	8.40	8.40	56	15.19 **	3.17	5.01	3.33	4.45
Moisture (%)	59.30	63.90	64.00	56	6.23 **	3.17	5.01	3.01	4.02
Proten (%)	17.63	20.26	20.22	56	9.18 **	3.17	5.01	1.08	1.45
Ash (%)	3.84	4.54	4.47	42	8.03 **	3.23	5.18	0.44	0.59
<u>Fat-Free Basis</u>									
Water (%)	70.70	69.69	69.80	56	1.72	3.17	5.01	--	--
Protein (%)	21.00	22.20	22.13	56	1.60	3.17	5.01	--	--
Ash (%)	4.57	5.05	4.88	42	2.16	3.23	5.18	--	--

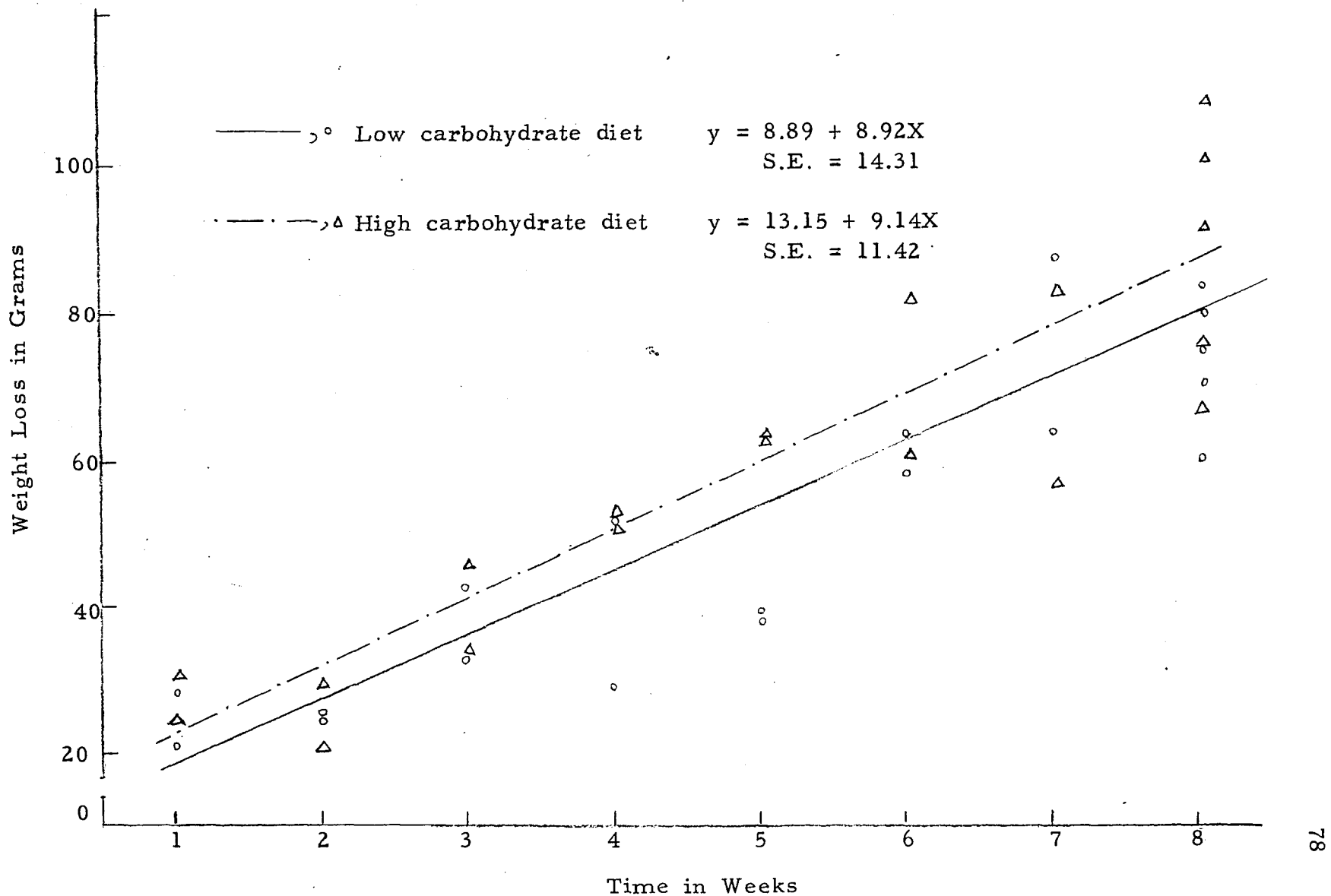
\*\* Highly significant.

for the two diets were similar (Figure 16). The loss of weight of the rats on the low-carbohydrate diet during the first four weeks was greater than that of the rats on the high-carbohydrate diet; while during the last four weeks, the reverse was the case. These results indicated that when rats are placed on a diet restricted to 12 calories per day, greater weight reduction will occur on a low-carbohydrate diet during the period when the animals are being reduced to what may be considered "normal" fat content. However, when the rats were kept on the highly restricted diet for a longer period of time, the rats were reduced below "normal" fat content, and both the low and high carbohydrate diets resulted in similar total weight losses. Although the salt content of the two diets differed, the rats on each diet received the same amount of salt daily and could be considered to be in equilibrium with respect to salt. The results obtained were in agreement with those of Anderson (2), who reported that over-all weight losses were similar on low- and high-carbohydrate diets that contained the same amount of salt and furnished the same number of calories, but that when the amount of salt in the high-carbohydrate diet was increased, less weight reduction resulted.

Body composition. The fat content of the rat carcasses decreased steadily throughout the time the rats were on the reduction



Figure 16. Regression of weight loss on time during period of weight loss.



diets (Figures 17 and 18). No significant differences were found between the fat content of the rats on the two different diets (Table 13). There was a highly significant correlation between fat loss and body weight loss of the rats during the weight-reduction period for both groups of animals (Table 14).

The moisture content of the rat body increased during the reducing period, and the rate of increase was similar for the rats on both the low-carbohydrate and high-carbohydrate diets (Figure 19). The moisture contents of the rat carcasses on the restricted diets were significantly higher than those on the control wheat diet when the moisture content was determined on the fresh basis (Table 13). However, when the moisture content was calculated on a fresh fat-free basis, there were no significant differences between the diets (Table 13). These results indicate that the rats placed on the low- and high-carbohydrate diets tended to replace the fat lost during the reducing period with water.

The protein content calculated on the fresh basis showed a significant difference between the rats on the wheat diet and those on the reducing diets (Table 13). However, the protein content of the rats was similar for both of the reducing diets and the control wheat diet when it was calculated on the fresh fat-free basis. When the

Figure 17. Regression of fat on time during period of weight loss.

