ABSTRACT

EFFECT OF FEEDING EXCESS THIAMINE IN THE BASAL RATION OF THE ALBINO RAT

by Esther L. Brown

Until recently it was difficult to consume excess amounts of vitamins, especially the water soluble ones. Today, the average consumer can purchase a considerable number of vitamin preparations which contain large amounts of the "B" vitamins. Many individuals consume vitamin preparations indiscriminately along with dietary sources of these vitamins such as enriched foods (bread, cereals, etc.). Hence the intakes of the water soluble vitamins by some individuals may become sizable.

The purpose of this research was to explore the possible toxicity that might be associated with excess consumption of thiamine in the ration of the albino rat. Four mg./of thiamine/kg. of diet is generally accepted as the requirement for the rat. The basal diet (containing 4 gamma of thiamine/gm. of ration) was supplemented with added thiamine to produce the following concentrations: 12, 21, 39, 74, 1004, 2004, 3004, 4004, 5004, to 10,004 gamma of thiamine/gm. of ration, a range of from 3 to almost 3000 times the thiamine requirement of the rat.

The addition of very large amounts of thiamine to rations containing various levels of protein had no deleterious nor beneficial effect on the growth of rats. Large doses of thiamine had no appetite-stimulating activity.

High levels of thiamine did not contribute to the deposition of fat in the liver, nor did they alter the concentration of nitrogen and moisture in the liver.

In so far as the protein, fat, ash, and moisture levels of the heart, kidneys, liver, and thigh muscle were concerned, very high levels of thiamine had no effect on the composition of these tissues.

The level of thiamine in the diet had no influence on the percentage of various protein fractions in blood serum.

Increased thiamine in the diet increased the thiamine level in all tissues examined but above 0.0035% (39 gamma of thiamine/gm. of ration) of thiamine intake thiamine deposition in the tissues did not increase to any extent. Greatest concentration of thiamine was found in the liver, heart, kidneys, and testes. In contrast to other species, thiamine content in rat adrenals was very low.

The blood level of thiamine reflected the dietary intake. Very little thiamine was excreted in the urine until the level of thiamine in the blood reached 0.09 gamma/ml. From that point urinary excretion of thiamine showed a straight line relationship to the dietary intake of thiamine suggesting that a level of 0.075 gamma of thiamine/ml. in the blood as the kidney threshold for thiamine.

With increased levels of dietary thiamine both urine and fecal thiamine excretion increased, but that for feces increased faster than that for urine.

The excretion of pyrimidine, thiazole, and thiamine in the urine increased as the dietary level of thiamine increased with the excretion of pyrimidine and thiazole following that of thiamine.

A significant observation was the inverse relation between the protein level in the ration and the thiamine concentration in many of the tissues. This was most obvious for the tissues from the rats fed the basal rations which contained 4 mg. of thiamine/kg. of ration.

The liver and thigh muscles showed a 10-fold reduction in thiamine content when the protein in the ration went from 0 to 20%. The heart showed a similar relation although not as pronounced as the liver and muscle.

In contrast to the above, the rats fed the rations containing different levels of casein showed blood levels of thiamine which were proportional to the protein level in the diet. The level increased from 0.01 gamma of thiamine/ml. for the 0% casein ration to 0.08 gamma for the 20% casein ration. The urinary excretion of thiamine in these rats followed the blood levels.

As measured by the concentration of thiamine in the tissues, thiamine metabolism depended on the protein level of the ration, but in the case of urinary thiamine excretion this relationship was reversed. If a similar situation holds true for man, this might mean that the evaluation of urinary excretion data should be considered from the standpoint of dietary protein levels when clinical analyses are conducted at the same time as dietary surveys.

There was a decrease in the growth of the rat the first two weeks following weaning when choline was omitted from the diet. The addition of high thiamine without choline did not improve the growth during these same two critical weeks.

Based on the studies carried out in this research the administration (intake) of high levels of thiamine above demonstratable physiological needs accrued no advantages to the albino rat.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Foods and Nutrition
College of Home Economics

1963

ACKNOWLEDGMENTS

The writer wishes to express her appreciation to: Dr. Thelma Porter, Dean of the College of Home Economics, who presented her application for the Dean Marie Dye Fellowship for 1959-1960, which made it possible for the writer to return to school; to Dr. Dena Cederquist, Chairman of the Department of Foods and Nutrition, for her suggestions and help throughout the writer's program; and to Dr. Dorothy Arata, Associate Professor, under whom this research was begun.

To Dr. Olaf Mickelsen, my major professor, the writer wishes to express grateful appreciation for his continued advice, valuable suggestions, and ready encouragement in the completion of this research and in the preparation of the thesis.

So many people, who cannot be listed individually, have contributed immeasurably, either directly or indirectly, to the final completion of this research. Without the invaluable support, continued interest, and encouragement of the laboratory technicians, fellow graduate students, friends, and especially family members the thesis on this research could not have become a reality.

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REVIEW OF LITERATURE

Introduction

About 20 years ago when thiamine (vitamin B-1) was isolated and its synthesis accomplished, studies were begun on its pharmacologic action. At that time it was believed that "relatively enormous doses were without effect in the normal organism. No toxic symptoms of any kind were observed when doses approximating 25,000 or more times the estimated daily requirement were administered" (Williams and Spies, '38). Today, as a result of a greater knowledge of metabolism, more refined tools for measurement, etc., one reads such statements as the following: "Toxic manifestations from thiamine administration in humans are encountered only rarely and are reported to be similar to anaphylactoid reactions. However there is pharmacologic evidence that thiamine overdosage produces a simple chemical toxicity thought to be due to the formation of excessive amounts of acetylcholine and histamine, and at least five deaths have been recorded" (Jaros et al., '52).

Until recently it was difficult to consume excess amounts of vitamins, especially the water soluble ones. Now as a result of the enrichment of foods (bread, cereals, etc.), or through indiscriminate use of vitamin preparations it may be possible to secure very large intakes of the water soluble vitamins. Many preparations today contain large amounts of the "B" vitamins. One preparation available without a prescription contains thiamine at a level of 1000 times the NRC allowances.

The few reports of animal studies involving the effect of massive doses of thiamine are inconclusive because of the variety of experimental conditions. Experiments have been conducted with cats, cows, chickens, dogs, pigeons, rabbits, guinea pigs, swine, mice, rats, even frogs and flies (Cerecedo et al., '44; Eisenstadt, '42; Haley and Flesher, '46; Smith et al., '48, '55; Kadner et al., '48; Science, '54). Different investigators used different criteria in defining an excess dose and used a variety of methods for administering the vitamin. The vitamin has been administered orally, intravenously, intramuscularly or subcutaneously. Injections were administered daily or every other day or several times a week. The size of the dose was arbitrarily decided upon or based on the weight of the animal or upon the amount of food or ration prepared for a certain period of time. One source (Barnes et al., '60) labels 4.0 mg. per kg. of diet excess thiamine. This amount is generally accepted as the requirement of thiamine for the albino rat. For no species has it been possible to find information on studies in depth.

Thiamine Toxicology

Acute Toxicity in Animals

Like the other water-soluble vitamins, thiamine seems to be well tolerated by the many species in which toxicity studies have been carried out. Hecht and Weese ('37) found that the ratio of the therapeutic dose to the minimum toxic dose in monkeys was approximately 1:5000.

The lethal dose in acute toxicity studies (Molitor and Sampson, '36) with mice, rats, rabbits, and dogs was reported to be 125, 250, 300, and 350 mg. per kilogram of body weight, respectively, when the thiamine was administered intravenously. Subcutaneously, the fatal doses were six times greater and orally, forty times greater. The signs of toxicity are shock, weakness, general muscular tetany, occasional cramp-like spasm, labored breathing and finally respiratory collapse. The heart continued beating from one to two minutes after respiratory failure, and the blood pressure generally fell only after breathing ceased completely.

The possibility of a cumulative toxicity from repeated administration of thiamine has been investigated by both Hecht and Weese ('37) and Haley and Flesher ('46). The daily administration of thiamine to rabbits, in doses of 50 mg. per kilogram for prolonged periods, produced no apparent abnormalities (Haley and Flesher, '46). These workers also found that doses of 200 to 300 mg. of thiamine given as a single intravenous injection resulted in collapse or death; however, recovery occurred if the injection was terminated before respiration ceased. They concluded that an injection of a sensitizing dose of thiamine one week before a toxic injection of thiamine apparently increased the resistance of the animal to the toxic thiamine injection. Their research showed: (1) the lethal dose of thiamine hydrochloride by intravenous injection into rabbits was approximately 126 mg. per kg. and (2) after a preliminary dose of 100 mg. of thiamine hydrochloride one week previous to the lethal dose, the lethal dose was then approximately 238 mg. per kg.

Chronic Toxicity in Animals

Female rats receiving approximately forty times the daily thiamine requirement throughout life exhibited a loss of maternal instinct and a failure of lactation in successive generations (Perla, '37; Richards, '45; Sure, '39; Scott and Griffith, '58; Morrison and Sarett, ' 59). Small quantities of manganese were found by Perla ('39) to reverse the latter effect of excess thiamine. Williams and Spies ('38) found the Sherman breeding diet (1/3 whole milk and 2/3 whole wheat bread) protective against a loss of maternal instinct and a failure of lactation, while

Richards found a white flour-casein diet unprotective. (Reproduction was affected adversely by high intakes of thiamine as evidenced by high mortality and low weaning weights of young.)

Scott and Griffith and Morrison and Sarett have investigated vitamin interrelationships when thiamine was given in large doses. Early findings indicated no effect on reproductive performance. Longer experimental periods are needed to investigate the same interrelationships for more conclusive evidence.

Acute and Chronic Toxicity of Thiamine in Human Subjects

Intolerance of human beings to thiamine develops relatively infrequently. The first report of a death in man (Mills, '41) followed an intramuscular injection of thiamine. In 1946 Reingold and Webb reported a death from the parenteral administration of 100 mg. of thiamine. Since the symptomatology of thiamine reactions was similar to some aspects of anaphylactic shock, the most likely explanation for the mechanism of the reactions seemed to be an anaphylactic one. In many cases immediate whealing on intradermal injection of thiamine occurred (Steinberg, '38; Kalz, '42; Eisenstadt, '42; Stein and Morgenstern, '44). Since the manifestations of the thiamine reactions were those known to occur with certain immunologic alterations and since these patients showed such immunologic alterations, it appeared reasonable to associate the two. It was conceivable that a combination of thiamine with protein developed which was antigenic to the host (Laws, '41; Schiff, '41; Stiles, '41; Stern, '38; Leitner, '43). The evidence at present, is not conclusive, and the anaphylactogenic properties of thiamine require further investigation.

In individuals, who appeared to be sensitive to thiamine, the pharmacological evidence came from the observation that thiamine overdosage produced a simple chemical toxicity thought to be due to the formation of excessive amounts of acetylcholine and histamine (Minz, '46; Jaros _et al._, '52). The sites of toxic action seemed to be both central and peripheral. Headache, hyperirritability, insomnia, rapid pulse, muscle tremor, weakness, and trembling were found (Stern, '38; Laws, '41; Schiff, '41; Stiles, '41; Leitner, '43). The toxicity of thiamine mononitrate was investigated in 1948 and was found to be essentially the same as that of the hydrochloride (Haley, '48).

In contrast to the above, no abnormalities were observed in normal individuals when doses were increased on a more or less experimental basis to a level as high as 500 mg. daily (Williams and Spies, '38) for one month. Jolliffe ('41) administered the vitamin to more than 3000 patients without mishap (parenterally, 10-30 mg. daily for 10 days to 3 months; parenterally, 20-50 mg. twice daily 3 to 6 weeks; parenterally, 20-100 mg. twice daily 3 to 6 weeks; 50-100 mg. three times daily two to seven days; if any disturbance were associated with the injection, thiamine was given by mouth, 2.5 mg. twice daily). Bicknell and Prescott ('46) have "given thiamine over a period longer than seven years without a case of intolerance developing."

Thiamine and Choline

In this research the purpose was to determine how dietary factors influence the toxicity of excess quantities of thiamine. One variable studied was dietary choline. A deficiency of choline induces fatty livers in albino rats, with the level of fat approaching three times normal. Not all species of animals need dietary choline but it has been shown to be essential in the ration of the rat (Best and Huntsman, '35). Choline deficiency in the rat results in growth retardation and in a marked accumulation of fat in the liver and an enlargement and hemorrhage of the kidney (Engel and Salmon, '41). The primary change in the liver is the uniform distribution of massive stores of fat in the liver cells.

Occasionally the central veins are extremely distended and filled with blood. Rats which survive an acute attack resume growth and survive.

The requirement for choline is related to the sex and age of the rat. Weanling female rats do not develop symptoms of choline deficiency. After thirty days of age, male weanling rats evidently begin to synthesize adequate amounts of choline from amino acids provided in the diet; the higher the casein level the more protective the diet. Griffith and Mulford ('42) indicated that a casein level of 30% or more would supply sufficient methionine to permit the omission of choline from the ration. However, when rats were fed an 18-24% casein ration, hemorrhagic degeneration could be prevented by one to two mg. of choline chloride daily and deposition of liver lipids prevented by four to six mg. of choline chloride daily. This indicated that the choline requirement for the prevention of fatty livers was two to three times that for the prevention of hemorrhagic degeneration. A choline deficiency has never been observed in man.

Scattered reports in the literature suggest a relation between choline and thiamine deficiencies. When an excess quantity of thiamine was fed, it also caused the deposition of fat in the liver (Whipple and Church, '36; McHenry, '35; McHenry and Gavin, '38; Gavin and McHenry, '40). Further observation indicated that as the thiamine stores were exhausted in a double deficiency of choline and thiamine, liver lipids decreased markedly. Thus, production of a fatty liver in choline deficiency depended on the presence of thiamine. Choline administration restored liver fat to normal levels.

Engel and Phillips ('39) observed that when thiamine was administered to vitamin B-1 deficient rats, hydropic degeneration and fatty metamorphosis occurred in the parenchyma of the liver. This histologic reaction in the liver was not prevented by choline. Chemical analyses on these livers showed that there was an increase in total fat, glycogen and moisture. The phospholipid and protein remained unchanged. Thiamine therapy in experimental vitamin B-1 deficiency causes an excessive production of free fat in the liver cell which disrupts normal cell structure. The question arose as to whether other B vitamins might be concerned with the metabolism of liver fat. Engel ('42) studied the relation of thiamine, riboflavin, pyridoxine, and pantothenic acid to the metabolism of choline by omitting them singly from the diet and comparing the results thereof with data obtained when all these nutrients were present in the diet. When thiamine, riboflavin or, pantothenic acid were omitted from the diet, approximately normal values for liver fat were obtained even though the daily choline chloride intake was only 2 mg. The need of additional dietary choline as a lipotropic agent was apparent when thiamine, riboflavin, and pantothenic acid were all present in the diet. The need for increased choline was correlated with the increased food consumption and increased body weight gains and reduction in liver fat. The omission of pyridoxine from the diet for a three-week experimental period had no effect on liver fat values.

Stetten and Salcedo ('44) regarded the quantity of fatty acids in the liver as the result of several processes all of which are presumed to be proceeding in the normal animal.

Depot Fatty Acids

Liver Fatty Acids

Synthesis   Diet Fat   Degradation

Any variation of experimental conditions may influence the rate of one or another of these processes. The fatty liver that is seen when thiamine is administered to rats fed a high carbohydrate diet may be assigned to an acceleration of fatty acid synthesis from carbohydrate precursors.

(That thiamine does indeed stimulate this process has been proven with the aid of isotopes (Boxer and Stetten, '44). Fatty liver of a choline deficiency has been shown to result from the impaired transport of fatty acids from the liver to the depots.

Addis *et al.* ('40) stated that the accelerating effect of vitamin B-1 on fat synthesis continued, although to a lesser extent, as amounts of the vitamin were ingested which greatly exceeded those generally considered as adequate.

According to Krider and Guerrant ('50) the deposition of fat in the carcasses of rats was influenced only by certain critical levels of thiamine which were related to the levels of fat in the ration. When they fed otherwise normal rations that contained either no or 10% fat, the levels of thiamine required for fatty carcass formation were 2-50 and 0-2 microgram per rat per day respectively. The results suggest that there is a certain level of thiamine intake, approximately 50 micrograms per day, at which a maximum deposition of fat occurred in rats and fat deposition was suppressed by high thiamine intakes (1000 micrograms daily) when the rats subsisted on the diets mentioned above.

Influence of Protein Level

Prior to the recognition of the choline-sparing effect of methionine, a number of studies indicated that the protein level of the ration influenced the formation of fatty livers in the absence of dietary choline.

The observation by Best *et al.* ('32) that fatty livers could be produced in rats which received a diet of mixed grains with 40% beef fat (contains enough choline to give each animal about 8 mg. per day) provided a means of studying the action of choline in preventing and curing such dietary fatty livers. A governing factor in fatty liver production when diets containing 40% fat are fed had been shown to be the choline content (Best and Huntsman, '35). With this finding in mind an attempt was made to produce dietary fatty livers in rats under conditions in which no loss of body weight occurred. For this purpose a diet of 20% casein, 30% starch, 40% beef fat, 5% salt mixture, 5% marmite and vitamins A and D was given. Except for the small amount of choline present in the marmite this diet was choline-free. Fatty livers did not result. These results suggested some factor other than the choline content influenced the production of fatty livers when diets contained 40% fat. Consideration of various possibilities suggested the level of protein intake as the most probable.

Channon and Wilkinson ('35) investigated the part played by protein in fatty liver production by feeding protein at 5, 10, 20, 30, and 50%. The fat content of the liver was 12.49%, 7.35%, 6.50%, 6.12%, and 5.60% respectively. These diets were substantially choline-free, for the choline intake of each rat in the different groups varied only between 1.42 and 1.70 mg. per day. It seemed clear that the amount of protein in the diet was a factor controlling the amount of fat appearing in the liver irrespective of any effect of choline, the degree of fat infiltration increasing with decreasing dietary protein content.