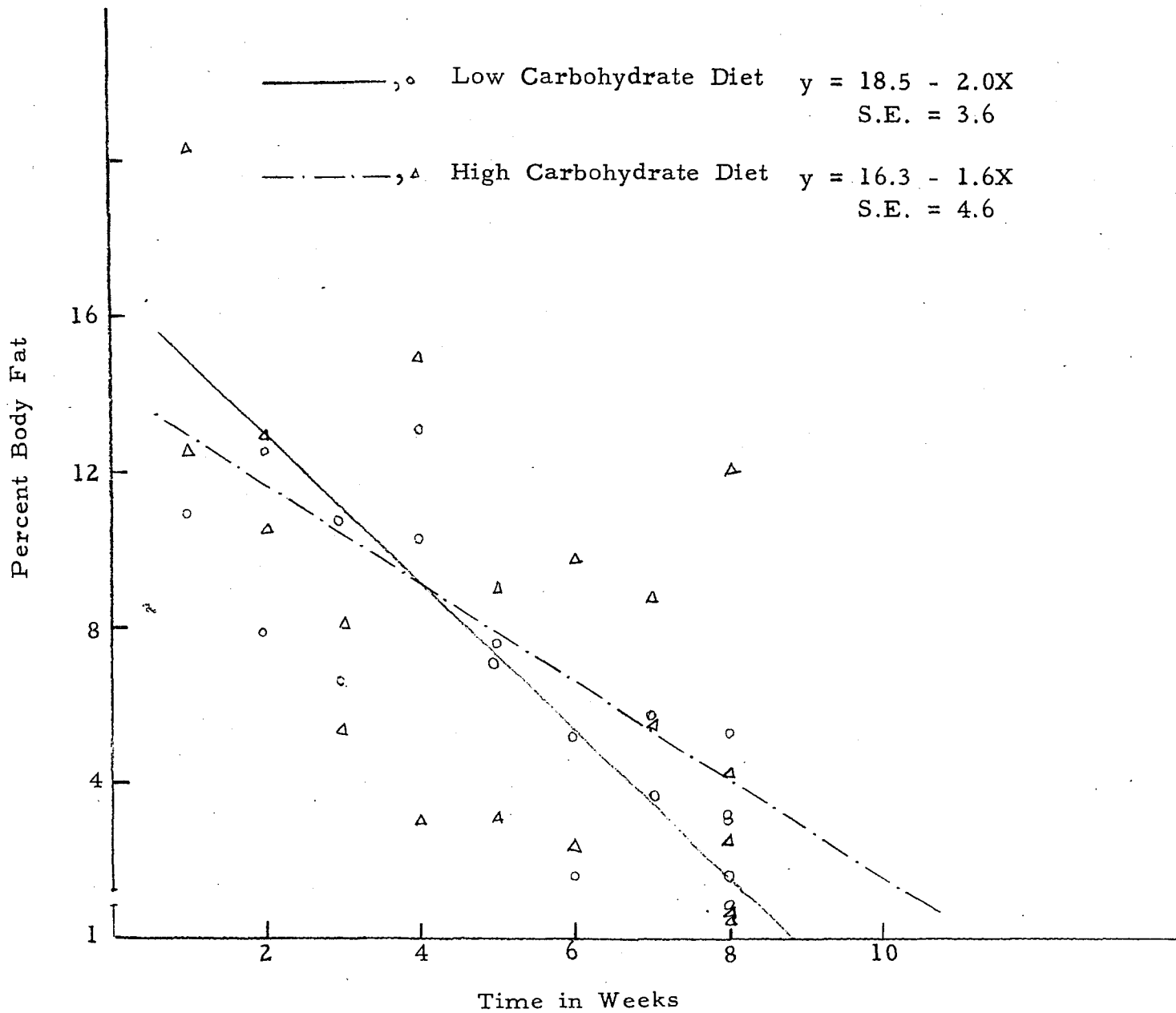


Figure 18. Regression of weight loss on fat content during period of weight loss.

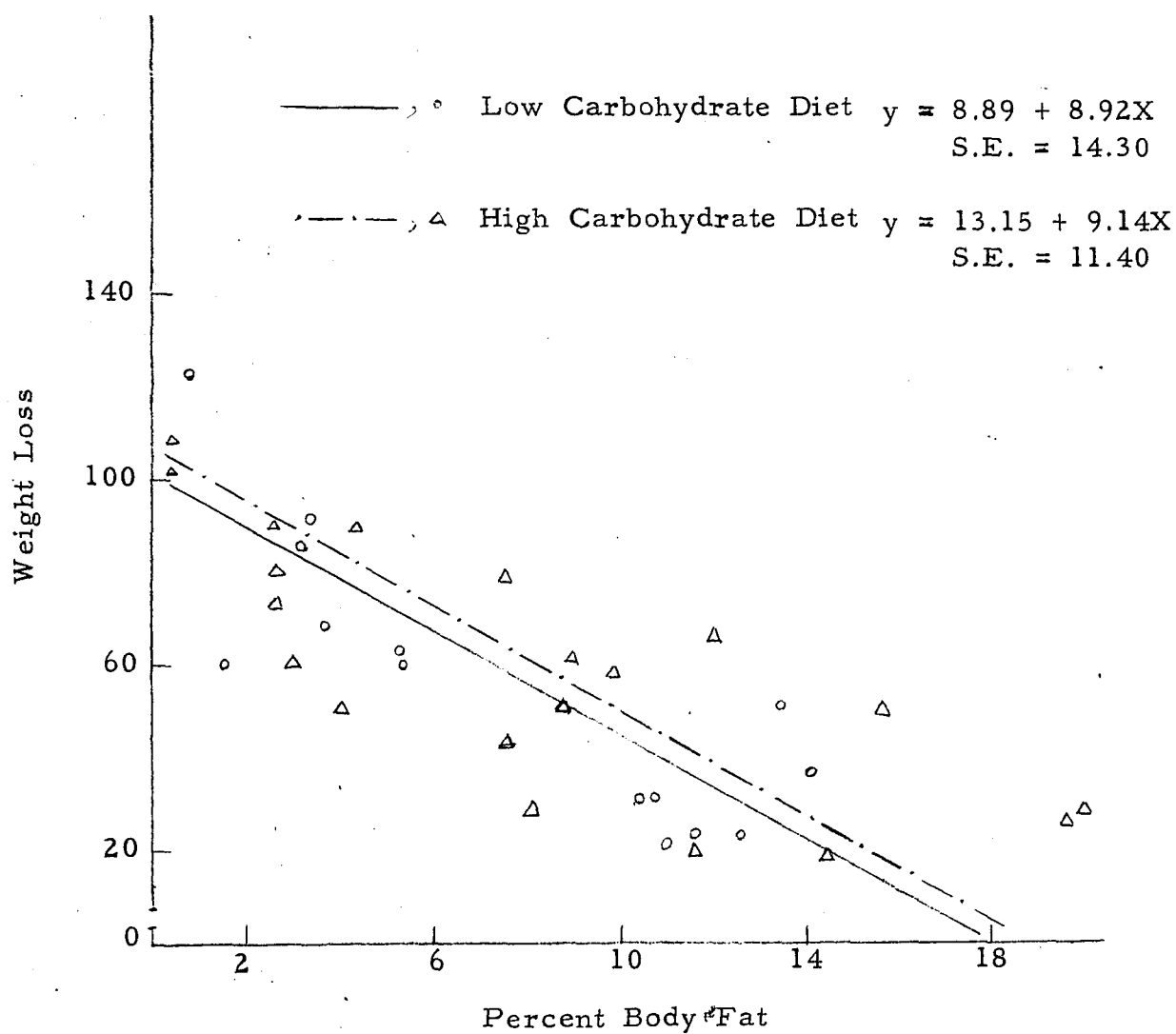


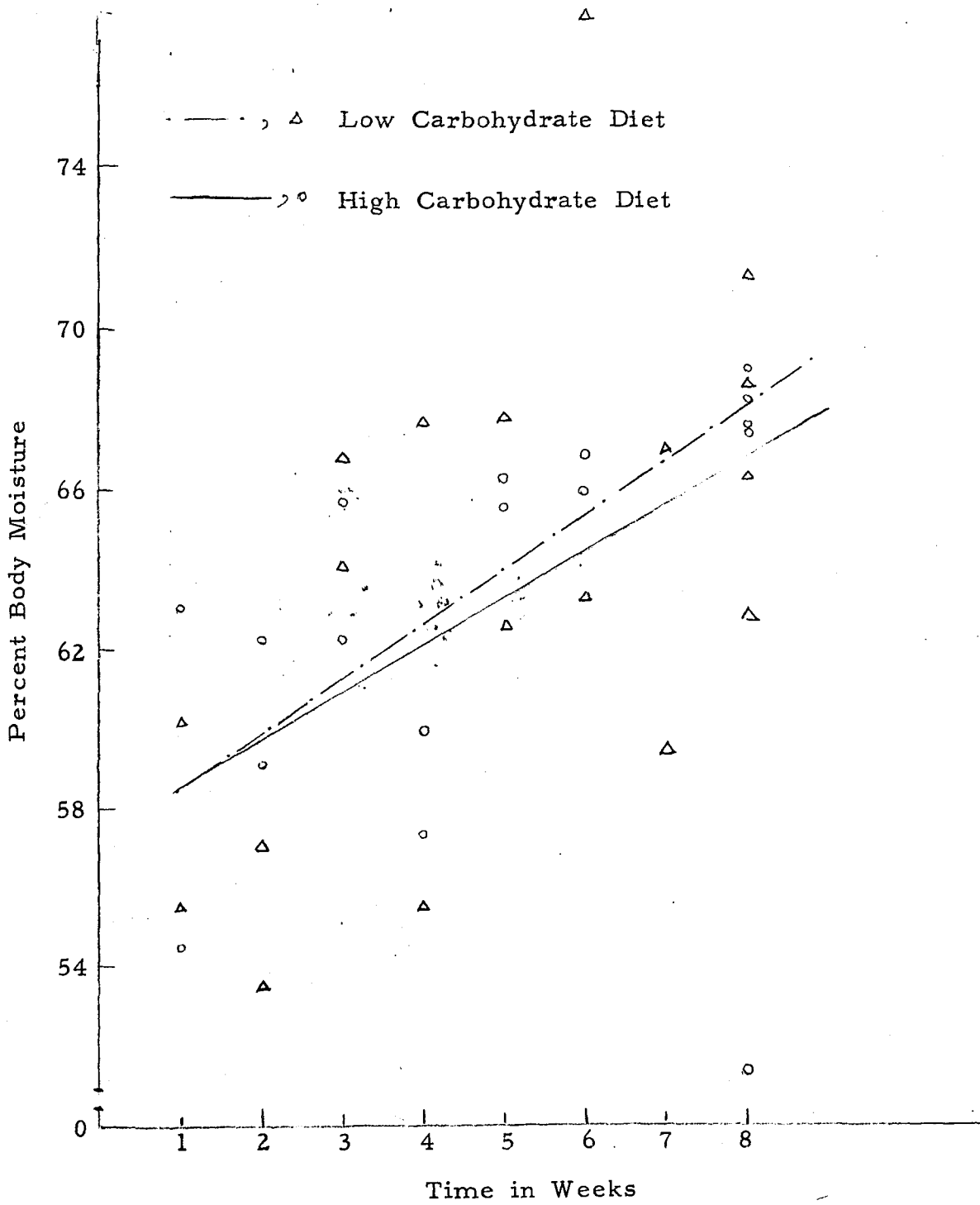
Table 14. Correlation coefficients of some body constituents during the period of weight loss.

Variables	Number of Observations	Degrees of Freedom	Sample Value of r	Predicted r Value	
				5%	1%
Weight loss and body content of fat					
Low carbohydrate diet	19	17	-0.753**	0.456	0.575
High carbohydrate diet	19	17	-0.756**	0.456	0.575
Time on diet and body fat content					
Low carbohydrate diet	19	17	-0.580**	0.456	0.575
High carbohydrate diet	19	17	-0.668**	0.456	0.575
Time on diet and body protein content					
Low carbohydrate diet	19	17	-0.691**	0.456	0.575
High carbohydrate diet	19	17	0.456*	0.456	0.575
Time on diet and mois- ture content of body					
Low carbohydrate diet	19	17	0.738**	0.456	0.575
High carbohydrate diet	19	17	0.59**	0.456	0.575
Time on diet and body content of ash					
Low carbohydrate diet	14	12	0.707**	0.532	0.661
High carbohydrate diet	14	12	0.165	0.532	0.661

\* Significant.

\*\* Highly significant.

Figure 19. Regression of moisture on time during reducing period.



protein content was calculated on a fresh weight basis, it increased proportionately throughout the period of weight loss on both reducing diets (Figure 20); there was not a significant difference between the slopes of the curves. When the data for the protein content were calculated on a fresh fat-free basis during the reducing period, there was an increase of protein up to the fourth week, then a gradual return to the average at the end of the experiment (Table 12 and Figure 21).

Rats on reducing diets showed a tendency to increase in ash content the longer they remained on the reducing diet (Figure 22). No significant difference between ash content of rats on the high-carbohydrate diet and those on the low-carbohydrate diet was found (Table 13). When the ash content was calculated on a fat-free basis, the increase in ash content became more apparent, and a significant difference between the rats and reducing diet and normal diet was shown (Table 13). This indicated that there was little loss of ash, if any, when the rats were put on the reducing diets.

Blood constituents. The blood cholesterol of the rats on the control wheat diet continued to increase with age (Figure 23). The average concentration of blood cholesterol for the rats during this period was 74.8 milligrams per 100 milliliters (Table 16), as compared

Figure 20. Regression of protein on time during period of weight loss.

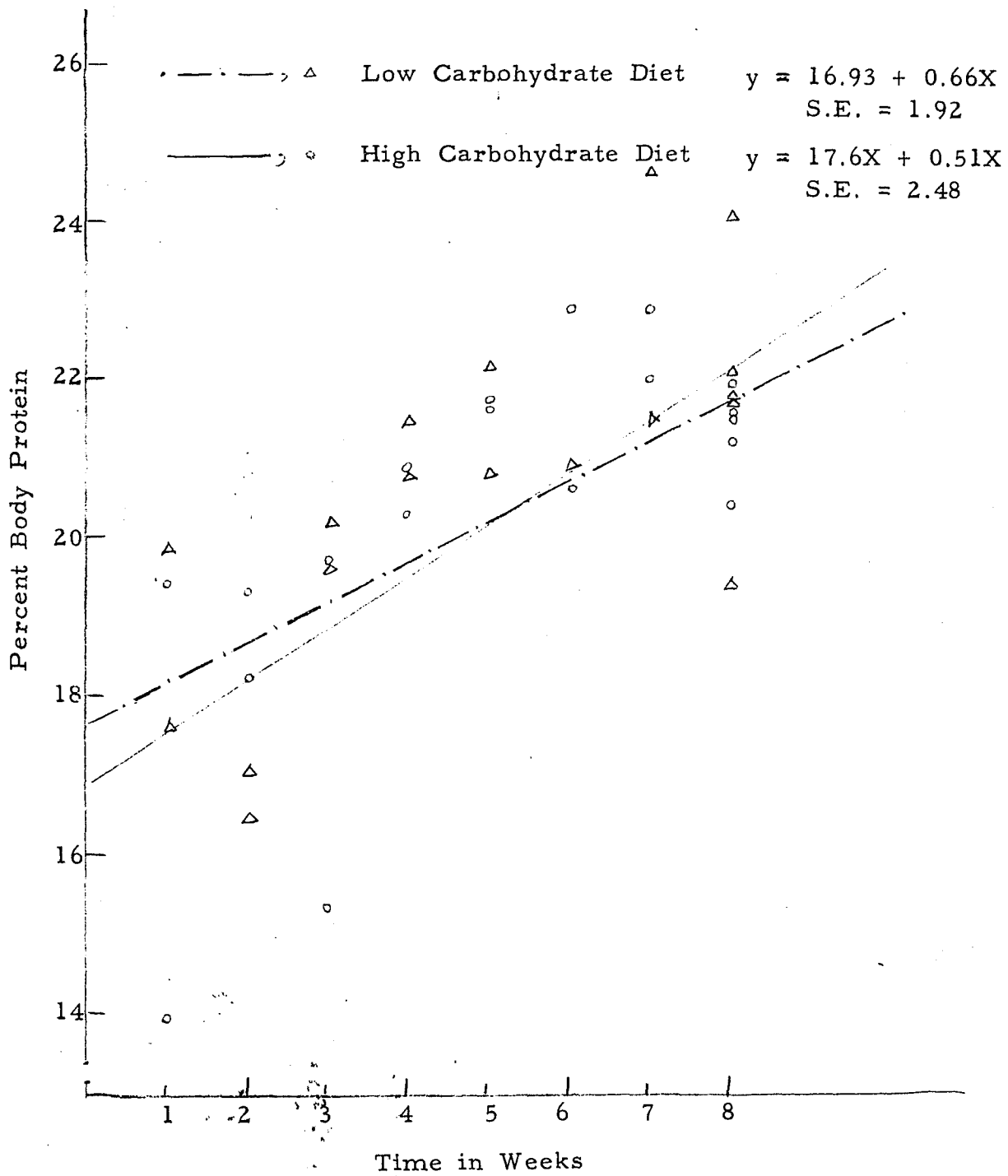




Figure 21. Protein changes (fat-free basis) during period of weight loss.