Such findings prompted the study of the components in the protein sources used which might contribute to the degree of fat infiltration into the animal tissues, especially the liver. It was found that a deficiency of the sulfur containing amino acids could bring about liver damage. It is not, however, the sulfur moiety in this instance which makes methionine, especially, important. Methionine contains methyl groups which are of importance to the animal economy. Until perhaps a decade ago it was assumed that animals required preformed methyl groups in their diet. This conclusion was based originally upon the observation that fatty livers and faulty growth which developed in animals lacking choline could be prevented not only by choline but also by methionine. Through isotopic studies it became clear that in living cells the methyl groups are transferred reversibly between methionine and choline. (Fischer and Garrity, '53; Griffith, '41; Griffith and Mulford, '41; Griffith and Wade, '39; Hale and Schaefer, '52; Harper et al., '54; Treadwell, '48; du Vigneaud et al., '40, '50, '51, '56), Therefore, when the ration contains low levels of protein, choline, in sufficient amounts, has a lipotropic effect as shown by the removal of fat from the liver. Choline does not produce any repair of the liver cells. When the ration contains low levels of choline and adequate protein, fatty livers do not develop.

Chemical Composition of the Liver

Although the resolution of the animal body into its components, both chemical and morphological, is generally regarded as only approximate because of individual variation there is an overall uniformity of mammalian tissues with respect to chemical composition. During the active growing period, the percentage of body fat generally increases, while the percentage of body water decreases. Moulton ('23) suggested that if the fat-free composition were considered, a point was reached when the concentrations of water, protein, and ash become more or less stationary, after which no further appreciable changes take place. He 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 animal to animal and from species to species. Spray and Widdowson ('50) reported that the mature female rat at the age of 200 days contained 23.7% fat; this percent of body fat was higher than that for the male rat of similar age. They suggested this value as the upper limit for the species since the percentage decreased in the older animals. Values of 13.4 and 12.8% for male rats at 246 and 321 days of age, respectively (Spray and

Widdowson, '50), are similar to the figure of 13.4% reported by Chanutin ('30) for adult animals. Hatai ('17) found only 5.7% fat in rats that were 294 days old. Donaldson ('24) and Deuel et al. ('44) reported that female rats contained more fat per 100 grams body weight than male rats (18.1% for males and 20.1% for females at the age of 84 days).

Spray and Widdowson ('50) 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. They found that the average content of water was 56% at the age of 120 days, with an increase to 60% at the age of 370 days. Hatai ('17) 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. ('44) reported that the average percent of water in rats studied in their experiment was 58.2 for males and 56.8 for females.

Spray and Widdowson ('50), Donaldson ('24), Chanutin ('30) and Deuel et al. ('44) showed that changes in protein values were reciprocal to those for water. This was demonstrated most clearly in the rat by Spray and Widdowson when the data for water and protein were calculated on a fat-free basis. Spray and Widdowson reported that rat carcasses on a fat-free basis contained 16.8% protein, a value similar to that reported by Hatai (15.7%) and by Chanutain (15.9%), a constant percentage being reached at an age of approximately 100 days. Deuel et al. found 17.3% protein in the males and 16.2% in the females. All workers agreed that the percent of protein remained relatively constant throughout the life of the mature rat.

Rathbun and Pace ('45) studied the water content and chemically combined nitrogen content of mammals fairly extensively. Their studies revealed that the proportion of these substances to body weight was constant, provided that the calculation was made on the fat-free basis.

No sex differences were found. They believed that the available experimental evidence supported the concept of a lean body mass that was relatively constant in gross chemical composition, in which body fat may be considered to act as a diluent. Spray and Widdowson ('50) confirmed this in their studies.

The ash content of the body of rats studied by Chanutin ('30) reached a maximum at the twentieth day of age, after which there was a gradual but marked decrease. Hatai ('17) reported that the mature rat contained 3.7% ash, which was similar to the results obtained by Chanutin, 3.8%. Deuel et al. ('44) found that the females had a higher percentage of ash than the males: 3.26 and 2.95% respectively.

The effect of dietary protein intake on the body composition of rats has been studied by Addis et al. ('36) and Kosterlitz ('47). They compared the chemical composition of the livers of rats fed an adequate stock diet with those of fasted rats and rats fed diets qualitatively and quantitatively deficient in protein. The percentage of water in the livers showed no significant difference among the individual groups of rats. The percentage protein in the livers decreased as the dietary protein level was reduced; the percentage glycogen increased. As the percentage protein content of the liver decreases the water-to-protein ratio increases.

The present research investigated the effect of massive doses of thiamine in the ration of the rat fed various protein levels for any possible effect on the chemical composition of the various tissues and also on the water-to-protein ratio in the various tissues of the rat.

Influence of the Carbohydrate

A limited study was undertaken to investigate the influence of dietary carbohydrate on the toxicity of large doses of vitamin B-1 because a number of investigators had shown the superiority of dextrin and cornstarch on the growth of rats and chicks as compared to sucrose (Lepp *et al.*, '47; Monson, Dietrich and Elvehjem, '50; Krehl, '46; Henderson, '47; Hankes, '48; Lyman and Elvehjem, '51; Hall and Sydenstricker, '46; Litwack *et al.*, '52; Harper *et al.*, '53). In many instances these investigators found that polysaccharides in the diet reduced the amount of fat in the liver compared to sucrose (Lepp *et al.*, '47; Monson, Dietrich and Elvehjem, '50; Krehl, '46; Henderson, '47; Hankes, '48; Lyman and Elvehjem, '51; Hall and Sydenstricker, '46; Litwack *et al.*, '52; Harper *et al.*, '53). These workers suggested that the action of dextrin and cornstarch was due to some effect on the gastrointestinal tract promoting more efficient utilization of the protein. Greater quantities of certain essential amino acids or other nutrients seemed to be made available to the rats and chicks (Elvehjem, '48; Baxter, '47).

Yoshida, Harper and Elvehjem ('58) and Harper and Spivey ('58) suggested that the osmotic differences of certain dietary constituents, dextrin for example, affected the amount of water retained in the stomach. When the osmotic effect of the diet was reduced young rats ate, digested, and assimilated a greater quantity of the diet and hence, gained weight more rapidly during the early stages of growth.

Litwack *et al.* ('52) and Harper *et al.* ('53) investigated the liver xanthine oxidase activity of rats fed various protein diets containing either dextrin or sucrose. They measured liver xanthine oxidase activity as an index of protein utilization. Protein sparing effect was shown in the xanthine oxidase values they secured.

Thiamine Excretion and Tissue Deposition

Influence of Dose

Thiamine is excreted in the urine in amounts that are dependent upon a number of factors. Thiamine has been reported to be a diuretic

(Hecht and Weese, '37) possibly through a central rather than renal effect (Fiorio, '38). Outstanding among the early results described was the condition of excretion equilibrium (Westenbrink and Goudsmit, '37, '38; Light _et al_., '38). Light and his group showed a straight line relationship between intake and excretion of thiamine over a range of 15 gamma to 500 gamma of thiamine intake. Leong ('37) using the bradycardia method of assay, reported balance studies in which adult rats were given, in their diet as well as subcutaneously, doses of thiamine ranging from 0 to about 700 I. U./rat daily.[1] The quantity in the feces was 0.5 to 1.5 I. U. per day, depending on the bulk of the feces. But urinary excretion became larger as the intake increased. When scarcely any thiamine was given the urinary excretion amounted to 0.1 to 0.4 I. U. of thiamine daily. When the intake ranged from 7.5 to 31 I. U. daily, urinary output increased from 0.6 to 12.8 I. U. When 250 I. U. were injected subcutaneously the amount excreted was about 75% of the intake. A large percentage of this was in the urine. But with oral ingestions of 100-700 I. U. daily, about 30 I. U. were unaccounted for in the excreta, thus indicating the approximate amount of destruction in the body. Leong estimated, however, that in cases of thiamine deficiency of the diet a greater percentage of the vitamin withdrawn from the tissues was destroyed.

The significance of these data is indicated in relation to the utilization and retention of thiamine in man. For example, Knott ('36), on the basis of twenty-three balance studies conducted with eight children from four to seven years of age, concluded that the body is not capable of building up a significant reserve of thiamine. The results indicated a definite trend toward higher apparent retentions of thiamine when the level of intake was relatively high. The levels which resulted in the highest

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[1] 1 I. U. = 3 micrograms B-1 (thiamine).

retentions were found to be about 27 I. U. per kilogram of body weight. These findings have been supported by those of Roscoe ('36) and Harris and Leong ('36). Roscoe, using rat curative tests, could not find more than traces of thiamine in the urine from normal adults receiving a hospital diet. The addition of 720 I. U. of thiamine daily to this diet was followed by the daily excretion of 167 to 333 I. U. in the urine. Owing to the inadequacy of the hospital diet in meeting the tissue needs for thiamine Roscoe believed was the reason more of the supplemented thiamine was not excreted. Harris and Leong, in searching for a simple method of estimating the state of thiamine nutrition in the human subject also found a marked correspondence between the amount of thiamine in the diet and the level of excretion in the urine.

Benson et al. ('42) stated that the best indication of tissue saturation or unsaturation was obtained by determining the percentage of dietary thiamine intake excreted in the urine. Children on an adequate diet (45 mcg. thiamine per 100 calories) who excreted less than 20% of the thiamine intake, required supplementation of the diet with thiamine to secure tissue saturation. Children convalescing from acute illnesses and some children with chronic illnesses excreted less than 20% of the thiamine intake in the urine. These children required thiamine supplementation for short periods.

Stearns et al. ('58) in their studies with children found that the excretion of thiamine varied directly with the intake. They found evidence of storage in growing children if the thiamine intake was ample. The criterion used was: if the urinary excretion of thiamine was held at or above 20% of the intake of thiamine this was evidence that there had been ample intake for some time; urinary excretion of 15% or more of the intake of thiamine indicated immediate adequacy of ingestion; there should be concern if urinary excretion were less than 10% of intake.

Influence of Mode of Administration

The size of the dosage and the method of administration of thiamine seem to be important factors in the level of thiamine excretion. Mason and Williams ('42) believe that the urinary excretion of thiamine reflects the intake of thiamine during and just prior to the time of a test unless there has been an abrupt change of some magnitude in intake. They suggest that a test dose should be near the individual's physiologic requirement for thiamine and that it should be given parenterally to insure complete and prompt absorption. Mason and Williams also found the intramuscular injection of 1000 gamma satisfactory for complete and prompt absorption. Chaney ('60) indicated that experiments performed on human subjects suggest a 5 mg. dose of thiamine as the maximum dosage for complete absorption (Melnick et al., '45; Alexander et al., '46; Friedemann et al., '48). Some of the vitamin is normally excreted in the feces; some is destroyed in the colon.

Ritsert ('38, '39) in studying the excretion of thiamine administered perorally and parenterally found that on a normal diet fecal excretion varied between 40 and 170 gamma/100 gm. feces or 100-400 gamma in 24 hours with an average of 180 gamma in 24 hours. When man was given 10 mg. thiamine orally, he excreted 4-6.5% of the thiamine in the urine and 20-25% of it in the feces. After a parenteral administration of thiamine, man excreted 25% in the urine, the greater part in one to two hours. Fecal excretion of thiamine did not increase.

Rats excrete, according to Ritsert, 78-84% of one mg. of thiamine in the urine after an intravenous injection. When rats are killed five minutes after an injection of 100 gamma and 1000 gamma, 85 to 90% can be recovered, with the largest increases in the liver, kidneys, muscle, testes, skin and the gastrointestinal tract with little or none of it in the heart, brain, spinal cord, lungs, and spleen.

Since 1960 Morrison with various associates has pursued the problem of administering thiamine for most complete and prompt absorption. In animal studies, Sarett and Morrison ('60) found that, in growing rats, the utilization of B vitamins was similar if the vitamins were given in the diet, or once daily by stomach tube. Later Morrison and Campbell ('60) reported a study on the effects of the size of the thiamine dose and the mode of administration on the urinary excretion of thiamine in normal male subjects, receiving nutritionally adequate diets. The men were given oral doses of one to 20 mg. of thiamine. The urinary excretion of the vitamin was determined until excretion levels returned to those found prior to dosing. The excretion of thiamine, expressed as percentage of dose, decreased markedly with doses greater than 2.5 mg. Increasing the dose from 2.5 mg. to 20 mg. increased the amount of thiamine excreted by only 0.2 mg. Excretion of thiamine after a 10 mg. dose was increased threefold by giving the vitamin in four doses of 2.5 mg. each, at two-hour intervals. Preparations designed to produce sustained release of thiamine in a single dosage form were found to vary markedly in availability and showed no evidence of sustained-release properties.

Middleton and Morrison ('62) gave thiamine-deficient weanling rats thiamine by stomach tube or subcutaneous injection at one, two or four-day intervals. The animals given thiamine daily or every other day, by oral or subcutaneous administration, grew at comparable rates. Although rats given thiamine subcutaneously every four days grew at similar rates to those receiving it daily, oral administration at four-day intervals significantly reduced weight gains. Less liver and carcass thiamine was found when thiamine was digested and absorbed through the normal body processes.

Pollack _et_ _al._ ('41) studied the urinary excretion of thiamine and pyrimidine of a group of patients under various conditions. Complete deprivation of thiamine changed the urinary excretion ratio of thiamine to pyrimidine from 9:1 to 1:9. The amount of pyrimidine excreted remained about the same while free thiamine almost disappeared--indicative of a recent deprivation. If both had been below normal this would have indicated a protracted insufficiency of thiamine. A load test was used before the 10 day deficiency study and after 10 days of deficiency and the excretion ratios were exactly the same. This showed that the basic state of nutrition for thiamine was unchanged. This group felt that pyrimidine excretion also indicated how thiamine was utilized. When large doses were given there was a decrease in thiamine utilization. Therefore the dosage should be divided when thiamine is administered.

Borsook et al. ('40) and McCarthy et al. ('54) studied the course of thiamine metabolism by use of thiamine prepared with radioactive sulfur. They found that there was a rapid interaction of injected thiamine with thiamine pyrophosphate present in the blood and tissues, and a rapid destruction of thiamine. This destruction yielded neutral sulfur compounds and inorganic sulfate in the urine. Normal rats maintained on a complete thiamine-sufficient diet excreted in the urine 64.38% of a dose of thiamine during a period of 10 days after an intramuscular injection. Sixty-two percent of the radioactivity was recovered in the neutral sulfur fraction, while the remainder was oxidized. Thiamine-deficient rats in the same period of time excreted in the urine 52% of the original radioactive sulfur, 46.5% being found as neutral sulfur compounds. Very low recoveries (1.4% of the dose administered) were obtained from the feces. The results of Borsook et al. were essentially the same when feeding labeled thiamine to humans.

Both Ochoa and Peters ('38) and Westenbrink and Goudsmit ('38) investigated the thiamine-cocarboxylase content of animal tissues. The liver of rats fed a thiamine-free diet for three weeks, contained only a trace of cocarboxylase. Very shortly after a subcutaneous injection of thiamine, however, the liver contained a large amount of cocarboxylase. The cocarboxylase content of the kidneys increased rapidly also. The increases in cocarboxylase were observed in the liver and kidney only. No other tissues showed an increase during the short interval between injection and measurement.

Influence of Dietary Constituents

Robinson et al. ('40) studied urinary thiamine excretion in various clinical conditions before and after an oral test dose of 5 mg. of thiamine. Excretion was low in alcoholic beriberi, cardiac decompensation and peptic ulcer conditions. Excretion of thiamine was normal in patients with regulated diabetes, hyperthyroidism and peptic ulcer patients given an oral test dose of thiamine.

The type of dietary intake has been studied as a possible factor governing thiamine excretion by Reinhold et al. ('44). They investigated the utilization of thiamine in human beings living under carefully controlled conditions in which the alteration in the carbohydrate-fat relationship of the diet was the only variable. The results showed that increased amounts of thiamine were utilized when the proportion of carbohydrate in the diet was high. Any "sparing action" of fat appeared to be related to a coincident decrease in the carbohydrate content of the diet. Meghal and Nath ('63) in a recent study of the effect of different food fats and the essential fatty acid, linoleic acid, on tissue thiamine content found hydrogenated ground nut oil plus linoleic acid produced the greatest deposition of thiamine in tissues with the cecum containing the highest amount and the liver next, heart and finally muscle.

Earlier Nath and Meghal ('61) fed male weanling rats thiamine-free diets for a time, then divided them into the following groups: control, 80% protein in the form of casein; 70% protein plus either 10% honey, glucose, sucrose, cellulose or pectin. Coprophagy was not prevented. There was a decrease in urinary and fecal thiamine in the glucose, sucrose and honey-fed rats; the first two groups also experienced effects of a thiamine deficiency. Cellulose and potato starch-fed animals grew well, showing no signs of a thiamine deficiency. The pectin-fed group showed a constant rise in urinary and fecal excretion of thiamine showing an enhanced effect of pectin in the biosynthesis of thiamine. Evidently when the hydrolysis products of pectin digestion were liberated they had a favorable effect on the intestinal flora, increasing fecal thiamine which was available to the rats after coprophagy.

Williamson and Parsons ('45) in general, found no correlation between variations in urinary and fecal thiamine output in humans while eating high and low fiber diets. Hathaway and Strom ('46) reported similar data on human subjects using natural diets.

Using the paired feeding technique in adult albino rats, Sure and Ford ('42, '43) studied vitamin interrelationships as observed in urinary excretion during periods of a deficiency of one vitamin. In thiamine deficiency there was a pronounced disturbance in riboflavin metabolism mainly because of poor absorption. However, in riboflavin deficiency there was no disturbance in thiamine metabolism. Subcutaneous injections of synthetic thyroxine to adult albino rats for 17-21 days resulted in the excretion of large amounts of riboflavin in the urine. Since hyperthyroidism also produced large losses of body weight and of riboflavin from many of the tissues (expressed as percentage of change in riboflavin content in experimental animal over control animal), the increased excretion of riboflavin in the urine may have been caused by body tissue catabolism. Hyperthyroidism, however, caused negligible changes in the level of thiamine in the urine and much smaller losses of this vitamin from the tissues.