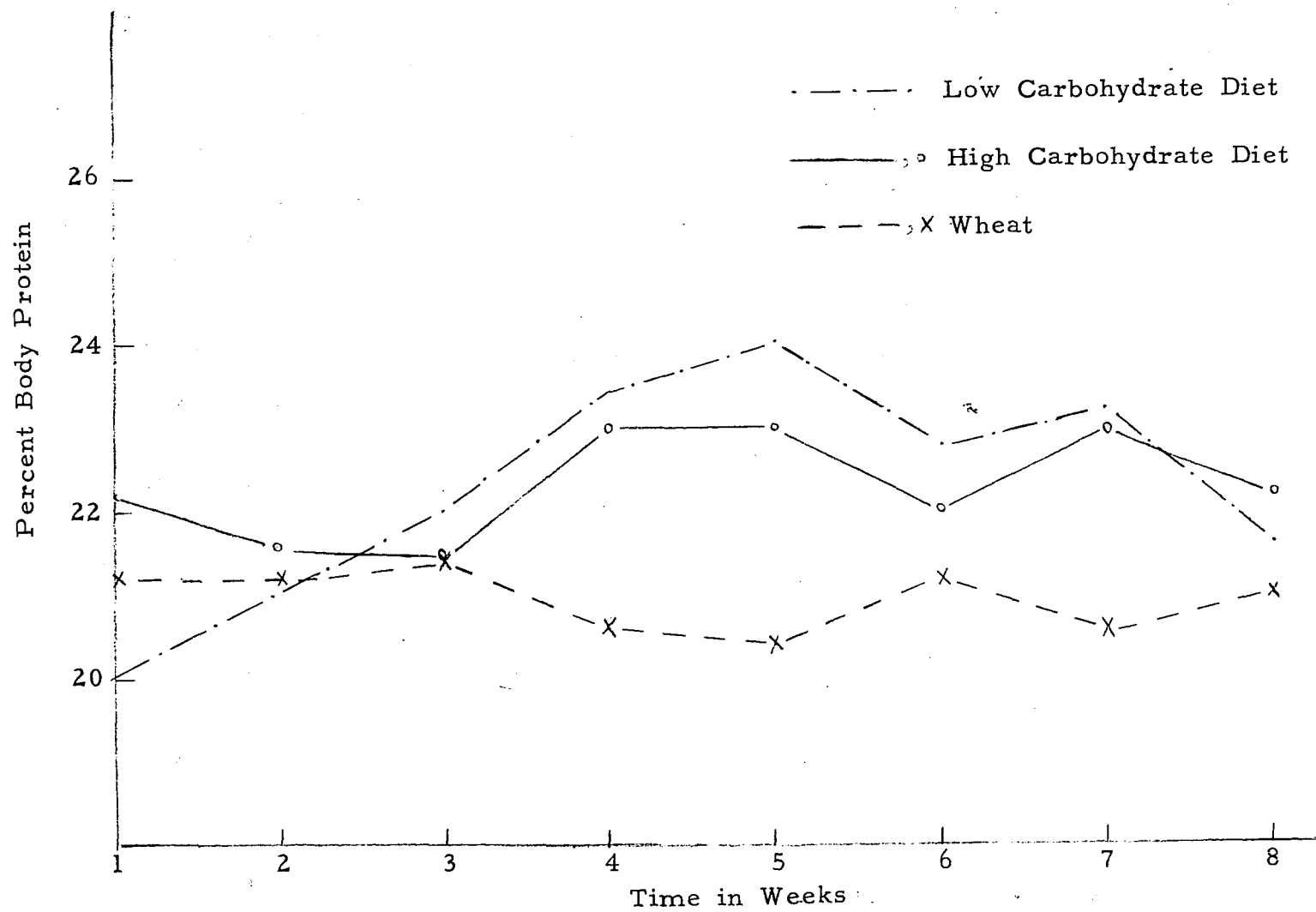


Figure 22. Regression of ash on time during period of weight loss.

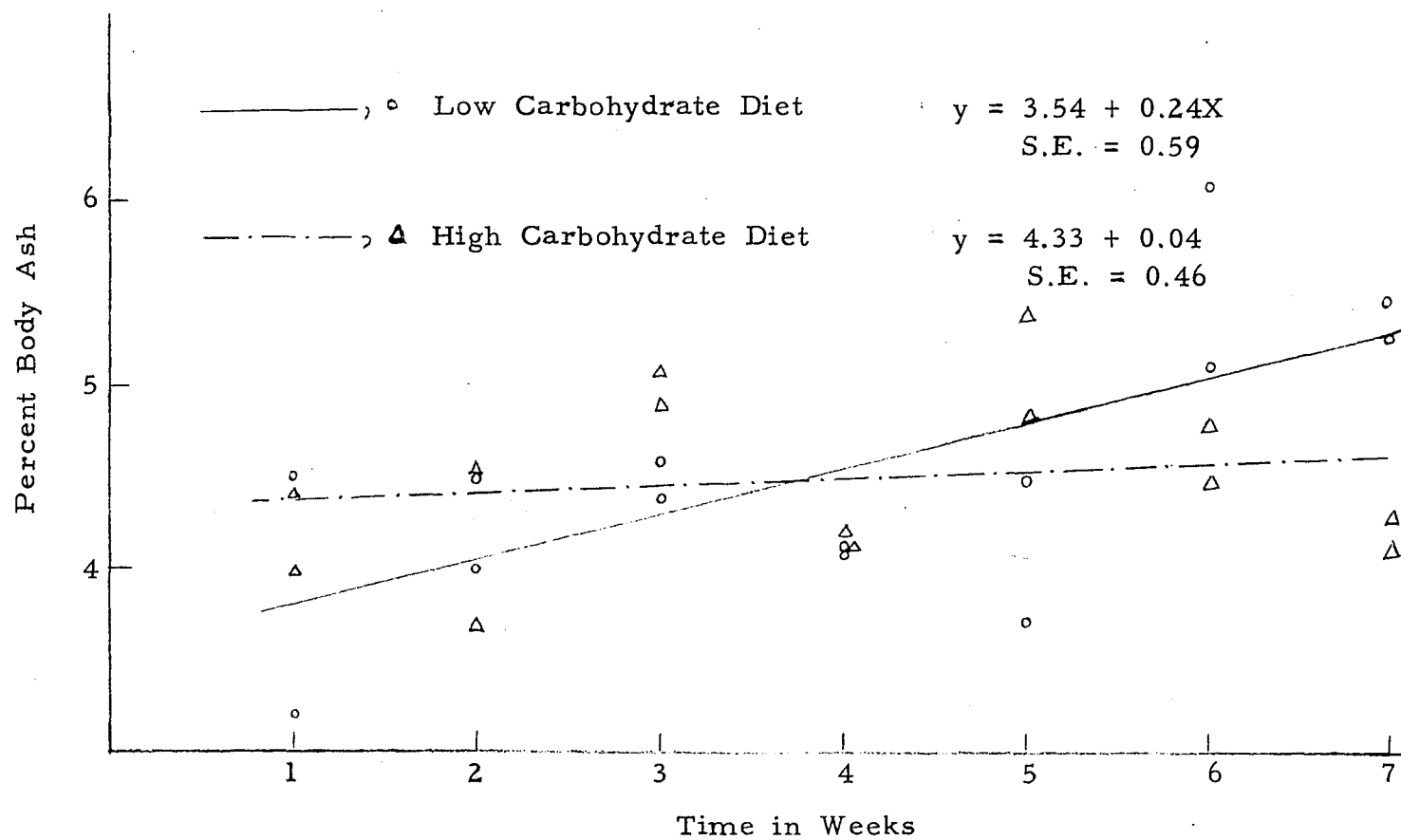


Figure 23. Regression of cholesterol on time during period of weight loss.

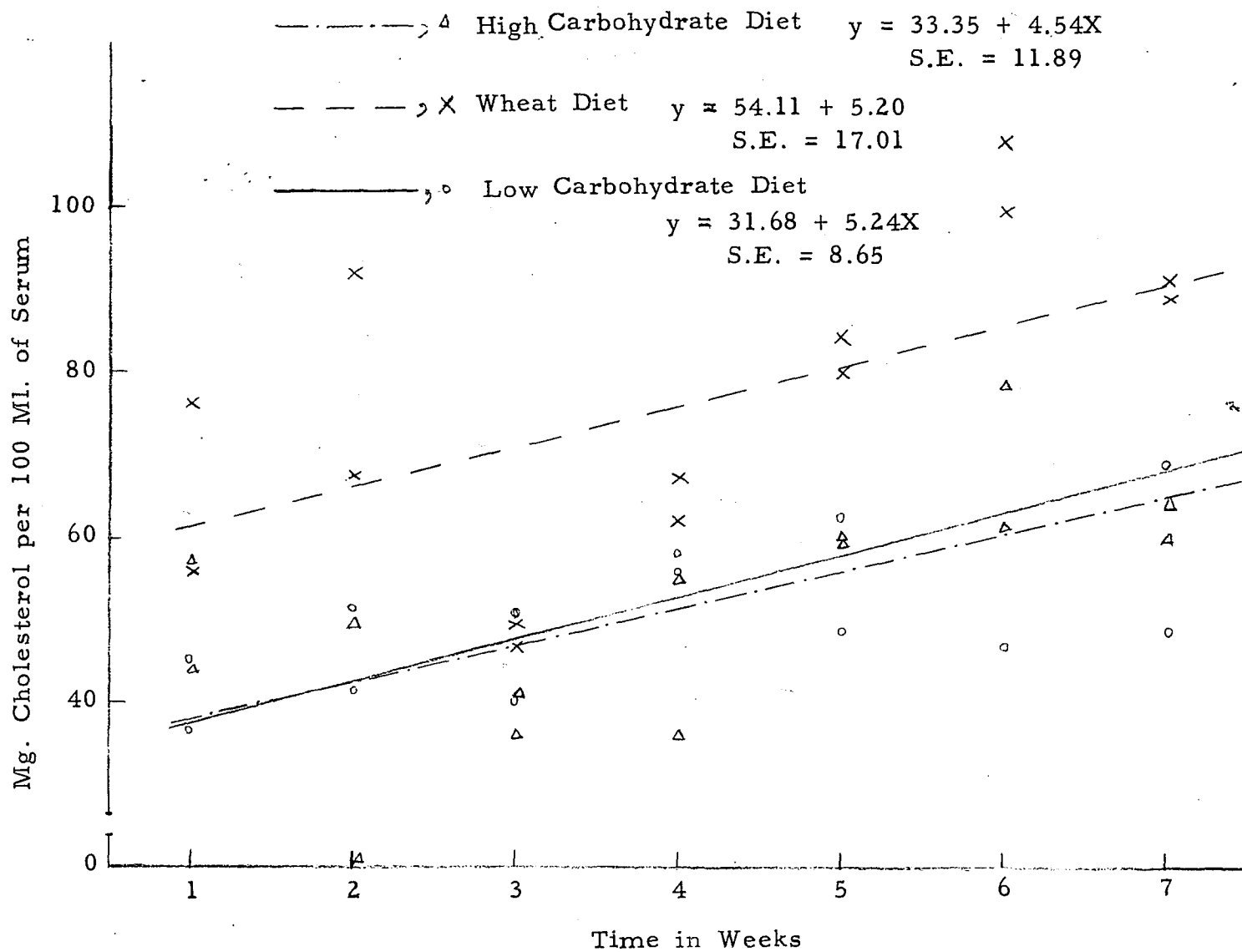


Table 15. Cholesterol, pyruvic acid, and potassium content of blood of rats during the period of weight loss.

	Week								
	1			2			3		
	Diet			Diet			Diet		
	A	C	D	A	C	D	A	C	D
Cholesterol (mg/100 ml)	60.8	45.8	50.7	79.5	46.7	34.3	47.8	45.5	38.1
Pyruvic acid (mg/100 ml)	1.4	2.2	2.1	1.7	1.7	1.5	1.8	1.6	2.0
Potassium (mg/100 ml)	170	214	173	158	172	180	179	183	182

Table 15 (Continued)

Week											
4			5			6			7		
Diet			Diet			Diet			Diet		
A	C	D	A	C	D	A	C	D	A	C	D
64.6	56.9	45.4	72.0	55.5	59.1	103.9	63.8	69.9	90.0	58.6	62.1
1.4	1.6	1.6	1.4	1.2	1.4	1.5	1.7	1.6	1.1	1.6	1.6
171	167	182	184	190	170	193	199	200	195	173	192

Table 16. Analysis of variance of blood constituents of rats during the period of weight loss.

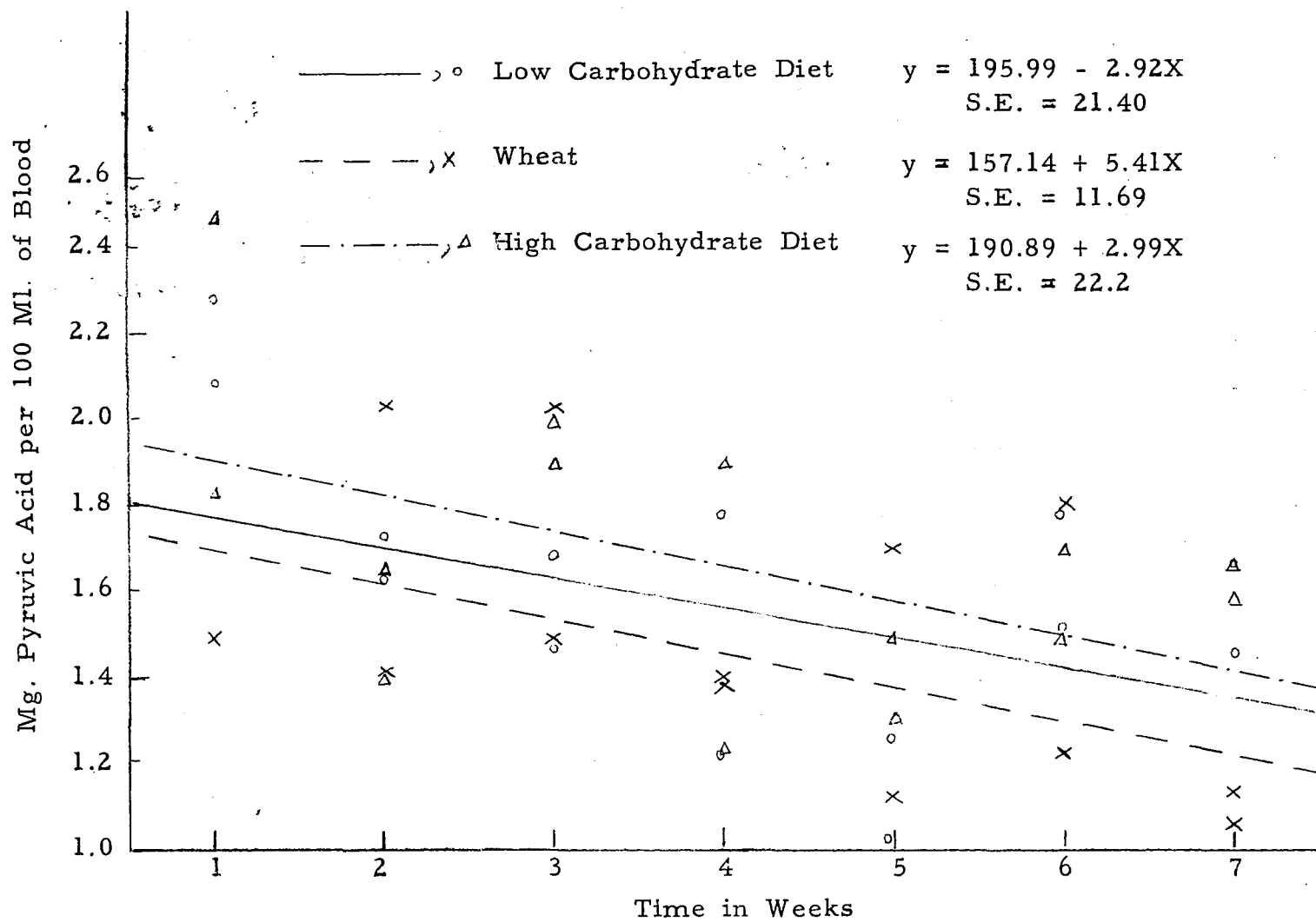
	Diet A	Diet C	Diet D	No. of Ob- ser- va- tions	Sample Value of F	Predicted Value of F		L.S.D.	
						5%	1%	5%	1%
	(av.)	(av.)	(av.)						
Choles- terol (mg/100 ml)	74.80	53.80	51.41	41	9.5**	3.25	5.21	12.00	16.00
Pyruvic acid (mg/ 100 ml)	1.5	1.6	1.60	40	1.4	3.25	5.21	--	--
Potas- sium (ppm)	178.70	186.60	182.20	41	5.97**	3.25	5.21	2.49	3.33

\*\* Highly significant.

to 69.8 during the gaining period. The same rate of increase was found to be true for the rats that were fed the two restricted diets (Figure 23). The average concentration of cholesterol in the blood of the rats fed the restricted low-carbohydrate diet was 53.8 milligrams and 51.36 milligrams for the restricted high-carbohydrate diet. The difference in concentration of cholesterol between the normal fed rats and the restricted ones was found to be highly significant (Table 16). The difference between the two restricted diets was found to be not significant. This indicated that the percentage of fat in the diet did not have any effect on the cholesterol concentration in the blood. However, restricting the caloric intake lowered the cholesterol concentration in the blood. This agrees with the work of Bose (14), who attributed this to be the effect of hunger. The data obtained showed that during the reducing period, the concentration of blood cholesterol was lowered. This is in conflict with the work of Poin-dexter and Brunger (83), who reported that the cholesterol content of the plasma was not altered by reduction in weight.