Influence of Bacterial Synthesis

It has been suggested by Najjar and Holt ('43) that the biosynthesis of thiamine in man must be considered a possible factor in the balance of thiamine intake-thiamine excretion. They studied nine sedentary young men between the ages of 16 and 23 in an institution for eighteen months, on a diet of 40 calories/kg. and reduced the thiamine intake gradually from 1 mg./day to 0.2 and 0.1 mg./day. Thiamine excretion in the urine fell to negligible figures at 0.4-0.6 mg/day intake yet no deficiency of thiamine was encountered. When thiamine was completely withdrawn, four of the men still developed no deficiency. Free thiamine was found in their stools. Najjar and Holt believed the thiamine in the stools could be from bacterial synthesis of thiamine in the intestinal tract or that the body stores were not completely exhausted. One subject was given sulfathiazole by mouth (1.5 gm. every four hours for one week). There was a prompt reduction in free thiamine in the feces. Najjar and Holt do not believe that thiamine requirements can be met for an indefinite period by thiamine formed by intestinal bacteria, but that certainly this carries an implication for human nutrition and the discrepancies in individual thiamine requirements. Others have shown practically no absorption of thiamine given in an enema retained for a number of hours, and given as far up the colon as possible (Alexander and Landwehr, '45).

Level of B-1 Absorption from the Intestine

It was mentioned previously that Schultz *et al.* ('39) had tried to establish for the rat a level at which the rat would not absorb further thiamine from the intestine. Schultz, Light and Frey in 1938 had shown that such a point existed in man, but for the rat these workers found a straight line relationship between intake and excretion. Mickelsen, Caster and Keys ('47) showed that in normal young men the relationship between thiamine intake and excretion began to level off (24 hour excretion) at a thiamine intake of approximately 2-3 mg. per day. It would seem that the rat has a different mechanism for handling thiamine absorption and excretion than does the human.

Storage of Thiamine in Body Tissues

The amount of thiamine stored in the body of the rat is relatively small (Leong, '37). Most of the reserves are lost in about ten days on a deficient diet. In a fully saturated rat, the liver, heart, and brain contain similar amounts of thiamine, approximately 4.3 gamma per gram of tissue. The concentration found in muscle (1.2 gamma per gram) is considerably lower. Nevertheless, the combined musculature contains about 50% of the total thiamine content of the animal and the liver only 25% (Leong, '37).

The University of Texas group gave the following thiamine content in micrograms per gram of moist tissue of normal rats:

Tissue	Male	Female
Liver	7.8	7.4
Kidney	3.9	4.4
Spleen	3.0	2.5
Heart	7.0	7.6
Lung	2.3	2.6
Brain	4.8	4.1
Muscle	1.3	1.2

Although this group stated that rat testes were abnormally high in thiamine content they did not indicate a value for this tissue.

The thiamine content of blood as examined by various workers follows. All values have been converted to gamma/100 ml.

Investigator	Method	Species	Whole Blood
Deutsch ('45)	Phycomyces	Man	9 - 16
Meiklejohn ('37)	"	"	6.5-14
Rowlands and Wilkinson ('38)	"	"	6.5-16.5
Sinclair ('39)	"	"	5.5-10.5
Benson *et al.* ('42)	Thiochrome	"	4.8-12.3
Friedemann and Kmieciak ('39)	"	"	3.0-11.2
Hennessy and Cerecedo ('39)	"	"	9 - 12
Pence *et al.* ('45)	"	"	8 - 9
		Ox	5.7
		Pigeon	20.2
		Rat	7.0
		Pig, semi-fast.	17.0
		", non-fast.	21.0
Ritsert ('39)	"	Man, rabbit, and sheep	3.0-15
Goodhart and Sinclair ('40)	Cocarboxylase	Man	4.5-12
Westenbrink *et al.* ('38)	"	"	9.0-13.5

Baker *et al.* ('61) using a microbiological assay with 0. malhamensis indicated these values:

Whole blood from 28 subjects 2000 - 4100 gamma thiamine/100 ml.
Serum from 28 subjects 300 - 1500 gamma thiamine/100 ml.

All investigators, with the exception of Baker *et al.*, indicated a range of from 3 to 16.5 gamma thiamine/100 ml. of whole blood. Baker *et al.*, values were almost 1000 times higher than those reported by others. The use of a different analytical technique or an error in their calculation may account for the different values. Baker *et al.* made no comparison of their data with that of others.

Greenberg and Rinehart('45) and Burch *et al.* ('52) found that the blood of the rat reflected more sensitively the changes in the dietary intake of thiamine than the blood of man and also that the blood thiamine level of the rat paralleled tissue concentration of the vitamin during periods of depletion. Burch and her group found this to be true whether whole blood, the red blood cells or white blood cells and platelets were examined.

Kirk in examining middle aged and older individuals ('49) noted a slight tendency for the thiamine value of blood to decrease with age whereas the pyruvic acid level showed no change with advancing years. No significant correlation was observed between the thiamine and pyruvic acid values in the same individual.

When doses of thiamine were administered and the entire wet tissue examined the greatest amounts were obtained in the testes, liver, kidneys, heart, brain, and muscle (Schultz et al., '39; McCarthy et al., '54).

Pyke ('40) examined the thiamine content of various muscles and found the thismine content of muscle inversely related to the glycogen level. In the rat he found a range of 2 to 35 I. U. thiamine/100 gm. of various muscle tissue; in the cardiac muscle Pyke found 100 I. U. thiamine/100 gm. Unfortunately Pyke did not also include glycogen levels in his data. Leong earlier reported a concentration of 60 I. U. thiamine/100 gm. of muscle tissue.

Unlike other tissues studied, (Salcedo et al., '48) the brain maintained its thiamine concentration in the face of a deficit of thiamine for a considerable period after which an abrupt fall occurred. The critical point at which the brain began to lose thiamine corresponded to a minimum level of urinary thiamine excretion, a point of physiological significance and a useful criterion for measuring thiamine requirements under various conditions.

Dreyfus ('59) in his study of the quantitative determination of total thiamine in subdivisions of the nervous system found the thiamine content of the liver was the highest, followed by the kidney, heart, brain and muscle. Dreyfus stated that there appeared to be little, if any, correlation between total thiamine content of any given subdivision of the brain and its susceptibility to thiamine deprivation. The heart, brain and peripheral nerves are known to be exceedingly vulnerable to thiamine deficiency, yet only the heart nerve contains a high concentration of total thiamine, the brain and peripheral nerves, respectively, reveal intermediate and low concentrations.

EXPERIMENTAL PROCEDURE

Male weanling rats of the Sprague-Dawley strain weighing between 45 and 55 grams were used in these experiments. The rats were housed individually in cages with one-half inch raised wire-mesh bottoms. The rats were allowed food and water ad libitum. The room was air conditioned and maintained at a temperature between 74 and 76° F.

Approximately 600 animals were used in these studies. There were eight to thirty rats to a group. They were distributed by weight so that the average weight of the animals in each group in each experiment did not exceed that of any other by more than one to 1.5 grams. Individual weights, in grams, were obtained twice a week for each animal. These were averaged for each group.

The composition of the diets fed the rats is given in Table 1. In addition to the carbohydrate, casein, choline and thiamine each diet also contained the following: corn oil, 5%;[1] salts W, 4%;[2] vitamin mix, 0.25%. The composition of the vitamin mixture in milligrams per kilogram of ration appears in Table 2. All changes in the diets were compensated for by adjusting the level of carbohydrate as shown in Table 1. The protein content was kept the same as the basal diet for each particular series.

At the end of an experimental period the rats were stunned, decapitated, blood was collected and the tissues removed and weighed.

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[1]Containing 75 mg. of alpha-tocopherol.

[2]L. G. Wesson, 1932. A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. Science 75:339. Purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

Table 1. Composition of Diets

Diet	Carbohydrate	Casein %	Choline[1] %	Thiamine γ/gm.
1	Sucrose	6	0.15	4.0
2	Sucrose	6	0.15	2004.0
3	Sucrose	6	0.15	3004.0
4	Sucrose	6	0.15	4004.0
5	Sucrose	6	0.15	5004.0
26	Sucrose	6	0.15	10,004.0
6	Dextrin	6	0.15	4.0
7	Dextrin	6	0.15	2004.0
8	Dextrin	6	0.15	3004.0
9	Dextrin	6	0.15	4004.0
10	Dextrin	6	0.15	5004.0
11	Sucrose	12	0.15	4.0
12	Sucrose	12	0.15	2004.0
13	Sucrose	12	0.15	4004.0
14	Sucrose	12	0.15	5004.0
15	Sucrose	12	0.15	10,004.0
16	Sucrose	30	0.15	4.0
17	Sucrose	30	0.15	2004.0
18	Sucrose	30	0.15	4004.0
19	Sucrose	30	0.15	5004.0
20	Sucrose	30	0.15	10,004.0
21	Sucrose	20	0.15	4.0
22	Sucrose	20	0.00	4.0
23	Sucrose	20	0.15	1004.0
24	Sucrose	20	0.00	1004.0
25	Sucrose	20	0.15	4.0 + 1005.0 niacin
27	Sucrose	20	0.15	74.0
28	Sucrose	20	0.15	39.0
29	Sucrose	20	0.15	21.0
30	Sucrose	20	0.15	12.0
31	Sucrose	0	0.15	4.0

[1]1.0 milliliters of 15% choline solution.

Table 2. Vitamin Mixture[1] (mg./kg. ration)

Vitamin A	25.0
Calciferol	1.0
Thiamine	4.0
Riboflavin	8.0
Niacin	5.0
B-6	2.5
Ca Pantothenate	20.0
Inositol	10.0
Folic Acid	0.2
B-12	0.02[2]
Biotin	0.1
PABA	2.0
Menadione	4.0

The above vitamins are mixed with enough sucrose to make the total weight 2.5 gms. To insure accurate weight of such small quantities of vitamins, 50 or 100 times each quantity was weighed. Then the mixture was placed in a brown bottle and stored in the refrigerator until used.

[1]R. A. Brown and M. Sturtevant, 1949. The vitamin requirement of the growing rat. Vitamins and Hormones 7:171.

[2]20 mg. of a 0.1% trituration with mannitol.

Experimental periods were two, three, four and six weeks in length. In the longer experimental period, approximately five rats from each group were sacrificed each week for a period of six weeks.

Most of the studies were two weeks in length. Animals fed 20% casein rations with and without choline and with and without a 0.1% level of thiamine in the first study (Diet Nos. 21, 22, 23, and 24) showed adaptation to the various dietary variables (excess thiamine, deficiency of choline) when sacrificed at the end of three weeks. No excess deposition of fat in the liver was found. The next study was designed to sacrifice a certain number of rats on each diet every two weeks. From the analyses made on the livers of rats receiving choline-free rations (Diet Nos. 22 and 24) it was learned that the greatest deposition of fat occurred during the second week. Thereafter most experiments were set up for a period of two weeks.

When the tissues were removed from the animal, they were rinsed in water, blotted free of excess moisture and weighed. They were then homogenized with water in a Potter-Elvehjem homogenizer or a Micro-Blender and frozen until analyzed for total fat, nitrogen, ash, and thiamine. The frozen homogenates, prior to analysis, were thawed at room temperature. Except when analyzing for thiamine, the tissues were transferred quantitatively to an evaporating dish and evaporated to dryness in twelve hours at 90° C. The dried residues were weighed and ground in a Wiley mill with a 40 mesh screen. One gram samples were weighed for fat extraction, with diethyl ether as the solvent, in the Goldfisch apparatus.

Twenty-five hundredths of a gram of the fat extracted liver was weighed for nitrogen determination by the macro-Kjeldahl method; three to seven milligrams of the fat extracted liver was weighed on a micro-balance (Cahn Electro-Balance) for nitrogen determination by the micro-Dumas method. The latter method was used one term when the macro-Kjeldahl equipment was not available for use. The manufacturer states that "the instrument permits nitrogen determinations down to the 0.01% nitrogen level and yields results within ± 0.20% of theory for routine materials."[1] The instrument was checked by the writer with ammonium sulfate, acetamide, and samples previously determined by the macro-Kjeldahl method. In all instances the results obtained by the two methods were comparable.

One gram samples were weighed into vicor crucibles and placed in a cold muffle furnace, then heated to 525° C. They were maintained at this temperature overnight. The crucibles were then transferred to a desiccator, cooled before weighing, and the percent ash was determined.

The determination of thiamine in urine, feces, blood and tissues was carried out by means of the Thiochrome Technique (Association of Vitamin Chemists, Methods of Vitamin Assay, second edition, 1951). The thiochrome procedure depends upon the oxidation of thiamine to thiochrome which fluoresces in ultraviolet light. Under standard conditions and in the absence of other fluorescing substances, fluorescence is proportional to the thiochrome present, hence to the thiamine originally in the solution. Successive steps involve extraction of thiamine from the tissues, purification, conversion to thiochrome, extraction of the thiochrome into isobutyl alcohol, measurement of the fluorescence and calculation of the thiamine. This method eliminates all or most of the interfering fluorescent substances through use of an alkaline solution at the final extraction stage with isobutanol. This method never yields negative values and allows satisfactory recovery of added thiamine.

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[1]From "Operating Directions for the Coleman Model 29 Nitrogen Analyzer," Coleman Instruments, Inc., 42 Madison St., Maywood, Ill. May, 1960.

Blood also was examined for the various protein fractions (total protein, albumin, gamma globulin, alpha 1 and 2 globulins and beta globulin). Blood was allowed to clot and placed in a warm room at 40°C. for a short time, then refrigerated overnight to allow complete clot retraction. The serum was removed by pipette. If the serum was to be used within one week, it was stored in a walk-in cooler at 40° C. If the length of storage was to be longer than one week, the samples were stored at minus 20° C.

The protein fractions were separated on a Spinco, Model R, paper electrophoresis system (Spinco Technical Bulletin 6027 A) at room temperature. A constant current of five milliamperes per cell was maintained for sixteen hours on Spinco no. 300-846 paper strips using veronal buffer of pH 8.6 and ionic strength of 0.075. This buffer was made up of 2.76 gms. of diethyl barbituric acid and 15.4 gms. of sodium diethyl barbiturate in one liter of distilled water. The buffer solution may be used twice in the same cell by reversing the current within the cell.

After sixteen hours, the strips were dried for 30 minutes in a forced draft oven at 110° C. in order to denature and fix the position of the proteins. The temperature and time were kept constant because these factors were found by Henry et al. ('57) to influence the results secured. These workers reported that for every degree change in temperature between $100-120^{\circ}$ there was a one percent increase in the albumin/globulin ratio. Approximately 0.008 ml. of serum was applied to each strip. One serum sample was run from each animal.

The staining method used was that described in the Spinco Technical Bulletin 6027 A. Bromphenol blue was used to render the proteins visible. The rinse consisted of five percent glacial acetic acid. The strips were then blotted and placed in the oven at 110° C. for fifteen minutes. After development of basic color by use of NH_4OH, relative intensities or concentrations of the separated proteins were determined by scanning the stained strip with the Spinco Model RB Analytrol.

In urine the major metabolites are thiamine itself, pyrimidine and thiazole. Through the use of radioactive sulfur Borsook et al. ('40) and McCarthy et al. ('54) have reported others: inorganic sulfate, ethereal sulfate, and neutral sulfur components. Presently there is no way of checking the latter three. To determine pyrimidine and thiazole the yeast resynthesis method was used. According to this procedure, yeast cells are incubated with the urine sample in one case with an excess of the thiazole component of thiamine and in another with an excess of the pyrimidine component. By subtracting the thiamine originally present in the urine and in the yeast, values can be secured for the pyrimidine and thiazole components, respectively, present in the urine. The thiamine thus formed may be assayed by the thiochrome procedure, already described. The advantage of this method is the simultaneous measurement of all three moieties made possible by the yeast's synthesis of thiamine from pyrimidine and thiazole.

Data were expressed as means for weight gains per week, ration eaten per week, and the composition of the wet tissues (heart, kidneys, muscle, mainly liver). The values for the latter are presented as percent fat, moisture, nitrogen, and ash. The nitrogen was multiplied by the factor 6.25 to convert these values to protein. The difference between the sum of the above values and 100 was considered to be glycogen.

Standard errors were calculated for all means and the Student "t" test was used as a measure of significance.

RESULTS AND DISCUSSION

Introduction

The purpose of this research was to explore the possible toxicity that might be associated with excess consumption of thiamine in the ration of the albino rat. Four mg./kg. of diet is generally accepted as the requirement of thiamine for the rat. The basal diet (containing 4 gamma of thiamine/gm. of ration) was supplemented with added thiamine to produce the following concentrations: 12, 21, 39, 74, 1004, 2004, 3004, 4004, 5004, to 10,004 gamma of thiamine/gm.of ration, a range of from 3 to almost 3000 times the thiamine requirement of the rat.

Evidence of toxicity was studied in the rate of weight gain (growth), food intake, changes in the fat, nitrogen, and moisture content of the liver, changes in the composition of other tissues in addition to the liver (moisture, protein, fat and ash in heart, kidneys, liver and thigh muscle), and changes in the serum protein composition. In addition a study was made of the excretion pattern of thiamine and its two main metabolites in the urine, of the percent of recovery of thiamine in urine and feces, and of the concentration of thiamine in certain body tissues: adrenals, brain, heart, kidney, liver, muscle, testes and blood. Briefly, the following conclusions regarding the toxicity of large doses of thiamine were drawn from this research:

1. The addition of very large amounts of thiamine to rations containing various levels of protein had no deleterious nor beneficial effect on the growth of rats.
2. The large doses of thiamine had no appetite-stimulating activity.

3. High levels of thiamine did not contribute to the deposition of fat in the liver, nor did they alter the concentration of nitrogen and moisture in the liver.

4. Very high levels of thiamine had no effect on the composition of tissues as measured by the tests used in this research.

5. The level of thiamine in the diet had no influence on the percentage of various protein fractions in blood serum.

6. Increased thiamine in the diet increased the thiamine level in all tissues examined except the adrenals. Greatest concentration of thiamine was found in the liver, kidneys, and testes.