There was a tendency for the amount of pyruvic acid in the blood to decrease during the period that the rats were on the low-calorie diets (Figure 24). However, no significant correlation was found to exist between age and pyruvic acid concentration in the

Figure 24. Regression of pyruvic acid on time during period of weight loss.





blood (Table 17). Rats fed the control wheat diet ad libitum had an average of 1.45 milligrams pyruvic acid per 100 milliliters in the blood (Table 15). No significant difference was found between the blood pyruvic acid of rats fed ad libitum and that of rats given the low-calorie diets. There was an average of 1.60 milligrams per cent of pyruvic acid in the blood of rats in both the high- and low-carbohydrate-restricted diets (Table 16). It appeared that the concentration of pyruvic acid in the blood of rats was not affected by age, fat content of the diet, nor by the number of calories consumed (Table 17), and that burning body fat during weight loss did not have an effect on the pyruvic acid concentration.

The concentration of potassium in the blood of the rats was 179, 187, and 182 parts per million, respectively, for the control wheat, low-carbohydrate and high-carbohydrate diets (Table 16). There was an indication that the potassium concentration in the blood of the high-carbohydrate fed rats and the wheat-fed rats increased with the length of time on the diet (Figure 25). However, none of these correlations was found to be significant (Table 17). The rats fed the control wheat diet maintained a blood concentration of potassium (178.7 ppm), which was significantly lower than the average blood potassium of rats on the restricted diets (Table 16). No significant

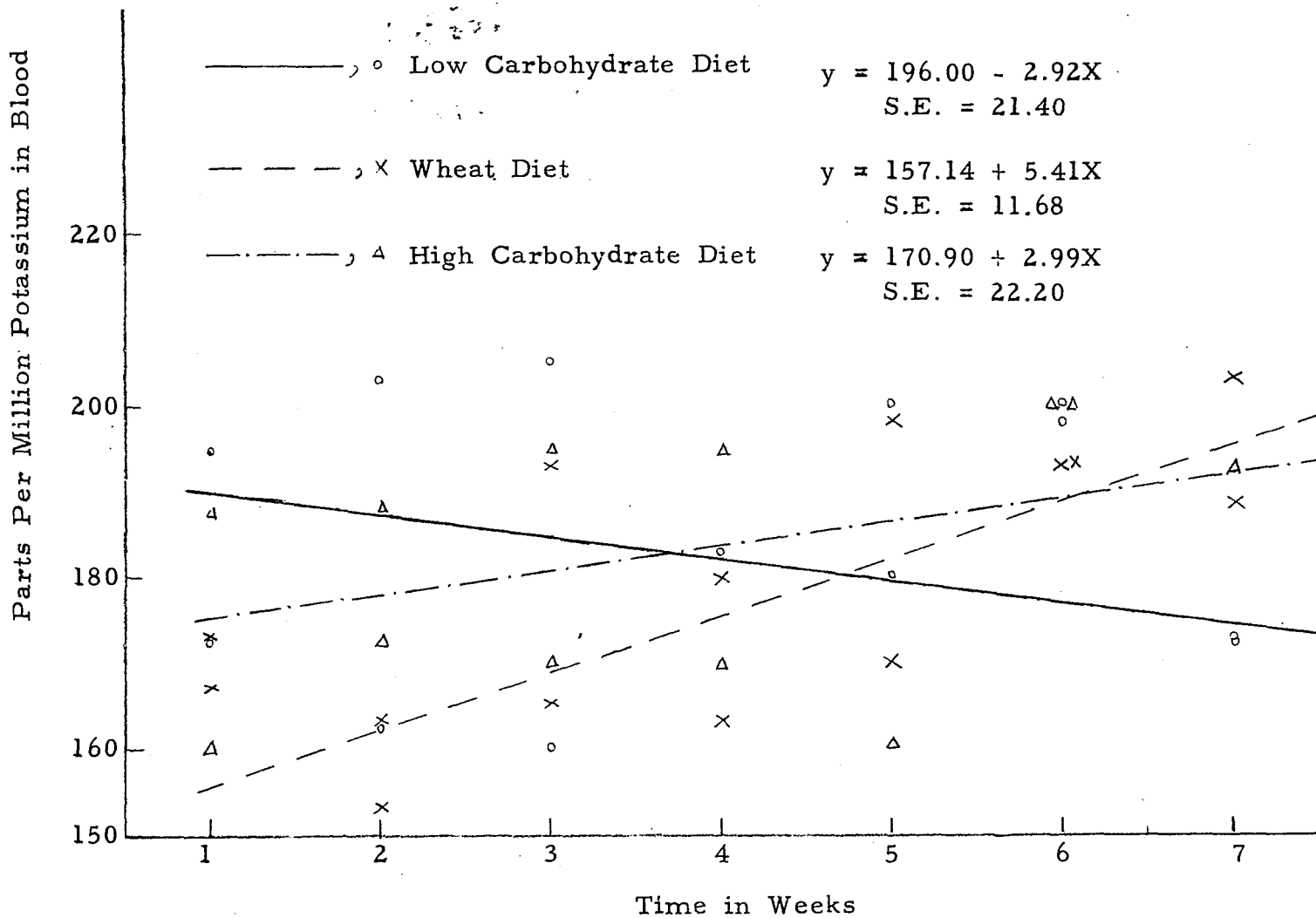
Table 17. Correlation coefficient of certain blood constituents and time of experiment for rats on low calorie diets.

Variables	No. of Observations	Degrees of Freedom	Sample Value of r	Predicted Value of r	
				5%	1%
Time on diet and blood cholesterol					
Wheat diet	14	12	0.550*	0.532	0.661
Low carbohydrate diet	13	11	0.895**	0.553	0.684
High carbohydrate diet	14	12	0.764**	0.532	0.661
Time on diet and blood pyruvic acid					
Wheat diet	14	12	-0.463	0.532	0.661
Low carbohydrate diet	14	12	-0.558*	0.532	0.661
High carbohydrate diet	13	11	-0.409	0.553	0.684
Time on diet and blood potassium					
Wheat diet	14	12	0.744**	0.532	0.661
Low carbohydrate diet	13	11	-0.283	0.553	0.684
High carbohydrate diet	13	11	0.247	0.553	0.684

\* Significant.

\*\* Highly significant.

Figure 25. Regression of potassium on time during period of weight loss.



difference was found between the concentrations of potassium in the blood of rats on the high- or low-carbohydrate diet (Table 16). Just as the data obtained during the weight-gain period showed that there was a relation between the blood potassium and body content of fat, the data obtained during the weight-loss period indicated a relation between the amount of potassium in the blood and the amount of fat burned in the body. This suggests that potassium had a role during the process of fat deposit and fat burning in the body. This also suggests that potassium has a role in fat metabolism as well as in carbohydrate metabolism as reported by many investigators (33, 85, 63, 39).