7. Tissues from the rats fed the basal rations which contained 4 mg. of thiamine/kg. showed an inverse relation between the protein level in the ration and the thiamine concentration in the tissues. This was most dramatic in the liver and thigh muscle and less pronounced in the heart tissue.

8. There was a direct correlation between the level of thiamine in the blood and the protein level in the ration. The concentration of thiamine in the blood followed the increased intake in the diet.

9. With increased levels of dietary thiamine both urine and fecal thiamine excretion increased, but that for feces increased faster than that for urine.

10. The excretion of pyrimidine, thiazole, and thiamine in the urine increased as the dietary level of thiamine increased, but there was a dietary level of thiamine below which very little urinary thiamine was seen. The protein level in the ration had a pronounced effect on urinary pyrimidine, thiazole, and thiamine excretion.

11. There was a decrease in the growth of the rat the first two weeks following weaning when choline was omitted from the diet. The addition of high thiamine without choline did not improve the growth during these same two critical weeks.

These points will be developed more fully in the pages to follow.

One variable studied in this research was dietary choline. A deficiency of choline induces fatty livers in albino rats. Up to three times the normal quantity of fat is deposited in livers of rats fed a choline deficient diet. Since scattered reports in the literature suggested an excess quantity of thiamine also caused fatty livers, a study was designed to determine whether there was any relation between dietary thiamine and choline.

Various protein levels were studied to determine whether large doses of thiamine would be more toxic when consumed with a low protein or a high protein diet. Casein was chosen as the protein source. In addition, observations made in the first low protein experiment led to questions which appeared important for further exploratory purposes. Observations to be further pursued were: growth of the rat, deposition of fat in the liver, the concentration of nitrogen and moisture in the liver, the concentration of protein fractions in the blood serum, the chemical composition of tissues, urinary and fecal thiamine and urinary pyrimidine and thiazole excretion.

Average Gains in Weight in Grams per Week

Growth data as affected by various supplements of thiamine added to diets containing various levels of casein with sucrose or dextrin as the carbohydrate are shown in Tables 3, 4, 5, 6, 7, and 8 and Figures 1, 2, 3, 4, 5, and 6.

Three problems were investigated in the several experiments using 20% casein diets (Table 3). The first was that of the effect produced by the supplementation of thiamine in the ration at levels above the 4 mg./kg. in the vitamin mix. Although one of the functions of thiamine is to promote growth in rats, there are no indications from this study that excessive intakes of this vitamin produced any growth stimulation in weanling rats over and beyond that produced by a ration containing what is considered an adequate intake. For the first two weeks, there were variations in the weight gains of the rats fed the rations containing

Animals		Diet		Weeks				
		No.	Description	1	2	3	4	Total
Trial 1	10							

Table 3. Average Gains in Weight (gms. per week) of Rats Fed 20% Casein Diets with Supplements of Thiamine and/or Niacin; With and Without Choline. In All Rations the Carbohydrate was Sucrose.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total
Trial 1 10 Trial 2 31 Trial 3* 10/51	21	Basal, 20% casein	26.8±2.6	41.8±2.0	40.4±2.5	34.2±2.3	143.2±9.4
Trial 1 5 Trial 2 33/38	22	Basal, no choline	23.8±2.3	35.6±2.6	41.1±2.3	37.8±2.4	138.3±9.6
Trial 1 10 Trial 2 31/41	23	Basal, + 0.1% B_1	31.2±2.8	42.8±1.9	42.3±2.2	34.9±3.7	151.2±10.6
Trial 1 8 Trial 2 32/40	24	+0.1% B_1 Basal, no choline	22.8±2.0	35.0±2.3	42.9±2.4	34.5±3.3	135.2±10.0
31	25	Basal, + 0.1% niacin	34.4±1.3	40.4±1.5	42.8±1.7	36.6±2.2	154.2±6.7
10*	21	Basal, 20% casein	23.5±2.8	41.2±1.8	37.5±2.4	32.9±2.5	135.1±9.5
10	30	Basal, +0.0008% B_1	22.7±2.7	37.8±2.2	32.5±3.4	36.7±3.2	129.7±11.5
10	29	Basal, + 0.0017% B_1	20.7±3.1	40.0±1.8	35.7±3.0	37.4±4.5	133.8±12.4
10	28	Basal, + 0.0035% B_1	28.5±3.0	41.4±2.2	32.2±3.9	33.9±4.2	136.0±13.3
10	27	Basal, + 0.007% B_1	24.5±3.6	43.0±1.7	38.9±2.1	33.6±3.7	140.0±11.1

*The results for trial 3 are included with those of Trials 1 and 2 in the first line and listed separately in line 6 to permit comparison with the other groups in Trial 3.

Level of Statistical Significance of Differences Between Groups

	Weeks 1, 1%	Weeks 1, 5%	Weeks 2, 1%	Weeks 2, 5%	Weeks 3, 1%	Weeks 3, 5%	Weeks 4, 1%	Weeks 4, 5%
Groups Compared	22 with 23	27 with 23	22 with 21, 23	30 with 23, 27	28 with 21, 23, 25	27 with 28, 30		
	23 with 24, 30	28 with 30	24 with 21, 23		29 with 25	29 with 21, 23		
	25 with 21, 22, 24, 27, 28, 29, 30	29 with 21, 23			30 with 21, 23, 25			
	28 with 29							

high levels of thiamine. Some levels of thiamine appeared to depress growth slightly (Table 3, Week 1: Diets 27, 29 and 30 vs. Diets 28 and 23; Week 2: Diet 30 vs. Diets 27 and 23). These growth gains were not consistent throughout the four weeks of the study. There was no difference in the total weight gains of any group receiving the various levels of thiamine. The thiamine content of ration 30 is three times that of ration 21; the thiamine content of ration 29 is almost twice that of ration 30; that of ration 28 almost twice that of ration 29; that of ration 27 almost twice that of ration 28; and that of ration 23 almost 250 times that of ration 21.

The ration supplemented with a 0.1% level of thiamine (Diet 23) produced a greater weight gain than the basal ration during the third week as well as the first two weeks. However, during the fourth week the weight gains of the rats receiving all supplements of thiamine leveled off except for Diets 29 and 30. These variations in weight gains are probably due to some factor(s) other than thiamine since the rats receiving 4 mg. thiamine/kg. (or 0.0004%, Diet 21, Table 3, first line) gained almost as much weight as those rats receiving a thiamine supplement equal to 0.007% of the ration (Diet 27). Except for the second week their weight gains (Diet 21) were superior to those of the rats fed Diet 27.

The similarity in the weight gain curves (Figure 1) of the rats fed the 20% casein ration to which various levels of thiamine were added was additional proof for the absence of any growth changes among weanling rats resulting from the addition of high levels of thiamine to the diet.

A second aspect of this study was the effect produced by the addition of a 0.1% level of niacin above the 5 mg./kg. provided by the vitamin mix. The addition of this amount to the ration improved the growth of the rats especially the first week following weaning when the rate of growth was

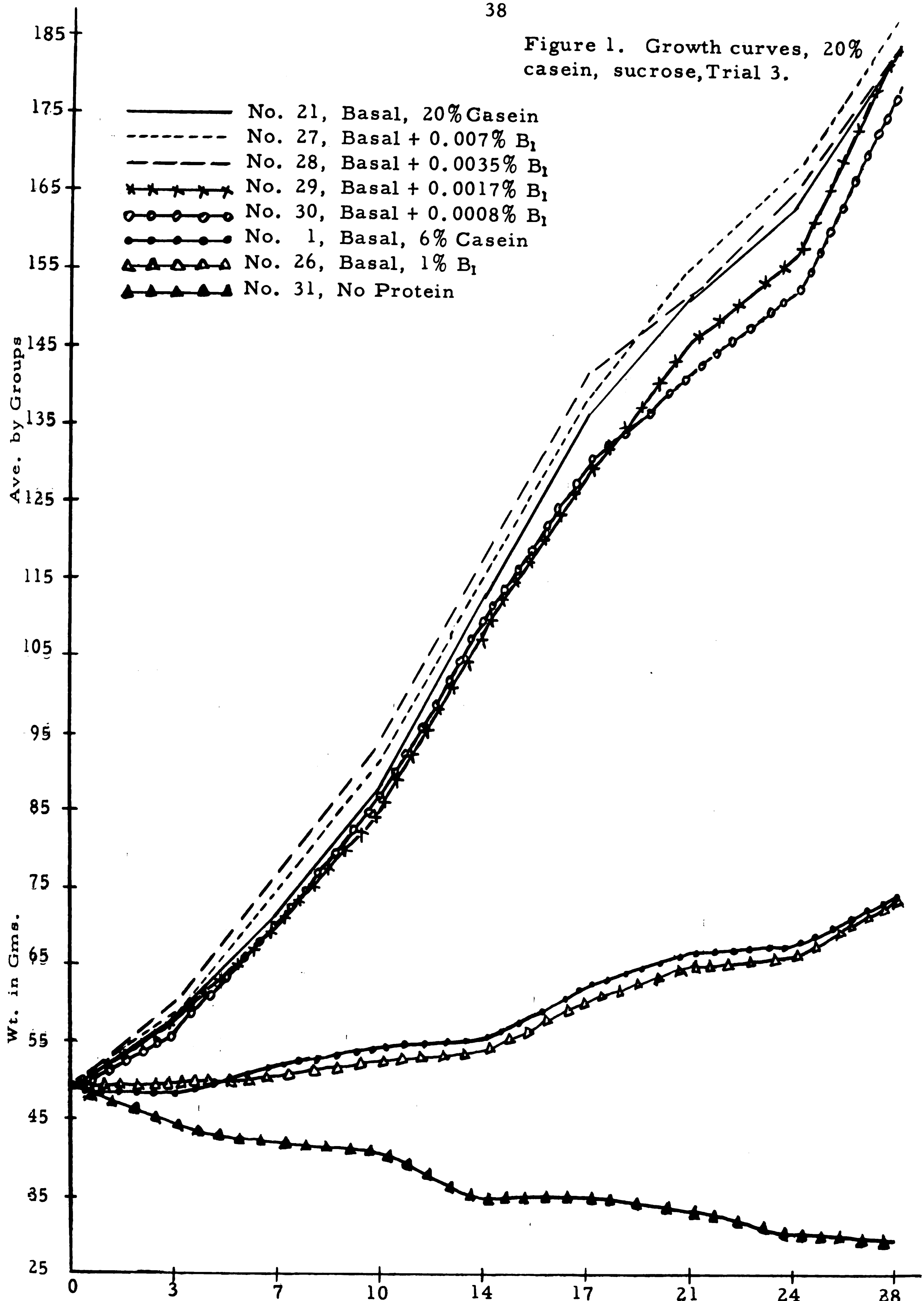


Figure 1. Growth curves, 20% casein, sucrose, Trial 3.

compared to the growth of all other weanling rats in this particular study. However, the rate of the gains were significant only during the first week.

The subject of choline was the third aspect of this study. In the first fourteen days following weaning, when choline was omitted from the diet (Diets 22 and 24) there was a decrease in growth. This difference was significant at the 1% level of probability. Although the weight of the choline deficient animals improved during the second week there was still a difference which was statistically significant at the 1% level. This significance disappeared during the third and fourth weeks. Diet 24 contained a 0.1% level of excess thiamine but its addition without choline in the ration did not improve the growth of the rats during the first two weeks following weaning (Diet 24 vs. Diet 23). After the rats were able to synthesize their choline needs weight increases in both groups were identical (Figure 2).

Results for the choline-deficient animal substantiate those found in the literature--that after thirty days of age, male weanling rats begin to synthesize adequate amounts of choline from the amino acids in the diet provided the ration contains an adequate level of methionine; the higher the casein level the more protective the diet. The literature further indicates that the choline requirement for the prevention of fatty livers is two to three times that for the prevention of hemorrhagic degeneration of the kidney. Griffith ('41) suggested that neither condition occurred if there was a restriction of the intake of the ration which produced severe damage when fed ad libitum. Such restriction produced less damage to both the liver and kidneys than when the ration was fed ad libitum. Griffith thought this was due to either a relation between the rate of metabolism or of growth and the need of the animal for choline.

The results of the preceding studies stimulated an interest in the use of both lower and higher casein levels in the rations as a critical test for the toxic effects that might result from feeding rations containing various high levels of thiamine (Tables 4, 5, 6, 7 and 8).

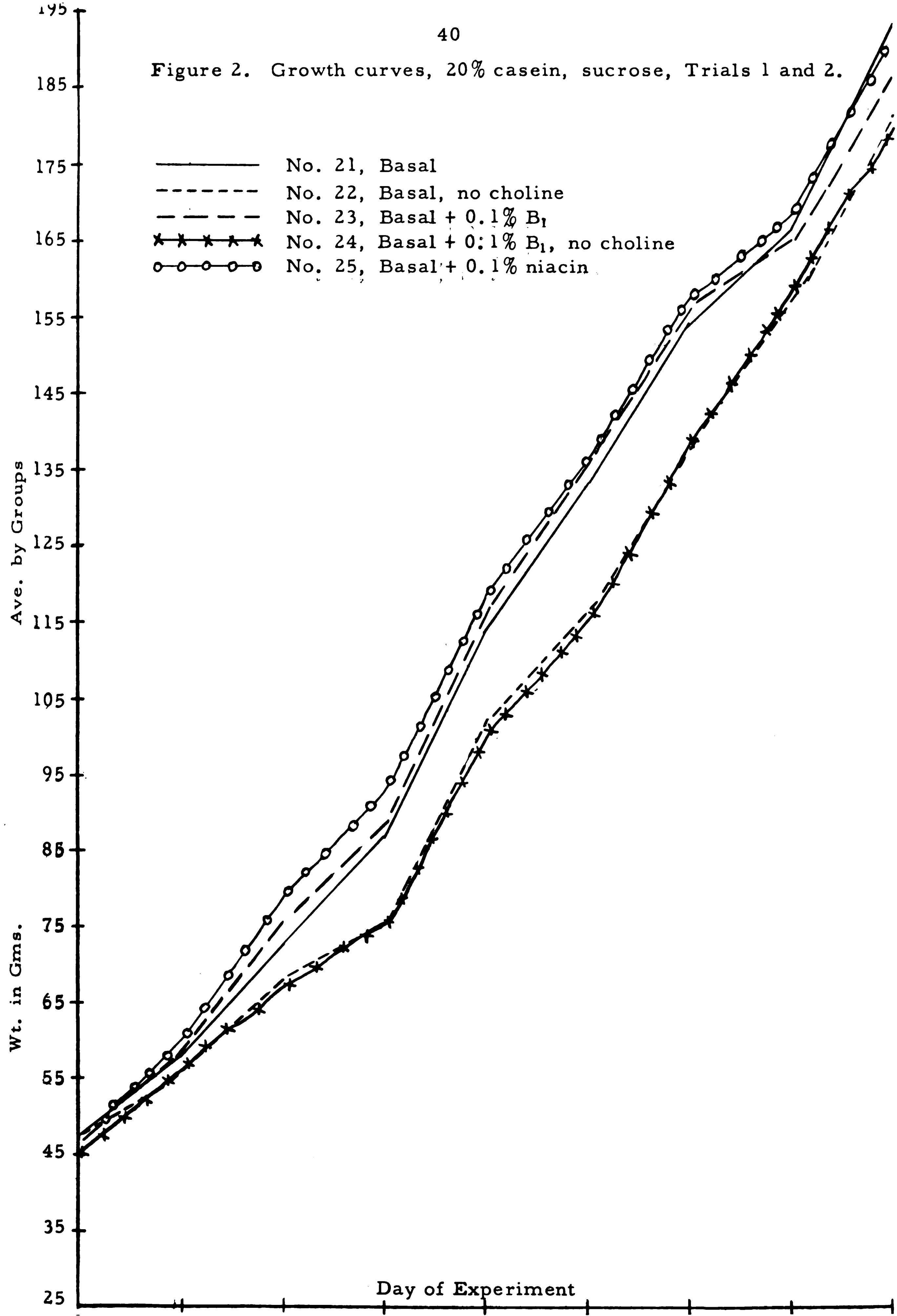


Figure 2. Growth curves, 20% casein, sucrose, Trials 1 and 2.

The average weight gain in grams per week for the rats on Diets 1, 2, 3, 4, 5, and 26 are presented in Table 4. Statistical analyses have been applied only to Diets 1, 2, 4, and 26 as the animals on Diets 3 and 5 were maintained on them for only two weeks. In addition, only 10 animals fed Diet 4 were maintained beyond two weeks and only 20 animals fed Diet 26 beyond two weeks.

Although the weight gains at the end of three weeks suggested an inhibitory action of the high levels of thiamine, the increased weight gains in the fourth week implied that it must have taken the rats slightly longer than usual to become accustomed to the taste of the rations with the high levels of the vitamin. These rations had a definite bitter flavor. On the other hand, these very large dietary supplements of thiamine did not suppress growth. It would appear from this that the addition of very large amounts of thiamine to a low protein ration had no deleterious nor beneficial effect on the growth of rats. This was true when the rations heavily supplemented with thiamine were fed over a four week period (Figure 3).

In comparing the data in Tables 4 and 5 where the ration in each case contained 6% casein, but different carbohydrates (sucrose vs. dextrin) it was apparent that when sucrose was used in the ration, the rats barely maintained their weight during the first week following weaning. All differences in weight gains of the rats fed dextrin and those fed sucrose are significant at the 1% level for the first week. However, during the second week, all rats were evidently able to adjust to the sucrose-casein diet and the slight differences observed in the second week, were not significant, except between Diets 4 and 9 (significant at the 5% level). This needs further research to justify such a conclusion.

There are no food intake data for the rats fed the dextrin rations. Therefore, it is not known if the rats on sucrose consumed more food

Table 4. — Average Gains in Weight (gms. per week) of Rats Fed 6% Casein Diets with Supplements of Thiamine

Table 4. Average Gains in Weight (gms. per week) of Rats Fed 6% Casein Diets with Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose.