Since, at the end of the eight-week period, the weight of the rats on the restricted diets was 36 per cent below the weight of the control rats and below what is considered the normal for their age (60), and since the carcass content of fat reached an average concentration of 3.4 per cent, which was far below that of the control rats (16.4 per cent) and that reported in the literature as the normal fat content of rats (24, 28, 47), it would appear that the restricted diets used resulted in starvation. This severe deprivation may have masked the differences effected by the two restricted diets which might have been apparent if less severe calorie deprivation had been imposed on

the animals. Starvation is also indicated by the fact that the blood cholesterol values were found to be lower in the reduced animals than in those of the control and lower than those reported as the normal for the blood of rats. Man and Gildea (66) found that starvation caused a decrease in the concentration of blood cholesterol. Poindexter (83) and others (111) reported that there was a slight increase of blood cholesterol values during the initial period of weight reduction. This was followed by a return to normal values. In this study, the blood cholesterol values decreased during the initial period of weight reduction and remained lower throughout the entire experiment than those of the control rats on the wheat diet. The blood potassium values of the rats on the restricted diets also were found to be lower than those of the control rats--a phenomenon which Mellinghoff (68) had observed during starvation.

## SUMMARY AND CONCLUSION

The influence of diets of different composition on certain body and blood components and on the rate of weight gain and weight loss of rats was studied. One hundred and forty-four mature female rats, approximately 120 days of age, were used in the experiment. In the first period, one-third of the rats were fed a wheat diet ad libitum; the remaining rats were fed a corn diet, ad libitum, in an attempt to produce obese animals. Both groups of rats gained weight throughout a period of approximately 140 days. No significant differences were found between the final weight of the rats on the two diets, indicating that obesity, interpreted as excess body weight, did not occur as had been expected in the rats fed the corn diet.

The protein, fat, moisture, and ash contents of the rat carcasses were determined at periodic intervals. Of these components, the only factor that varied greatly during weight-gain period was the fat. The average fat content of the rats fed the control wheat diet was 12.8 per cent, and that of the rats fed on the corn diet was 17.2 per cent. The protein, moisture, and ash contents of the body were found to be relatively stable when calculated on a fat-free basis.

However, when the components were calculated on a fresh basis, an increase in fat content was associated with a decrease in the moisture content.

Body analyses indicated that the weight increase of rats between the ages of 120 and 260 days represented adult growth and was not a deposition of adipose tissue as believed by Spray (101), since no correlation between age or body weight and body composition was found.

The blood cholesterol of rats fed ad libitum was affected by diet, age, and body fat content. Rats fed the corn diet had lower blood cholesterol values than the rats fed the wheat diet. Older rats also had higher cholesterol concentrations than younger rats, and rats that had high body fat content had lower blood cholesterol values than the rats that had low fat content.

Blood potassium was affected by the type of diet and the amount of fat in the body. Corn-fed rats had higher blood potassium values than the rats fed the wheat diet, and the blood potassium values tended to increase with an increase in body fat.

Neither age nor diet was found to have any effect on the blood pyruvic acid concentration. Body fat content was the only factor that was found to have a relation to the blood pyruvic acid

concentration. The blood pyruvic acid content increased with the increase in the fat content of the body.

At the end of the weight-gaining period the rats fed the corn diet were paired according to weight. One group was fed a high-carbohydrate diet and the other a low-carbohydrate diet. Both groups were fed 12 calories per day. The control rats were continued on the wheat diet fed ad libitum.

No difference was noticed between the effect of the high-carbohydrate and the low-carbohydrate restricted diet on the rate of weight loss of the rats, and the total loss in weight was approximately the same for rats on the two diets. The two restricted diets had nearly the same effect on the body composition of the rats. Great loss of body fat was accompanied by little change in the water and protein content calculated on a fat-free basis, and no change in the ash content was observed.

The rats fed the two restricted diets had lower blood cholesterol values than the rats fed the control wheat diet. No significant difference between the effect of the two restricted diets was noticed.

Rats fed the restricted diets and the control wheat diets had nearly the same blood pyruvic acid values. With both restricted



diets an increase in the blood potassium was observed. The rats fed the restricted diets showed higher potassium values than those fed the control wheat diet.

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APPENDIX

Table 18. Data for rats fed wheat diet during the period of weight gain.

Rat No.	No. of Weeks on Diet	Original Weight	Wt. of Rat When Killed	Total Gain in Weight	Total Food Consumed
96	7	148	178	30	355
95	7	169	206	37	398
94	8	156	191	35	424
93	8	176	200	24	435
92	9	163	173	10	409
90	9	136	170	34	454
89	10	125	180	55	594
88	10	152	221	69	624
87	12	155	198	43	659
86	12	171	224	53	710
85	14	151	197	46	723
84	14	167	202	35	672
83	16	147	200	53	758
82	16	164	218	54	747
81	18	131	204	73	868
80	18	150	222	72	939
79	20	144	197	53	1,271
78	20	164	214	5-	1,076

Table 18 (Continued)

Body Constituents				Blood Constituents		
Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Potas- sium (ppm)	Pyruvic Acid (mg/100ml)	Cholès- terol (mg/100ml)
62.8	17.37	13.38	4.26	165.0	3.45	57.50
65.6	16.15	12.88	4.18	135.0	1.76	51.34
60.26	19.75	13.67	3.27	195.0	1.22	80.00
61.48	17.61	15.71	3.78	225.0	1.70	86.67
61.5	17.45	14.33	3.87	180.0	2.05	36.50
61.7	17.60	12.72	3.94	192.5	1.84	45.00
61.4	18.01	13.08	3.85	160.0	2.57	39.50
62.85	17.85	11.21	4.00	152.0	2.18	116.67
61.41	18.00	12.55	3.72	172.5	--	76.25
61.9	18.26	11.73	4.00	142.5	--	--
63.31	17.92	11.01	4.05	--	1.86	83.75
62.81	18.34	11.45	3.95	202.5	2.88	73.00
62.0	17.99	12.03	4.11	185.0	--	72.29
61.1	17.57	12.74	4.02	180.0	2.71	80.72
60.8	18.01	13.58	3.85	207.5	2.34	64.17
58.75	16.94	17.68	3.72	157.5	2.59	71.39
64.3	18.87	8.89	3.98	162.5	1.62	82.67
63.5	16.98	10.12	4.07	157.5	2.50	--

Table 19. Data for rats fed corn diet during the period of weight gain.

Rat No.	No. of Weeks on Diet	Original Weight	Wt. of Rat When Killed	Total Gain in Weight	Total Food Consumed
146	7	173	192	19	379
145	7	185	215	29	376
144	8	169	210	41	406
143	8	174	200	26	417
133	9	163	191	28	459
141	9	160	212	52	479
140	10	170	205	35	536
138	10	164	205	41	509
137	12	158	222	64	612
136	12	171	209	38	597
135	14	166	217	51	724
134	14	175	211	36	625
132	16	161	227	66	736
131	16	178	250	72	856
130	18	163	214	51	788
129	18	186	255	69	993
128	20	175	213	38	1,012
127	20	178	238	60	1,056



Table 19 (Continued)

Body Constituents				Blood Constituents		
Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Potas- sium (ppm)	Pyruvic Acid (mg/100 ml)	Choles- terol (mg/100 ml)
60.00	17.57	15.91	3.45	177.5	1.28	74.25
58.52	17.12	18.07	3.62	225.0	1.90	46.25
59.39	17.41	17.47	3.40	195.0	1.44	57.08
60.67	17.30	16.00	3.62	175.0	2.48	77.92
59.20	16.75	17.61	3.33	--	--	36.00
61.30	17.35	15.30	3.94	182.5	1.86	46.50
59.55	17.81	15.66	3.96	185.0	2.41	--
56.77	16.20	20.00	3.75	185.0	--	69.83
58.39	17.09	15.86	4.18	187.5	--	84.38
60.12	18.18	14.34	4.15	217.5	3.08	85.67
58.81	17.09	17.41	3.85	205.0	2.92	94.38
58.19	18.00	15.42	3.50	162.5	3.93	33.0
59.65	17.01	15.78	3.90	195.0	2.89	75.0
55.75	15.99	29.81	3.15	200.0	2.77	69.5
58.28	17.16	17.91	3.71	185.0	1.41	80.50
54.61	18.18	19.20	3.72	185.0	3.50	74.83
61.32	19.57	11.82	3.90	185.0	1.65	62.09
60.21	17.85	14.62	4.14	182.5	1.44	53.33

Table 20. Data for rats fed wheat diet during the period of weight loss.