No. of Animals		Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total 2 Wks.	Total 4 Wks.
Trial 1	8								
Trial 2	20								
Trial 3	10								
Trial 4	10/48	1	Basal, 6% casein	3.2±0.7	7.6±0.7	7.9±1.0	6.3±0.9	10.8±1.4	25.0±3.3
Trial 1	8								
Trial 2	20								
Trial 3	10/38	2	Basal + 0.2% B_1	3.4±0.8	8.0±1.0	6.7±0.6	7.8±0.6	11.4±1.8	25.9±3.0
8		3	Basal + 0.3% B_1	1.4±0.7	4.4±0.5			5.8±1.2	
Trial 1	8 *1								
Trial 2	20								
Trial 3	10/38	4	Basal + 0.4% B_1	2.9±0.5	8.5±0.7	3.3±0.5	13.3±1.2	11.4±1.2	28.0±2.9
8		5	Basal + 0.5% B_1	1.3±0.5	5.3±1.0			6.6±1.5	
Trial 1	20 *2								
Trial 2	10								
Trial 3	10/40	26	Basal + 1.0% B_1	2.1±0.7	6.6±0.6	6.7±0.7	10.4±0.8	8.7±1.3	25.8±2.8

*1 Only 10 beyond two weeks
*2 Only 20 beyond two weeks

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2		Weeks 3		Weeks 4	
	1%	5%	1%	5%	1%	5%	1%	5%
Groups Compared	0	0	0	0	4 with 1, 2, 26	0	4 with 1, 2	
							26 with 1	26 with 2, 4

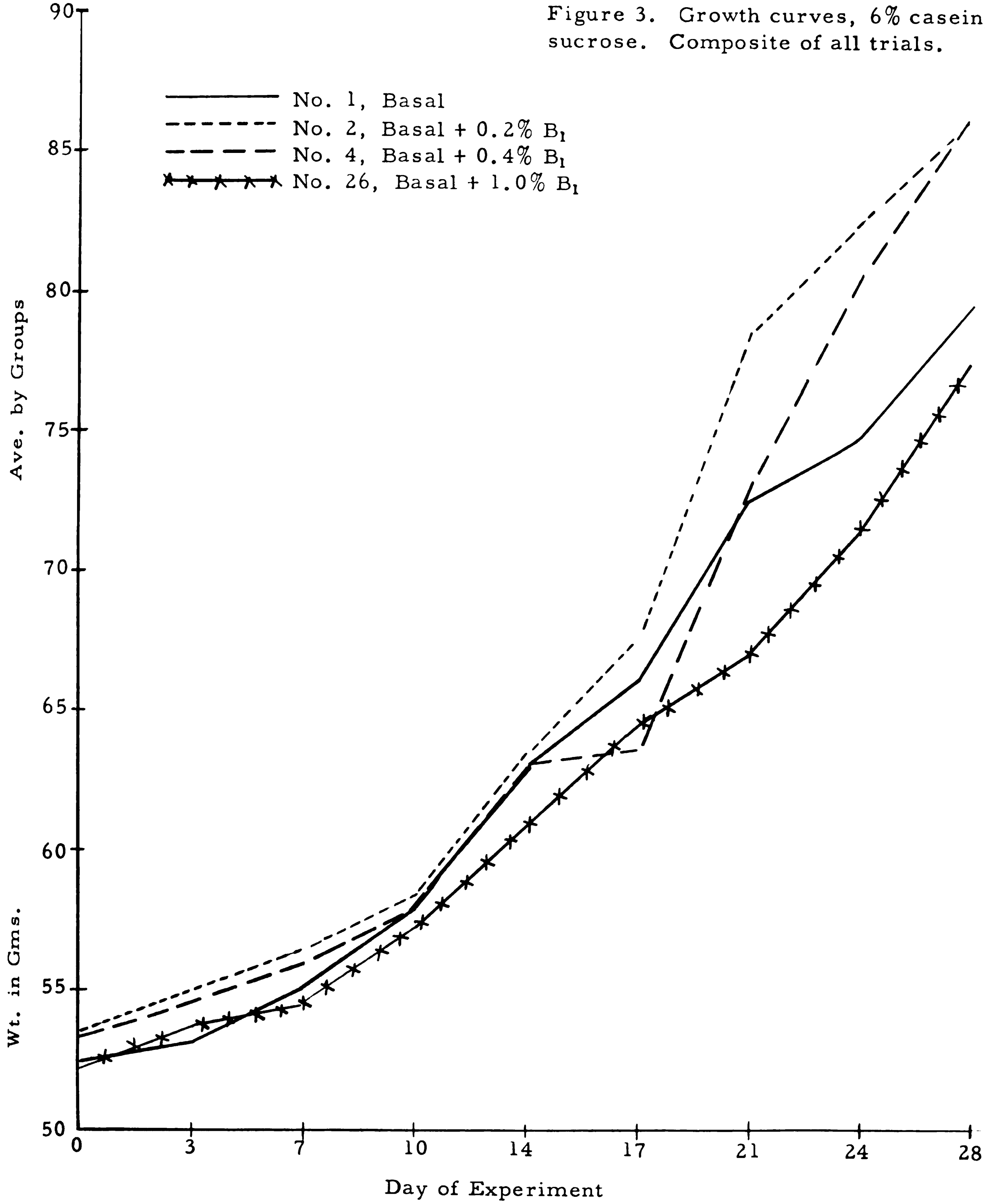
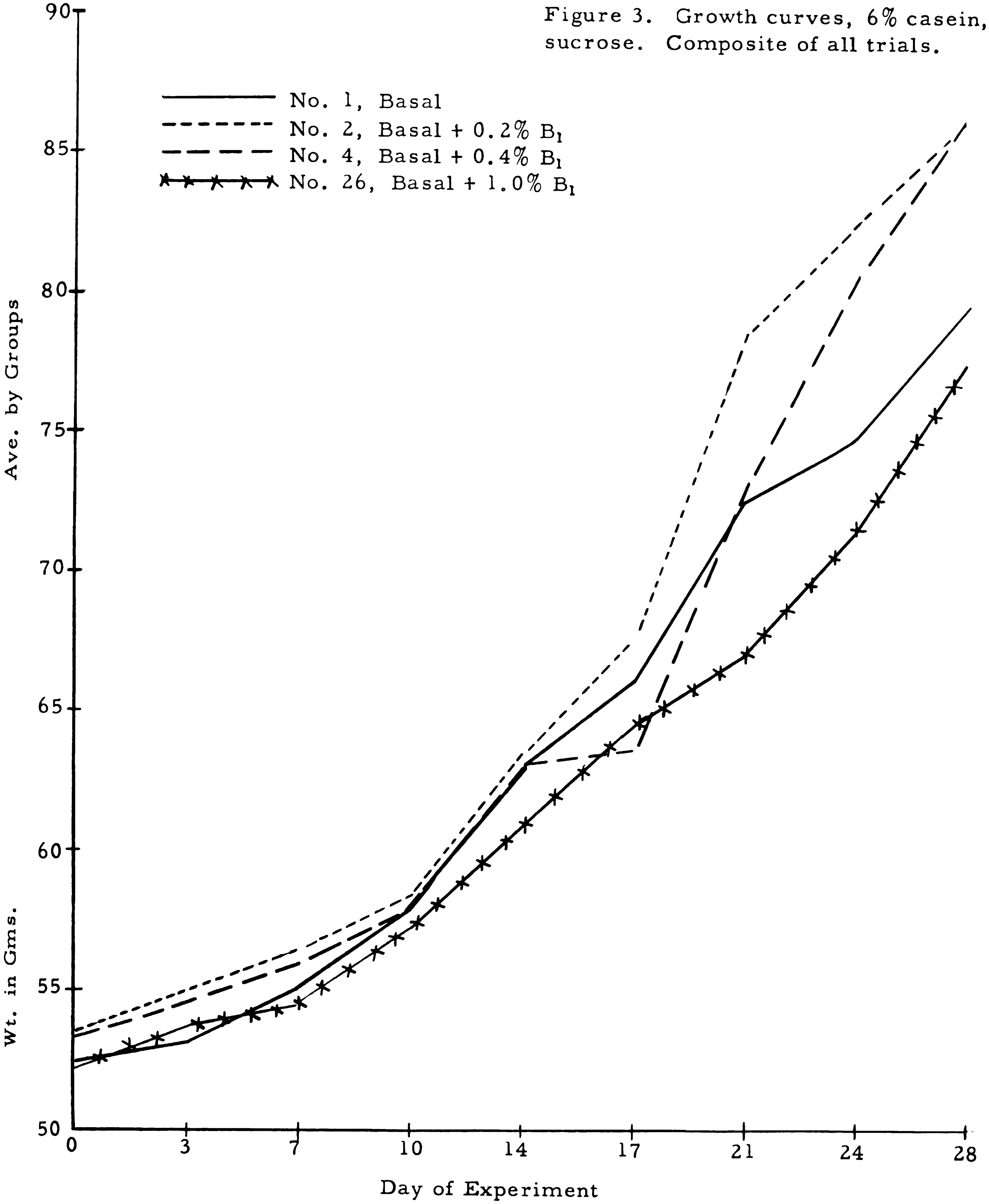


Figure 3. Growth curves, 6% casein, sucrose. Composite of all trials.

Figure 3. Growth curves, 6% casein, sucrose. Composite of all trials.



during the second week than the rats fed dextrin, which fact might have provided part of the answer. The superiority of growth of weanling rats fed dextrin as their source of carbohydrate does not seem to be related to the amount of thiamine in the ration. According to the work of many researchers (Lepp et al., '47; Monson, Dietrich and Elvehjem, '50; Krehl, '46; Henderson, '47; Hankes, '48; Lyman and Elvehjem, '51; Hall and Sydenstricker, '46; Litwack et al., '52; Harper et al., '53; Elvehjem, '48; Baxter, '47), the growth differences associated with dietary carbohydrates were due to the availability of greater quantities of certain essential amino acids when sucrose was replaced by dextrin or cornstarch. Although the requirement of the B vitamins in the animal depends in part upon the composition of the diet with respect to carbohydrate (amount and kind) and although the B vitamins promote growth in animals, the growth effect in this study could not be attributed to larger quantities of thiamine that were made available to the rats fed the dextrin rations. There was no growth response that could be traced to the large amounts of thiamine present in the 6% casein rations.

In Table 5 the differences in weight gains between the rats fed the different diets were very small and indicated no significant differences (also Figure 4). These findings suggest that if very large doses of thiamine have an adverse effect, the action will be less apparent when rats are fed dextrin as the source of carbohydrate--comparable to human diets where starch makes up the largest percentage of carbohydrate in the diet. It may also be of some consequence that in the second week the weight gains decreased with increasing thiamine supplements. These studies should probably be repeated and continued for four weeks to determine whether the difference is biologically important.

Some of the growth data in Tables 6 and 7 will be discussed in connection with the data in Table 8. It was apparent that as the level

Table 5. Average Gains in Weight (gms. per week) of Rats Fed 6% Casein Diets with Supplements of Thiamine. In all Rations the Carbohydrate was Dextrin.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Total
8	6	Basal, 6% Casein	6.6±0.7*1	7.1±0.6	13.7±1.3
8	7	Basal + 0.2% B_1	8.1±0.7*1	7.4±0.6	15.5±1.3
8	8	Basal + 0.3% B_1	7.1±0.8*1	6.4±0.8	13.5±1.6
8	9	Basal + 0.4% B_1	6.5±0.6*1	5.3±0.9*2	11.8±1.5
8	10	Basal + 0.5% B_1	7.5±0.7*1	7.0±1.3	14.5±2.0

*1 1% Level of significance between groups, Week 1, Table 4.

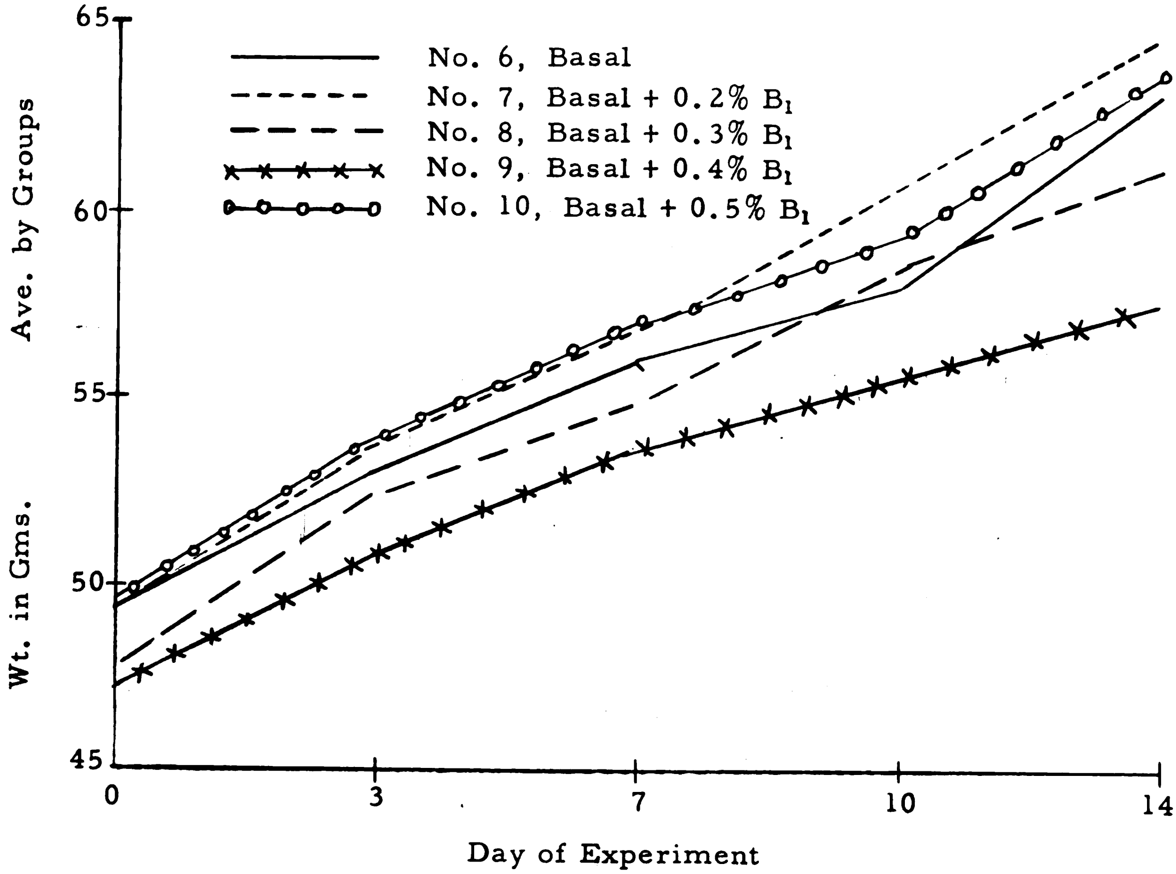
*2 1% Level of significance with Diet No. 4, Week 2, Table 4.

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2	
	1%	5%	1%	5%
Groups Compared	0	0	0	0

Wt. in Gms.   Ave. by Groups

Figure 4. Growth curves, 6% casein, dextrin.



of casein increased, which brought a change in the amount and proportion of amino acids available to the rat, the rats grew more rapidly. However, there were no statistically significant differences between the weights of the control groups (Diets 11 and 16, Tables 6 and 7) and those in the dietary groups receiving increased thiamine supplements, Diets 12, 13, 14, 15, 17, 18, 19, and 20 except in one instance (Figures 5 and 6 also). The rats fed Diet 15 showed a slight depression in growth during the second week. This difference was significant at the 5% level when compared with that for Diets 11 and 12, although there was no statistical significant differences in the food intakes for the rats in these groups (Table 11).

The weight gains of the rats in relation to the percent of casein in the diet are presented in Table 8. The growth promoting quality of the protein is evident from Table 8 for as the percent of casein in the ration increased the growth rate per week was greater. The "t" values obtained in the statistical analysis presented at the bottom of the Table indicated that the differences were highly significant.

These data indicated as others have shown that rats fed a ration devoid of protein lose considerable weight during the first week of such a dietary regime. The percent of body weight lost showed a less dramatic change than the absolute weight change. It is remarkable that weanling rats which initially weighed an average of 49.3 grams should be able to lose 19 grams during four weeks of eating a no-protein ration. This loss represented 38.5% of the weanling rats body weight.

The protein stores of the rats fed Diet 31 were apparently undergoing depletion during the four weeks they were on this diet. This loss was progressive the first two weeks; however, it was at a slower rate the last two weeks of the experiment. During the third week two of these rats gained two grams each, while four maintained weight and three others each lost only one gram. The decrease in rate of loss of

Table 6. Average Gains in Weight (gms. per week) of Rats Fed 12% Casein Diets with Supplements of Thiamine. In all Rations the Carbohydrate was Sucrose.

No. of	Diet		Weeks		
Animals	No.	Description	1	2	Total
8	11	Basal, 12% Casein	14.1±1.7	31.0±2.3	45.1±4.0
8	12	Basal + 0.2% B_1	11.6±1.5	29.6±1.9	41.2±3.4
8	13	Basal + 0.4% B_1	16.9±2.4	27.5±2.9	44.4±5.3
8	14	Basal + 0.5% B_1	14.6±2.7	25.0±3.0	39.6±5.7
8	15	Basal + 1% B_1	16.9±2.0	24.9±1.1	41.8±3.1

Level of Statistical Significance of Differences Between Groups

	Weeks			
	1		2	
	1%	5%	1%	5%
Groups Compared	0	0	0	15 with 11, 12

Figure 5. Growth curves, 12% casein, sucrose.

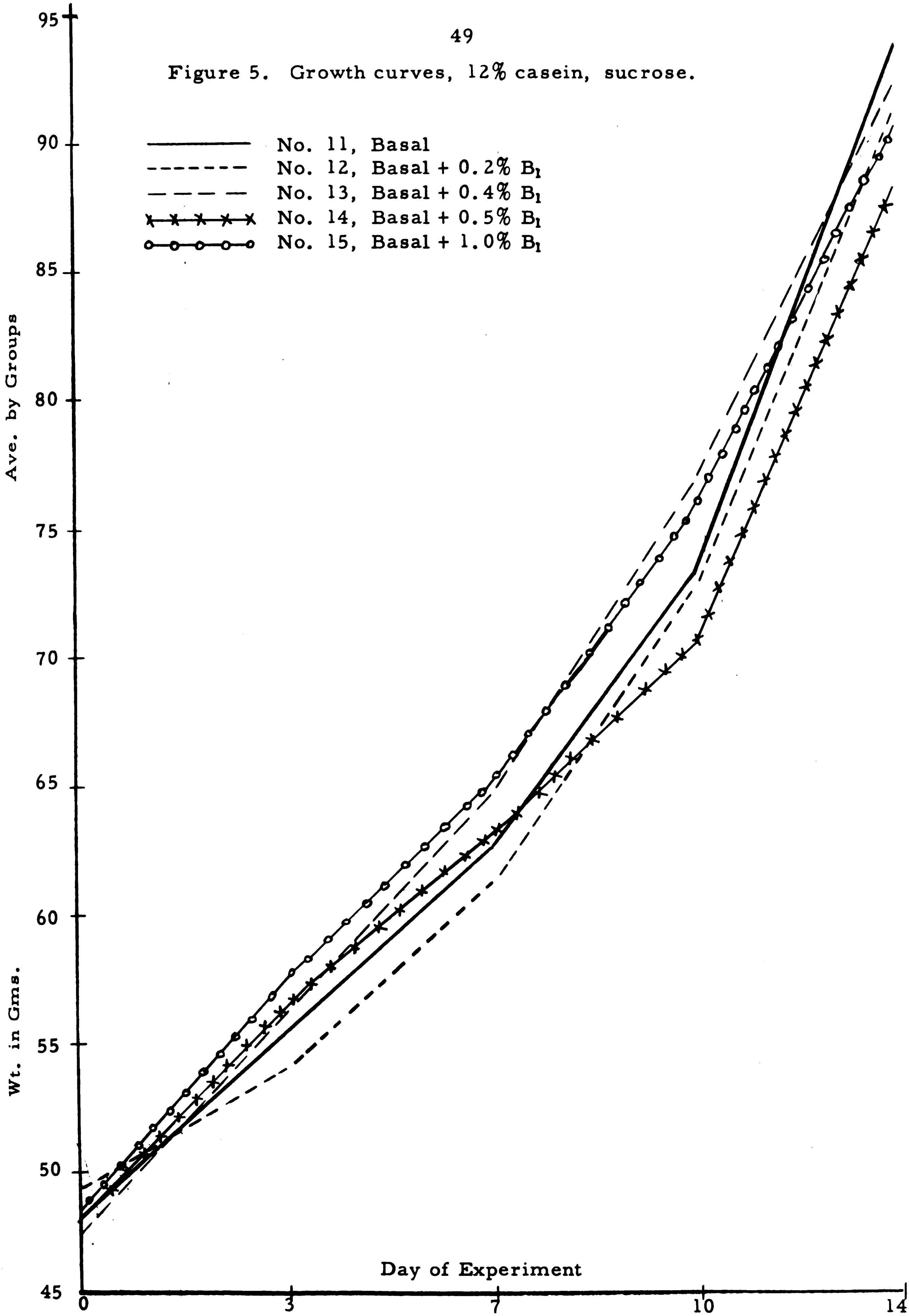


Table 7. Average Gains in Weight (gms. per week) of Rats Fed 30% Casein Diets with Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks Total
8	16	Basal, 30% Casein	27.1±2.2	37.1±3.0	64.2±5.2
8	17	Basal + 0.2% B_1	27.3±1.8	38.8±0.9	66.1±2.7
8	18	Basal + 0.4% B_1	26.5±2.4	33.5±2.5	60.0±4.9
8	19	Basal + 0.5% B_1	30.0±2.0	38.6±1.3	68.6±3.3
8	20	Basal + 1% B_1	24.3±3.0	34.3±2.3	58.6±5.3

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2	
	1%	5%	1%	5%
Groups Compared	0	0	0	0

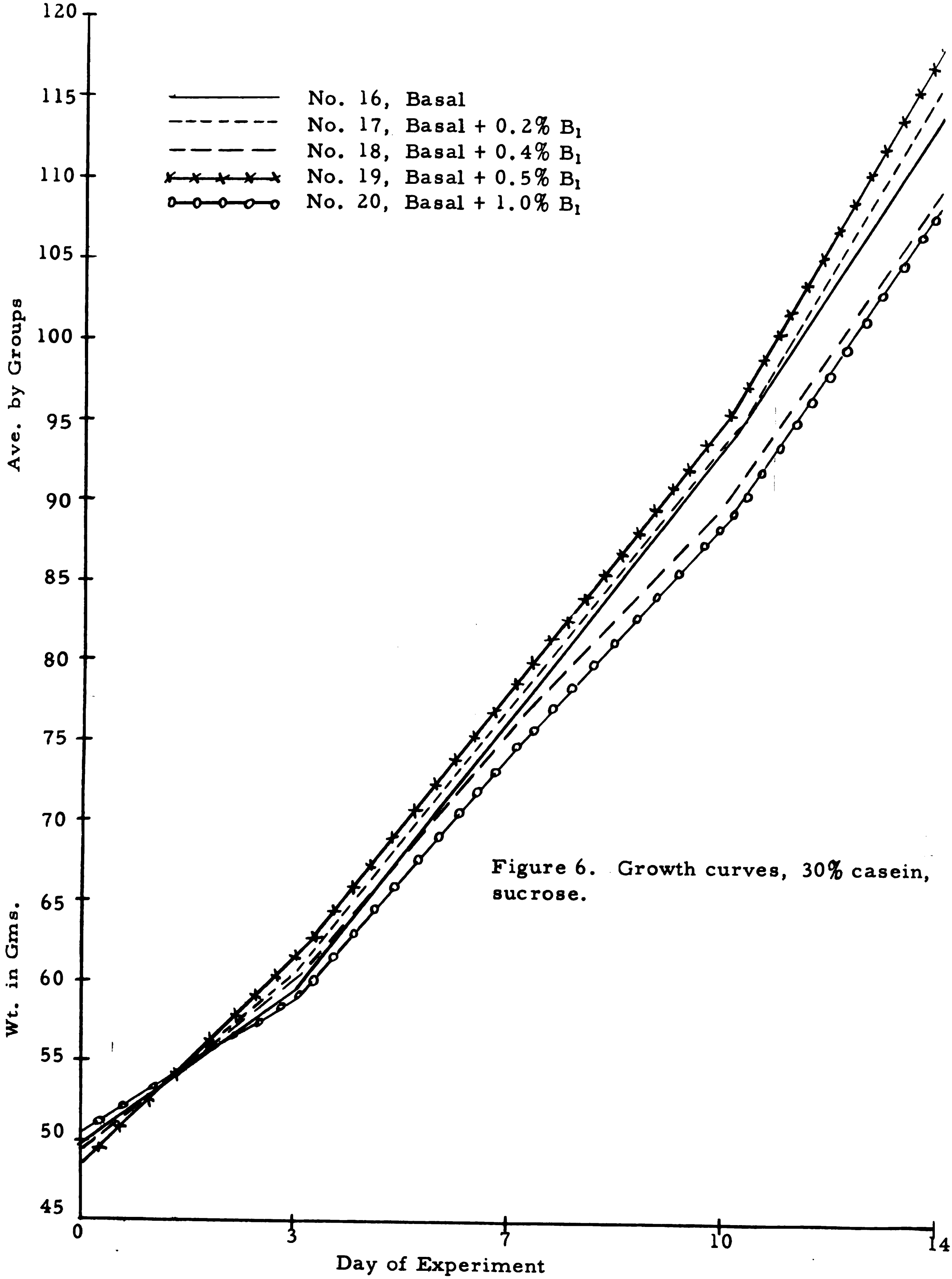


Figure 6. Growth curves, 30% casein, sucrose.

body weights was not compensated for by increased food consumption since the rats did not consume more ration during the third week (Table 12).

In starvation and undernutrition or malnutrition, edema can sometimes be observed clinically, although not always. Albanese ('52-'53), Allison ('60), Guggenheim ('56), Kahn ('59), Keys et al. ('50), and Williams ('61) indicated that the greatest effect of a protein-free diet was evidenced in the liver and that an increased extracellular fluid was associated with depletion of protein reserves even though clinical edema could not be observed. In general, the data of other workers showed that the protein, including enzymes, in the liver were the most labile proteins in the body while the protein, including the enzymes, of the brain were the most resistant. The protein, including the enzymes, of the ventricle of the heart were more resistant than those of the kidney, spleen, or skeletal muscle and almost as resistant as those of the brain. Frisch et al. ('29) found that in cases of malnutrition of any kind, the serum proteins were lowered and there was a tendency toward water retention. Guggenheim ('56) in his experiments, was able to show that hormonal factors were at least partially responsible for the water retention in protein deficient animals. Although no gross edema was observed in the rats fed Diet 31 it may have been that during the third week when the rats seemed to improve in appearance there was water retention in some tissues. At any rate it should be noted that during the third week when the rats appeared to be in better condition, the food intake was lower than at any other time.

There was an increase in weight gains with an increase in the dietary casein level up to 20%.(Table 8). The weight gains per day for the rats fed Diet 21 (almost 6 grams both the second and third weeks) were excellent. During the second week, the rats on Diet 1 (6% casein) tripled the weight gain of the first week; they consumed 1.3 times as

Average Gains in Weight (gms. per week) of Rats Fed Rations Containing Different Levels of Casein.
All Rations Contained Sucrose as the Carbohydrate; There was 4 mg. Thiamine in Each Kg. of Ration.

Table 8. Average Gains in Weight (gms. per week) of Rats Fed Rations Containing Different Levels of Casein. All Rations Contained Sucrose as the Carbohydrate; There was 4 mg. Thiamine in Each Kg. of Ration.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total 2 Wks.	Total 4 Wks.
10	31	No protein	-8.6±0.4	-6.7±0.5	-0.3±0.5	-3.4±0.4	-15.3±0.9	-19.0±1.8
10	1	Basal, 6% casein	1.4±0.7	4.7±0.5	11.1±0.9	5.5±0.6	6.1±1.2	22.7±2.7
8	11	Basal, 12% casein	14.1±1.7	31.0±2.3			45.1±4.0	
10	21	Basal, 20% casein	23.5±2.8	41.2±1.8	37.5±2.4	32.9±2.5	64.7±4.6	135.1±9.5
8	16	Basal, 30% casein	27.1±2.2	37.1±3.0			64.2±5.2	

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2		Weeks 3		Weeks 4	
	1%	5%	1%	5%	1%	5%	1%	5%
Groups Compared	31 with 1, 11, 16, 21	0	31 with 1, 11, 16, 21	0	31 with 1, 21	0	31 with 1, 21	0
	1 with 11, 16, 21	0	1 with 11, 16, 21	0	1 with 21	0	1 with 21	0
	11 with 16, 21	0	11 with 21	11 with 16	0	0	0	0

much food as they did in the first week. During the third week they doubled the weight gain of the second week while consuming more than 1.5 times as much food (Table 12). The rats fed Diet 11 (12% casein) doubled the weight gain during the second week while the rats on Diets 21 and 16 did not increase weight that rapidly. An increase in food intake may largely explain these changes (Table 12). The rats fed Diet 11 almost doubled food intake the second week compared with the first week. The rats fed Diet 21 ate no more during the first two weeks than the animals receiving the 12% casein diet yet the weight gains for these two groups were markedly different (Tables 8 and 12). This emphasized the findings of others, namely that the efficiency of a diet in producing body weight increases is related to the nutritional value of the diet--any significant dietary deficiency results in a high food intake and a relatively poor gain in body weight.

Supplements of thiamine seemed to have had no effect on the growth of rats fed 6%, 12%, and 30% casein diets. Weanling rats fed 20% casein rations showed stimulative growth effects from levels of 39, 74, and 1004 gamma of thiamine/gm. of ration for three weeks beyond weaning, then the effect of the thiamine leveled off, at which time doses of 12 and 21 gamma of thiamine/gm. in the ration indicated some stimulating growth effect.

Average Food Intake in Grams per Week

Average food intakes in grams per week as affected by the addition of various levels of thiamine to diets containing various levels of casein are shown in Tables 9, 10, 11, and 12 and Figure 7. Unfortunately food intake was not recorded for the rats fed the 6% casein-dextrin ration, the 20% casein rations with and without choline, nor the 30% casein ration.

Rats fed the 20% casein diets, Table 9, with supplements of thiamine gained weight and ate approximately the same amount of food. Although the differences in food intake and weight gains (Table 3) were small some statistical significance was found for individual weeks. Since the total overall differences for both food intake and weight gains were slight it seemed advisable to calculate the feed efficiency ratio of these animals to explore whether the differences observed for individual weeks were biologically important. The table below for the feed efficiency ratios indicates that there were still slight differences in groups from week to week, but the total overall differences were negligible. This data provides additional evidence of the safety of the high thiamine levels.

Diet	Dietary B-1 gamma/gm.	Week 1	Week 2	Week 3	Week 4	Total
21	4	62.1	58.6	28.9	29.3	38.6
30	12	64.1	58.3	26.6	32.3	38.6
29	21	56.1	58.3	28.8	31.1	38.3
28	39	65.8	56.0	25.3	29.3	37.7
27	74	63.7	60.8	29.1	29.5	39.5

$$F.\ E. = \frac{\text{weight gained}}{\text{food eaten}} \times 100$$

The food intake record for rats fed 6% casein with supplements of thiamine, Table 10, indicated that overall totals for the four diets were very similar. Although the statistical data showed significance for some differences between groups during the four week period, biological importance was doubtful because the total averaged weights were so similar. Except for week 3 supplements of 0.2% and 0.4% thiamine increased food intake.

A supplementary thiamine level of 0.4% may have had a stimulatory effect on food intake for the rats fed 12% casein (Table 11). However, none of the data in this Table had statistical significance at either the 1% or 5% level of probability.

Table 9. **Average Food Intake (gms. per week) of Rats Fed 20% Casein Diets with Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose.**

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total
10	21	Basal, 20% casein	37.8±2.2	70.3±2.9	129.7±5.1	112.2±4.8	350.0±15.0
10	30	Basal + 0.0008% B_1	35.4±2.4	64.8±4.0	122.0±4.8	113.4±6.0	335.6±17.2
10	29	Basal + 0.0017% B_1	36.9±3.0	68.6±2.3	123.7±5.0	120.2±6.1	349.4±16.4
10	28	Basal + 0.0035% B_1	43.3±3.3	73.9±3.1	127.3±5.9	115.6±7.9	360.1±20.2
10	27	Basal + 0.007% B_1	38.4±3.4	70.7±3.7	131.5±4.4	113.7±4.6	354.3±16.1

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2		Weeks 3		Weeks 4		Total	
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
Groups Compared	0	28 with 21, 29, 30	0	28 with 29	0	29 with 27, 28	0	21 with 29	0	30 with 21, 27, 28, 29
				30 with 21, 27, 28		30 with 21, 27				

Table 10. Average Food Intake (gms. per week) of Rats Fed 6% Casein Diets with Supplements of Thiamine

Table 10. Average Food Intake (gms. per week) of Rats Fed 6% Casein Diets with Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose.

No. of Animals		Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total
Trial 1	8							
Trial 2	20							
Trial 3	10							
Trial 4	10/48	1	Basal, 6% casein	41.3±1.9	56.2±3.2	66.4±3.7	64.1±2.6	228.0±11.4
Trial 1	8							
Trial 2	20							
Trial 3	10/38	2	Basal + 0.2% B_1	46.1±2.2	61.9±3.2	62.2±3.2	75.4±2.5	245.6±11.1
Trial 1	8							
Trial 2	20							
Trial 3	10/38	4	Basal + 0.4% B_1	45.9±2.4	66.3±4.0	53.7±2.5	77.9±2.9	243.8±11.8
Trial 1	20							
Trial 2	10							
Trial 3	10/40	26	Basal + 1.0% B_1	37.5±1.3	56.6±3.1	61.1±2.8	68.0±2.4	223.2±9.6

Level of Statistical Significance of Differences Between Groups

	Weeks 1, 1%	Weeks 1, 5%	Weeks 2, 1%	Weeks 2, 5%	Weeks 3, 1%	Weeks 3, 5%	Weeks 4, 1%	Weeks 4, 5%
Groups Compared	26 with 2, 4	1 with 2, 4, 26	4 with 1, 26	2 with 1, 26	4 with 1, 2, 26	1 with 26	2 with 1, 26	0
							4 with 1, 26	

Table 11. Average Food Intake (gms. per week) of Rats Fed 12% Casein Diets with Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Total
8	11	Basal, 12% Casein	38.8±2.0	74.4±4.1	113.2±6.1
8	12	Basal + 0.2% B_1	37.0±2.6	72.0±7.1	109.0±9.7
8	13	Basal + 0.4% B_1	40.3±2.7	81.4±7.1	121.7±9.8
8	14	Basal + 0.5% B_1	37.0±2.2	66.0±5.7	103.0±7.9
8	15	Basal + 1% B_1	40.7±2.3	72.5±8.2	113.2±10.5

Level of Statistical Significance of Differences Between Groups

	Weeks 1: 1%	Weeks 1: 5%	Weeks 2: 1%	Weeks 2: 5%
Groups Compared	0	0	0	0

Table 12 indicated that the food intake of rats fed the 12% and 20% casein rations were very similar during the first two weeks (Diets 11 and 21). It would have been interesting to have seen what effect the same high levels of thiamine would have had on food intake but unfortunately the data was not comparable (range of thiamine for 12% casein rations, 0.2% to 1.0%; range for 20% casein rations, 0.0008% to 0.007%).