Rat No.	No. of Weeks on Diet	Original Weight	Gain in Weight	Food Consumed	Weight at Start of Reducing Period	Weight When Killed	Food Consumed During Reducing Period
76	1	168	52	1,250	220	220	25
75	1	136	88	1,614	224	228	44
74	2	141	66	1,631	207	214	144
73	2	148	63	1,478	211	217	153
71	3	123	71	1,272	194	197	153
69	3	178	49	1,391	227	222	158
68	4	158	36	1,111	194	200	193
67	4	160	29	1,211	189	193	220
66	5	138	84	1,336	222 <sup>1/2</sup>	232	289
65	5	162	76	1,566	238	246	438
64	6	170	55	1,371	225	230	380
63	6	156	42	1,308	198	202	344
60	7	168	48	1,425	216	221	471
59	7	157	46	1,426	203	201	418
55	8	151	89	1,404	240	240	519
54	8	143	56	1,287	199	200	442
50	8	161	83	1,377	244	230	389

Table 20 (Continued)

Body Constituents				Blood Constituents		
Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Potas- sium (ppm)	Pyruvic Acid (mg/100 ml)	Choles- terol (mg/100 ml)
57.96	17.36	17.97	3.36	167.0	--	76.1
58.49	17.58	17.70	3.34	172.5	1.48	55.5
60.79	17.97	15.13	4.08	162.5	1.40	67.2
60.33	17.80	15.50	3.42	152.5	2.03	91.8
54.41	19.67	18.23	4.19	165.0	1.48	46.8
58.53	17.92	16.82	4.10	192.5	2.04	48.8
57.10	17.76	17.34	3.87	162.5	1.38	67.3
60.51	16.80	15.70	3.95	180.0	1.40	61.9
57.70	15.21	19.91	2.94	197.5	1.70	69.9
58.30	17.82	17.17	3.73	170.0	1.12	74.2
61.95	18.22	13.35	4.48	192.5	1.24	118.2
60.04	18.11	15.03	3.90	192.5	1.81	89.6
61.55	18.06	13.56	4.39	187.5	1.07	91.5
64.09	15.92	12.87	3.94	202.5	1.14	88.5
58.60	18.69	14.33				
57.89	17.33	18.66				
61.44	18.32	13.57				
57.48	16.25	21.35				

Table 21. Data for rats fed low-carbohydrate diet during the period of weight loss.

Rat No.	No. of Weeks on Diet	Original Weight	Gain in Weight	Food Consumed	Weight at Start of Reducing Period	Weight When Killed	Food Consumed During Reducing Period
2	1	162	98	1,735	260	232	20.0
16	1	172	47	1,612	219	196	20.0
8	2	190	57	1,401	247	222	37.5
33	2	155	62	1,518	217	192	37.5
7	3	162	52	1,420	214	181	45.0
12	3	188	57	1,668	245	202	45.0
3	4	173	89	1,653	262	210	62.5
31	4	166	53	1,350	219	190	62.5
4	5	175	66	1,520	241	202	80.0
5	5	172	78	1,643	250	212	80.0
6	6	171	51	1,479	222	163	97.5
10	6	168	74	1,585	242	178	97.5
11	7	172	50	1,507	222	156	115.0
29	7	162	98	1,655	260	172	115.0
14	8	170	62	1,515	232	157	132.5
17	8	172	60	1,449	232	148	132.5
19	8	182	40	1,325	222	162	132.5
24	8	208	48	1,732	256	134	132.5
27	8	182	44	1,377	226	155	132.5

Table 21 (Continued)

Body Constituents				Blood Constituents		
Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Potassium (ppm)	Pyruvic Acid (mg/100 ml)	Cholesterol (mg/100 ml)
54.59	13.90	25.57	3.16	232.5	2.08	45.8
63.08	19.38	11.03	4.50	195.0	2.28	--
59.15	18.16	12.68	4.45	172.0	1.74	51.9
62.20	19.30	11.79	4.01	--	1.63	41.5
62.28	19.66	10.74	4.55	205.0	1.46	51.0
62.66	19.35	10.45	4.39	160.0	1.68	40.1
60.11	20.28	13.45	4.13	182.5	1.87	55.9
61.22	20.91	10.48	4.10	152.5	1.23	57.9
61.03	20.55	11.03	3.69	200.0	1.02	62.7
57.40	21.71	13.98	4.45	180.0	1.25	48.3
66.34	22.88	1.62	6.04	200.0	1.54	46.6
65.64	20.57	5.36	5.07	197.5	1.76	80.9
66.01	22.93	3.78	5.25	172.5	1.66	68.5
66.85	21.96	3.51	5.50	172.5	1.45	48.8
68.32	21.51	3.32				
67.58	21.39	3.34				
67.46	20.39	5.36				
71.10	21.19	0.77				
69.10	21.90	1.64				

Table 22. Data for rats fed the high-carbohydrate diet during period of weight loss.

Rat No.	No. of Weeks on Diet	Original Weight	Gain in Weight	Food Consumed	Weight at Start of Reducing Period	Weight When Killed	Food Consumed During Reducing Period
38	1	171	89	1,615	260	230	24
103	1	162	56	1,247	218	194	24
104	2	171	73	1,393	244	215	45
114	2	167	47	1,218	214	194	45
42	3	170	44	1,390	214	180	66
106	3	164	79	1,393	243	198	66
45	4	159	104	1,499	263	212	87
41	4	159	59	1,660	218	166	87
116	5	189	53	1,484	242	180	108
118	5	174	71	1,397	245	183	108
100	6	180	44	1,260	224	164	129
30	6	190	42	1,639	242	171	129
120	7	160	60	1,257	220	164	150
37	7	168	90	1,623	258	176	150
112	8	170	62	1,286	232	132	171
44	8	156	74	1,444	230	122	171
123	8	168	56	1,388	224	150	171
99	8	173	83	1,508	256	165	171
119	8	163	63	1,262	226	160	171

Table 22 (Continued)

Body Constituents				Blood Constituents		
Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Potassium (ppm)	Pyruvic Acid (mg/100 ml)	Cholesterol (mg/100 ml)
55.70	17.56	20.40	4.00	187.5	2.76	57.6
61.06	19.86	11.69	4.43	157.5	1.54	43.8
53.48	18.45	19.63	4.51	172.5	1.64	49.5
57.10	16.48	14.51	3.74	187.5	1.39	19.1
64.13	20.19	8.20	4.94	195.0	1.90	35.8
66.81	19.73	5.65	5.12	170.0	2.00	40.4
55.5	20.84	15.00	4.15	170.0	1.90	35.8
67.11	21.46	4.03	4.25	195.0	1.20	55.0
66.82	22.15	3.12	5.36	180.0	1.48	59.4
62.60	20.75	9.08	4.81	160.0	1.30	58.8
63.43	19.86	9.84	4.78	240.0	1.49	78.5
80.48	12.55	2.39	2.96	160.0	1.72	61.2
59.39	24.73	8.84	4.10	--	--	64.2
64.88	21.45	7.63	4.26	192.0	1.58	60.0
71.46	21.75	0.58				
68.52	24.05	0.72				
69.08	22.08	2.53				
66.28	21.69	4.31				
62.81	19.35	11.96				

Table 23. Rats that died or were killed and the apparent cause.

Rat Number	Time on Experiment	Original Weight	Final Weight	Cause of Death
<u>Corn Diet</u>				
1	1	150	130	Unknown
1	16	168	210	Pneumonia
15	18	172	212	Bloody urine, abnormal kidneys
23	16	155	192	Pneumonia
39	3	141	154	Pneumonia
46	20	153	210	Bloody urine, abnormal kidneys
48	21	176	246	Pneumonia
101	21	191	200	Bloody urine, abnormal kidneys
111	4	162	131	Pneumonia
142	8	170	180	Pneumonia
<u>Wheat Diet</u>				
51	14	141	183	Pneumonia
56	20	154	216	Pneumonia
62	17	192	220	Pneumonia
70	21	155	160	Killed, abnormal growth of teeth. Loss of weight.



Table 23 (Continued)

Rat Number	Time on Experiment	Original Weight	Final Weight	Cause of Death
72	21	160	170	Killed, abnormal growth of teeth; loss of weight.
77	21	178	224	Killed. No upper teeth; lower teeth are white and brittle, loss of weight.

Rat Number	Diet	Cause
	<u>Reducing Diet</u>	
13	High carbohydrate	Pneumonia
18	High fat	Ear infection
20	High fat ad libitum	Ear infection