According to Donaldson ('24) who summarized the research of various workers, the food consumption of albino rats depends upon body weight: about 7 grams per day for a rat of 100 grams body weight; between 11 and 14 grams per day for a 200 gram rat. The relative weight of the food consumed decreases with the increase in body weight. Rats used in this research generally weighed around 50 grams when placed on the diets. Considering their average weight when sacrificed, Table 12, the rats consumed more than 7 grams per day after the first week following weaning except those rats fed 6% casein.

	Weeks							
	1		2		3		4	
Diet	Weight*	Food Eaten Gm./Day	Weight*	Food Eaten	Weight*	Food Eaten	Weight*	Food Eaten
1	51.4	4.8	56.1	6.5	67.2	10.1	72.7	9.4
11	64.1	5.5	85.1	10.6				
21	73.5	5.4	114.7	10.0	152.2	18.5	185.1	16.0

*Av. for Group; Actual body weight in grams per week

There was no evidence that excess thiamine in the ration stimulated the appetite of the rats. For all rations except those containing 6% casein, the food intakes were very similar regardless of the thiamine level in the ration. The rats fed the 6% casein ration consumed slightly more feed when the ration contained 0.2% and 0.4% thiamine (Table 10).

Table 12. Average Food Intake (gms. per week) of Rats Fed Rations Containing Different Levels of Casein. All Rations Contained Sucrose as the Carbohydrate; There was 4 mg. Thiamine in Each Kg. of Ration.

No. of Animals	Diet No.	Diet Description	Weeks 1	Weeks 2	Weeks 3	Weeks 4	Total
10	31	No protein	22.6±0.8	24.5±1.3	19.6±2.3	35.9±2.4	102.6±6.8
10	1	Basal, 6% casein	33.8±1.9	45.6±2.5	70.4±2.9	65.8±2.2	215.6±9.5
8	11	Basal, 12% casein	38.8±2.0	74.4±4.1			
10	21	Basal, 20% casein	37.8±2.2	70.3±2.9	129.7±5.1	112.2±4.8	350.0±15.0

Level of Statistical Significance of Differences Between Groups

	Weeks 1		Weeks 2		Weeks 3		Weeks 4		Total	
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
Groups Compared	31 with 1, 11, 21	1 with 11	31 with 1, 11, 21	0	31 with 1, 21	0	31 with 1, 21	0	31 with 1, 21	0
			1 with 11, 21		1 with 21		1 with 21		1 with 21	

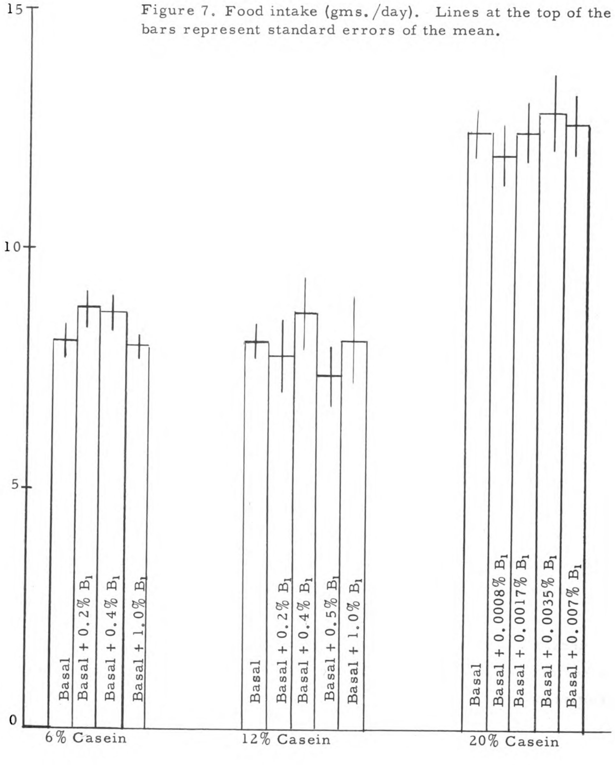
However, the increases in these cases were not statistically significant. These observations agree with the clinical findings that except for individuals who are deficient in thiamine, this vitamin has no appetite-stimulating activity. The more recent publications, especially treatises (Bicknell and Prescott, '53) state: "With a thiamine deficiency, there is a loss of appetite; when thiamine is returned to the diet, the appetite returns." No emphasis is placed on the use of thiamine to promote the appetite.

The rations containing the higher levels of thiamine had a bitter taste. Two weeks after preparation, the taste became less bitter. If the rat responded to this taste as human subjects did, they might eat larger amounts of food in the second week compared to the first. Enough ration was mixed just prior to the arrival of the rats to last through the experimental period regardless of the length chosen for the experimental period. These rations were kept at a temperature of 5° C. in the refrigerator. The rations in the food cups were in a room maintained at a temperature of 23-26° C. The ration in the food cup was regularly changed two to three times a week throughout the experiment. The pH of two of the rations, 6% casein basal (Diet 1) and the 6% casein basal plus 1% thiamine (Diet 26), was read immediately following preparation (pH week 1) and at the end of the experiment (pH week 4). The thiamine content of these diets (Diets 1 and 26) was also verified using the Thiochrome Technique (Association of Vitamin Chemists, Methods of Vitamin Assay, second edition, 1951) immediately following preparation [B-1 (1)], two weeks [B-1 (2)], and four weeks [B-1 (3)] later.

Diet	Diet. B-1 γ/gm.	pH at week 1	pH at week 4	B-1 (1) γ/gm.	Recovery	B-1 (2)	Rec.	B-1 (3)	Rec.
1	4	6.4	7.3	3.6	90%	2.8	69%	1.9	48%
26	10,004	5.9	7.8	9180	92%	8084	81%	5000	50%

These data indicated that during the four-week experimental period the rations became more basic. Evidently this flavor was more acceptable

Figure 7. Food intake (gms./day). Lines at the top of the bars represent standard errors of the mean.



to the rats as they consumed more food after the first week. The effect of flavor of these rations on food intake by rats might be investigated further.

Liver Fat, Nitrogen, and Moisture

This section will be restricted to a discussion of the level of fat, nitrogen, and moisture in the livers of rats fed various casein levels. The influence of thiamine and other nutrients on the composition of the liver and other tissues will be discussed with the ratio of water to protein in the next section.

The rats fed the 6% casein rations (sucrose) regardless of the level of thiamine had about the same amount of fat in the livers (Table 13 and Figure 8). (The term "liver fat" as used here refers to the total ether-soluble substances of the liver.) The type of carbohydrate had a great influence upon the amount of fat which was deposited in the liver. When dextrin was used in the place of sucrose less fat was deposited in the liver (significant at the 1% level). Although the growth curves for rats fed 6% casein with sucrose or dextrin were not very different, the liver fat levels were different (Figure 8). The action of dextrin on liver fat probably was not mediated through the gastrointestinal tract to make more thiamine or some other nutrient available to the rat. If this had been so, the growth rates should have reflected this. The mechanism whereby dextrin influences the levels of liver fat should be investigated further.

The rats fed the sucrose rations had larger livers and a higher percentage of lipids than those fed the dextrin rations. This combination produced livers in the sucrose-fed rats that had considerably more total lipids than the dextrin-fed rats.

Table 13. Liver Composition on the Basis of Fresh Tissue: Moisture, Fat and Protein, for Rats Fed 6% Casein Diets Supplemented with Thiamine. Dextrin or Sucrose was the Carbohydrate.

CHO	No. of Animals	Diet No.	Diet Description	Liver Component: Moisture %	Protein %	Moisture/Protein	Fat %
Sucrose	Trial 1 8 Trial 2 20 Trial 3 10 Trial 4 5/43	1	Basal, 6% casein	71.9	14.60	4.92	3.74
	Trial 1 8 Trial 2 20 Trial 3 10/38	2	Basal + 0.2% B_1	71.6	15.04	4.76	4.40
	8	3	Basal + 0.3% B_1	72.2	16.88	4.28	3.45
	Trial 1 8 Trial 2 20 Trial 3 10/38	4	Basal + 0.4% B_1	71.6	14.44	4.96	4.37
	8	5	Basal + 0.5% B_1	71.4	15.63	4.57	3.78
	Trial 1 20 Trial 2 10 Trial 3 5/35	26	Basal + 1.0% B_1	72.4	13.47	5.37	4.17
Dextrin	8	6	Basal, 6% casein	72.9	14.38	5.10	2.01
	8	7	Basal + 0.2% B_1	73.5	15.00	4.90	2.36
	8	8	Basal + 0.3% B_1	74.2	14.38	5.16	2.30
	8	9	Basal + 0.4% B_1	73.5	14.38	5.11	2.09
	8	10	Basal + 0.5% B_1	75.1	13.75	5.46	2.22

Sucrose	Liver Wts. (CHO-sucrose)	Dextrin	Liver Wts. (CHO-dextrin)
6% basal	3.35 gm. (48)*	6% basal	2.62 gm. (8)
" + 0.2% B-1	3.35 gm. (38)	" + 0.2% B-1	2.66 gm. (8)
" + 0.3% B-1	2.98 gm. (8)	" + 0.3% B-1	2.53 gm. (8)
" + 0.4% B-1	3.38 gm. (38)	" + 0.4% B-1	2.48 gm. (8)
" + 0.5% B-1	3.16 gm. (8)	" + 0.5% B-1	2.55 gm. (8)
" + 1.0% B-1	3.16 gm. (40)		

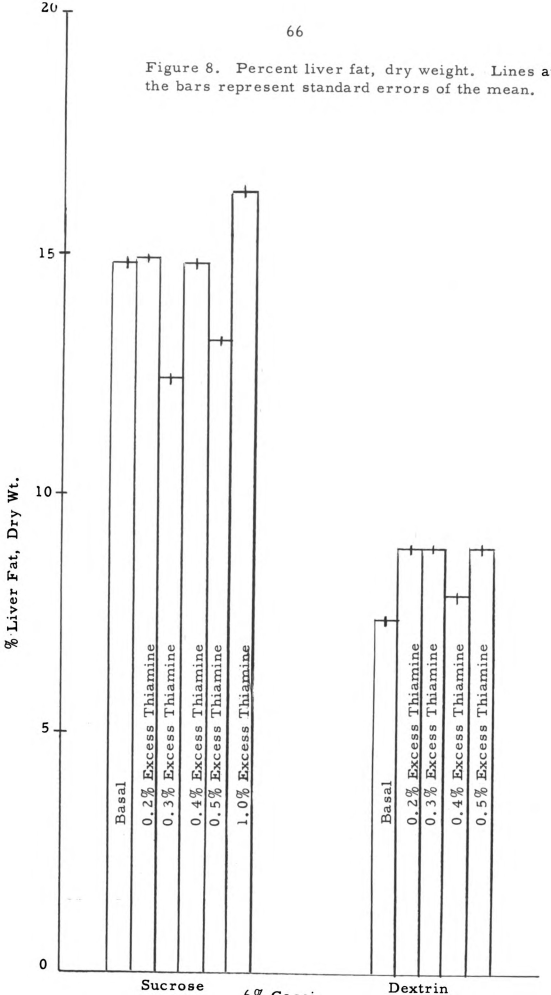
*Animals

No significant differences were observed in the concentration of liver nitrogen for the rats fed 6% casein rations containing either sucrose or dextrin as the only carbohydrate. The moisture content of the livers of rats receiving dextrin was greater than in the livers of rats receiving sucrose and was significant at the 5% level when Diets 2 vs. 7, 3 vs. 8, 4 vs. 9, and 5 vs. 10 were compared. In view of the findings of a higher moisture-protein ratio for the 6% casein-dextrin diets over the 6% casein-sucrose diets it would be interesting to determine what this ratio would be in 20% casein-dextrin diets since we have the data for 20% casein-sucrose diets.

The higher levels of casein in the diets (Table 14 and Figures 9 and 10) also influenced the amount of fat which was deposited in the liver. Regardless of the level of thiamine, less fat was deposited in the liver when the protein level was increased (20% and 30% casein diets) than when the rats received either a 6% or 12% casein ration. No significant differences were observed in either nitrogen or moisture concentration in the liver for any of the groups in Table 14.

All of the diets shown in Table 14 contained 0.15% choline except Diets 22 and 24. Despite its presence, the fat level was higher in the entire 12% casein group than in the entire 30% casein group in which the supplementary thiamine was the same. The fat level of the 20% basal casein group, Diet 21, was also lower than the fat level of the basal diet

Figure 8. Percent liver fat, dry weight. Lines at the top of the bars represent standard errors of the mean.



of the 12% casein group, Diet 11. Probably the lipotropic activity of the protein contributed to this factor.

When choline was omitted from the diet (Diet 22) a high quantity of fat was deposited in the livers of rats. An even larger quantity of fat was deposited in the liver when choline was omitted from the diet to which excess thiamine at the 0.1% level was added (Diet 24). The second week following weaning appeared critical for the deposition of fat in the liver of the choline-deficient animal. At that time, some adaptation, such as a reduction in dietary requirement took place. The final averaged figures (Table 14) indicated that the presence of choline in the diet decreased liver fat and masked the opposite lipogenic effect shown by the addition of a high level of thiamine (Diets 24 vs. 23). Choline and thiamine were antagonistic so that if choline was not present in the ration to help act as a lipotropic factor in the removal of fat from the liver and the level of thiamine was increased, a greater deposition of fat was observed. Differences in fat deposition were significant at 1% between Diets 21 and 22, between Diets 21 and 24, between Diets 23 and 24 and significant at 5% between Diets 22 and 24. As the protein content increased the deposition of liver fat approached the normal level for the rat. The effect of the dietary protein is probably due to the presence of the sulfur containing amino acids in the casein.

The difference in fat deposition when 0.1% excess niacin replaced 0.1% excess thiamine in the diet (Diets 23 and 25) was significant at the 5% level.

In all instances it should be noted that there was no significant differences in the liver nitrogen concentrations in these studies (Table 14).

The progressive decrease in moisture content of the liver (Figure 9) of the rats fed rations containing increasing amounts of casein (up to 12%) is in agreement with the previously mentioned work of other investigators. The increase in moisture content seen in the livers of the rats fed

Table 14. Liver Composition on the Basis of Fresh Tissue: Moisture, Fat, and Protein, for Rats Fed 12%, 20%, and 30% Casein Diets Supplemented with Thiamine and/or Niacin; With and Without Choline. Sucrose was the Carbohydrate.

Casein	No. of Animals	Diet No.	Diet Description	Liver Component Moisture %	Protein %	Moisture/Protein	Fat %
12%	8	11	Basal, 12% casein	67.5	16.25	4.15	4.32
	8	12	Basal + 0.2% B_1	69.5	15.00	4.63	3.48
	8	13	Basal + 0.4% B_1	69.5	15.63	4.45	3.51
	8	14	Basal + 0.5% B_1	68.9	15.63	4.41	3.61
	8	15	Basal + 1.0% B_1	70.4	16.25	4.33	2.96
20%	46**	21	Basal, 20% casein	71.7	18.13	3.95	2.75
	38*	22	Basal, no choline	71.3	18.13	3.93	4.31
	41*	23	Basal + 0.1% B_1	72.1	17.50	4.12	2.68
	40*	24	+ 0.1% B_1 Basal, no choline	70.9	17.50	4.05	5.09
	31*	25	Basal + 0.1% niacin	72.0	16.25	4.43	3.11
30%	8	16	Basal, 30% casein	71.1	18.75	3.79	2.75
	8	17	Basal + 0.2% B_1	71.6	18.75	3.82	2.13
	8	18	Basal + 0.4% B_1	71.9	18.75	3.83	1.71
	8	19	Basal + 0.5% B_1	71.5	18.13	3.94	2.02
	9	20	Basal + 1.0% B_1	72.1	18.13	3.98	2.01

*See Table 3 for number of trials.

**Includes only 5 of Trial 3; tissues of other 5 animals used in thiamine analyses.

Figure 9. Percent moisture and percent protein in liver for rats fed 0%, 6%, 12%, 20%, and 30% casein diets containing 4 mg. of thiamine/kg. of ration. Sucrose was the carbohydrate.

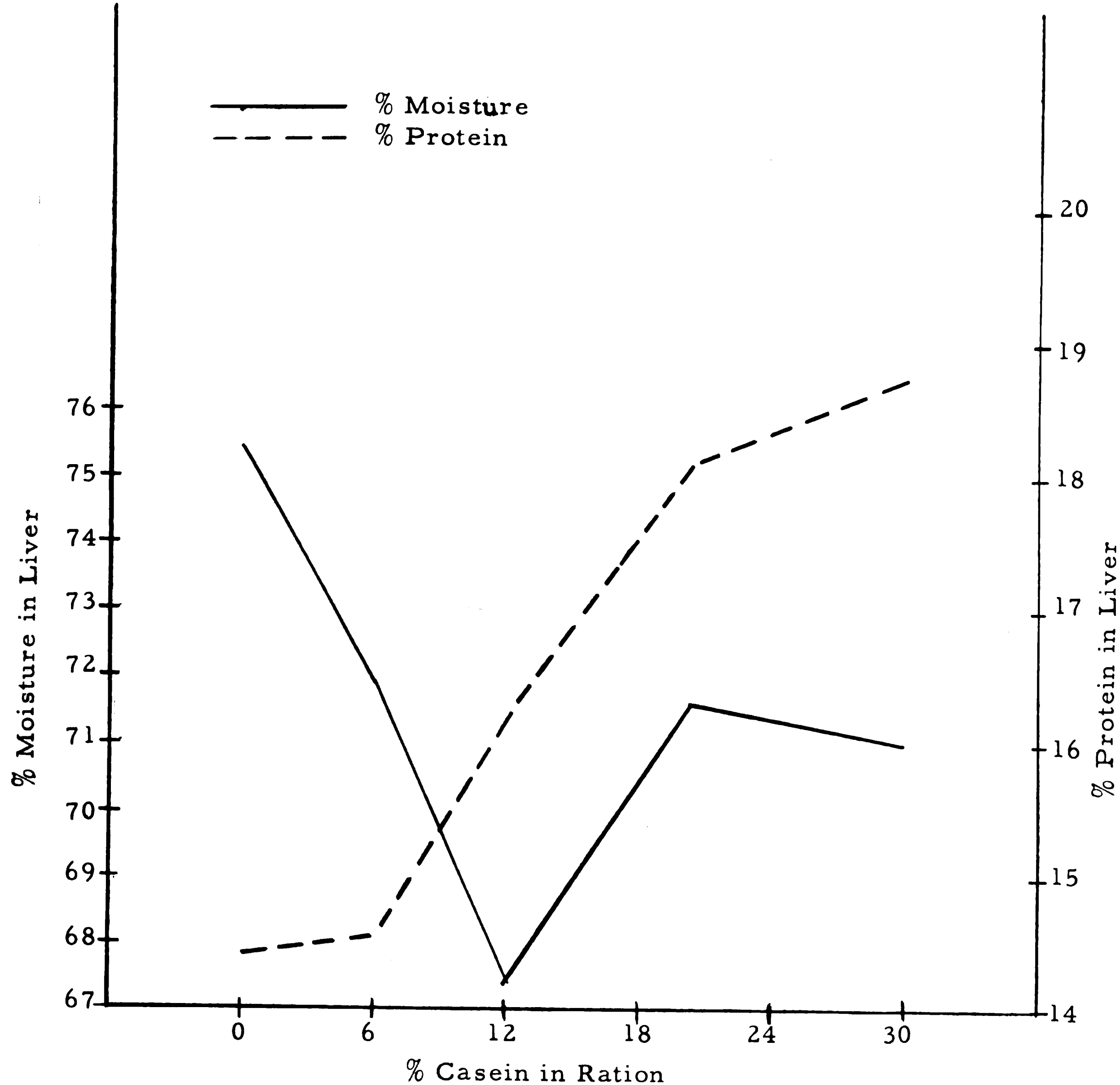
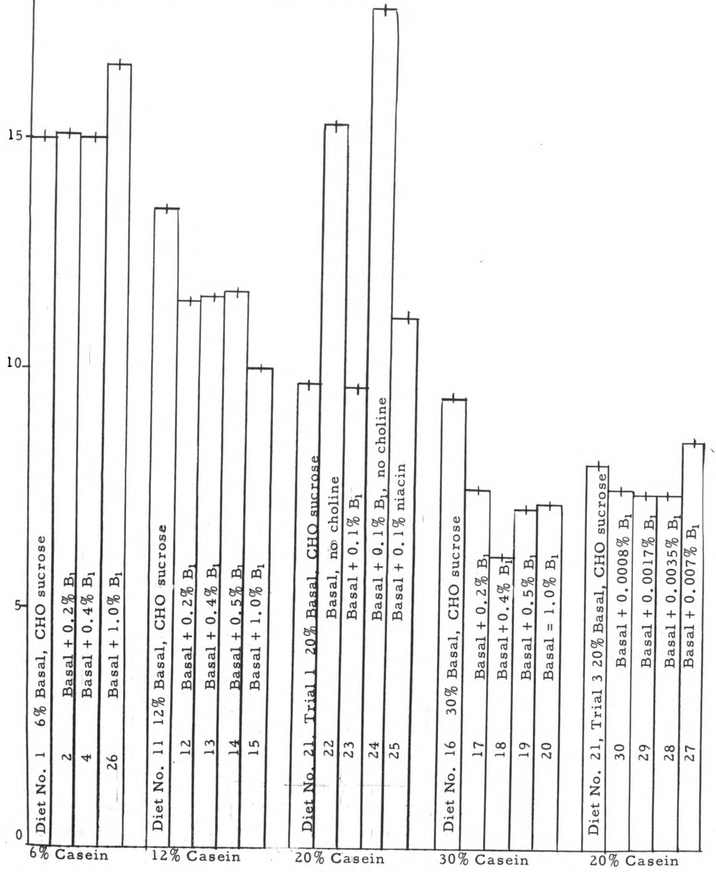


Figure 10. Percent liver fat, dry wt. Lines at the top of the bars represent standard errors of the mean.



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the higher levels of casein in their rations is unexplainable. The changes in the protein levels confirm the suggestion of Allison ('60) that this organ serves as a primary store for the labile protein reserves. (There were no comparable changes in the percent protein for the heart, kidneys, and muscle (Table 15).) It may well be that the changes observed here are a reflection of these reserves.

Chemical Composition of Certain Tissues

Another object of this experimental work was to determine the influence of the various diets upon the moisture, protein, fat and ash content of the liver, heart, kidneys and thigh muscle. It is known that the chemical composition of an animal's body is not constant, but varies throughout life. Although these variations occur, uniformity of mammalian tissue is recognized and animals of similar morphology have similar composition. Hatai ('17), Moulton ('23), and Chanutin ('30) analyzed rats in various stages of growth and in maturity for water, protein, fat, and ash. Deuel, et al. ('44) studied the effects of diet on the body composition of both sexes of the rat. Deuel, et al. ('44) and Spray and Widdowson ('50) have observed differences in body composition between the sexes of the rat. Addis et al. ('36, '40) and Kosterlitz ('47) were among the first to study in some detail the relationship between dietary protein and the protein content of the liver. They found that after feeding a protein-free diet for two days the liver of the rat loses more protein than any other organ while increasing somewhat in water content. Kosterlitz also found that when his studies were continued for as long as 24 days, the loss in nucleic acids started on the 4th day, the loss in stainable cytoplasm and mitochondria from liver cells started on the 7th day. He observed no change in liver cell size although the cells showed a loss in stainable cytoplasm with the formation of large clear spaces filled with glycogen.

Table 15. Composition of Tissues From Rats Fed Rations Containing Various Levels of Casein. The Carbohydrate in All Rations Was Sucrose. All Values Expressed on the Basis of Fresh Tissue. (Glycogen was Calculated as the Difference Between the Sum of the Other Percentages and 100.) There was 4 mg. Thiamine in Each Kg. of Ration.

Tissue	No. of Animals	Diet		Component					
		No.	Description	Moisture %	Protein %	Moisture / Protein	Fat %	Ash %	Glycogen %
Heart	5	31	No protein	78.24	16.75	4.67	1.54	1.87	1.60
	5	1	Basal, 6% casein	78.77	15.75	5.00	1.58	1.74	2.16
	5	21	Basal, 20% casein	78.79	16.38	4.81	1.34	1.44	2.05
Kidney	5	31	No protein	76.31	17.69	4.31	2.47	2.13	1.40
	5	1	Basal, 6% casein	76.72	16.00	4.80	3.77	1.62	1.89
	5	21	Basal, 20% casein	76.93	15.69	4.90	3.36	1.17	2.85
Liver	5	31	No protein	75.47	14.36	5.26	2.91	2.28	4.98
	43	1	Basal, 6% casein	71.9*	14.60*	4.92	3.74*	1.57	8.19
	46	21	Basal, 20% casein	71.7*	18.13*	3.95	2.75*	1.64	5.78
Muscle	5	31	No protein	76.77	18.94	4.05	1.29	1.86	1.14
	5	1	Basal, 6% casein	75.79	17.45	4.34	4.43	2.23	0.10
	5	21	Basal, 20% casein	75.22	17.39	4.33	4.78	1.87	0.74

*Average for all trials.

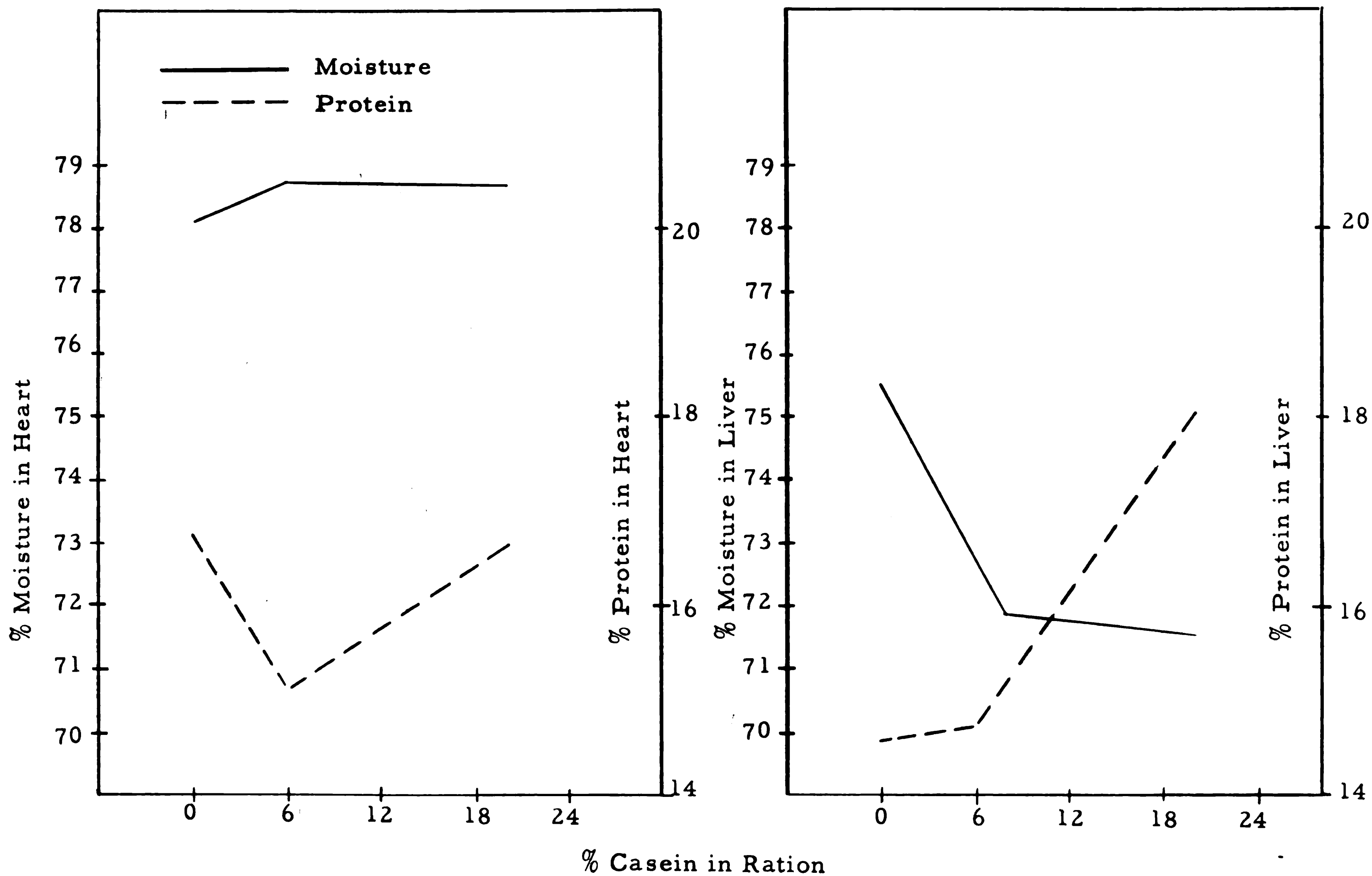
The different diets used in the present study (Table 15 and Figure 11) had no effect on the moisture content of the heart, kidney or muscle. However, the protein content of the ration had a marked influence on the moisture content of the liver (Figures 9 and 11). The variation in the ratios of moisture to protein was greatest for the livers from the animals fed different levels of casein in the rations. These ratios for the livers ranged from 3.79 to 5.26. They showed least variability for the muscles from the animal in the different casein basal groups--the range was 4.05 to 4.34.

The diets in this research did not duplicate those used by Kosterlitz in composition, but they did in that both used adequate vs. low and no protein rations. Kosterlitz' moisture-protein ratios for rat liver ranged from 3.92 to 5.70. These findings may have some implication for the seeming maintenance of weight by the rats fed Diet 31 during the third week following weaning. There may have been some edema in tissues which would have been observed only if the animals had been sacrificed that week. The percent of fat in thigh muscle increased with the increase in dietary casein. This is what one would expect--a better nutritional state results in a greater deposition of fat between the muscle fibers.

To evaluate the influence of the dietary thiamine excesses on the composition of the rat tissues, it was deemed advisable to analyze the tissues from the rats fed the 6% casein ration containing a normal thiamine level and those receiving the highest excess. Had any significant differences appeared in any of this work, the animals from the other groups would have been analyzed to determine whether the intermediate vitamin excesses produced appropriate changes.

The average moisture, protein, fat, and ash content for rats fed the 6% casein diets with and without 1.0% excess of thiamine is presented in Table 16 and that for rats fed similar rations (but lower levels of thiamine) containing 20% casein is presented in Table 17 and Figure 12.

Figure 11. Percent moisture and percent protein in heart, kidneys, liver and muscle for rats fed 0%, 6%, and 20% casein diets containing 4 mg. of thiamine. Sucrose was the carbohydrate.



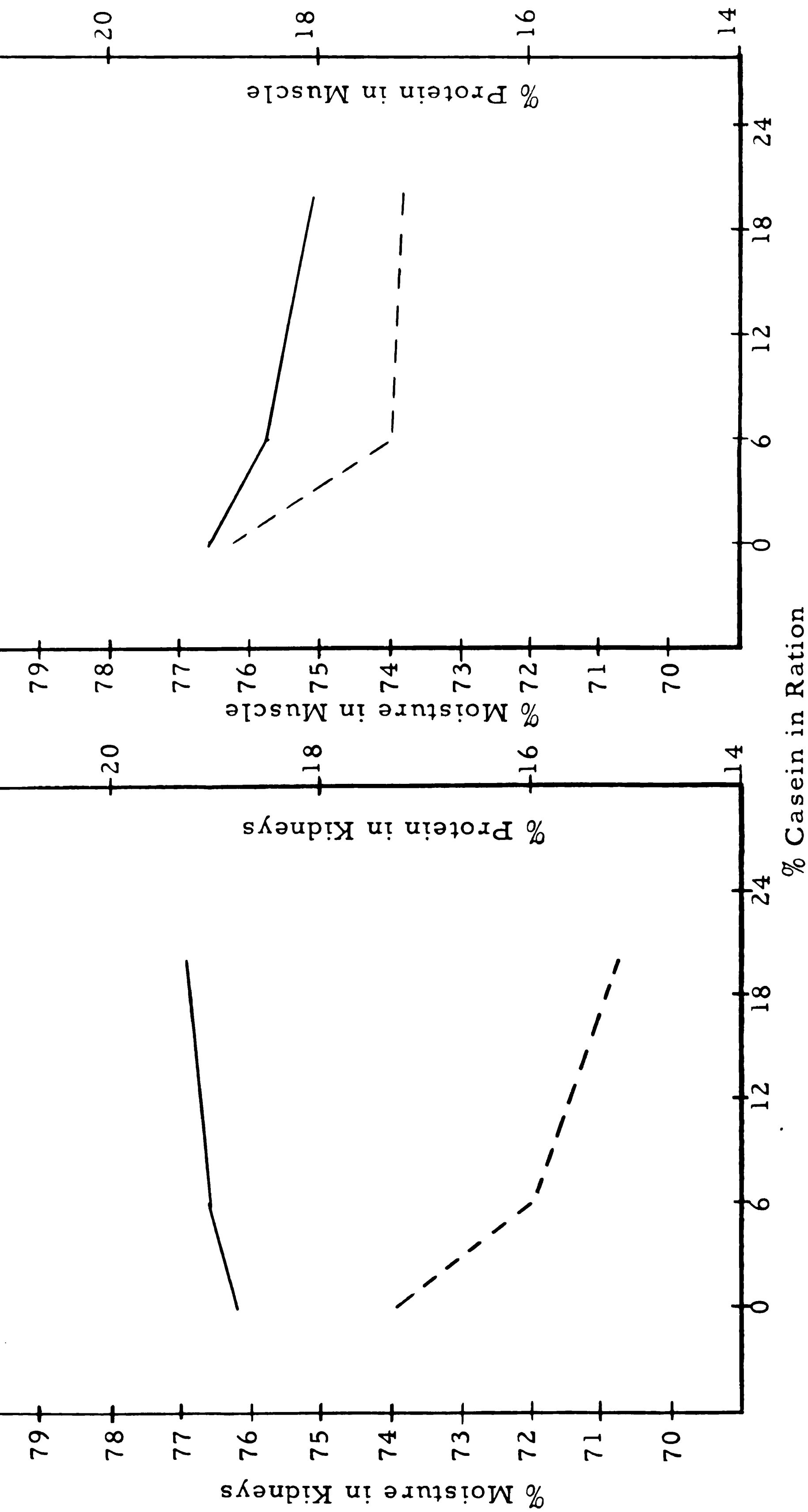

% Protein in Muscle
20
18
16
14
% Moisture in Muscle
79
78
77
76
75
74
73
72
71
70
% Protein in Kidneys
% Moisture in Kidneys
% Casein in Ration
0
6
12
18
24

Table 16. Composition of Tissues from Rats Fed Rations Containing 6% Casein with Supplements of 4 mg. and 10,004 mg. Thiamine in Each Kg. of Ration. The Carbohydrate in All Rations was Sucrose. All Values Expressed on the Basis of Fresh Tissue. (Glycogen was Calculated as the Difference Between the Sum of the Other Percentages and 100.)

Tissue	No. of Animals	Diet No.	Diet Description	Moisture %	Protein %	Moisture/Protein	Fat %	Ash %	Glycogen %
Heart	5	1	Basal, 6% casein	78.77	15.75	5.00	1.58	1.74	2.16
	5	26	Basal + 1% B_1	78.73	15.81	4.98	2.09	1.74	1.63
Kidney	5	1	Basal, 6% casein	76.72	16.00	4.60	3.77	1.62	1.89
	5	26	Basal + 1% B_1	78.59	14.88	5.28	3.14	1.89	1.50
Liver	43	1	Basal, 6% casein	71.9*	14.60*	4.92	3.74*	1.57	8.19
	35	26	Basal + 1% B_1	72.4*	13.47*	5.37	4.17*	1.83	8.13
Muscle	5	1	Basal, 6% casein	75.79	17.45	4.34	4.43	2.23	0.10
	5	26	Basal + 1% B_1	73.46	18.81	3.91	5.68	1.86	0.19

*Average for all trials; only 5 of Trial 3; see notes, Table 14.

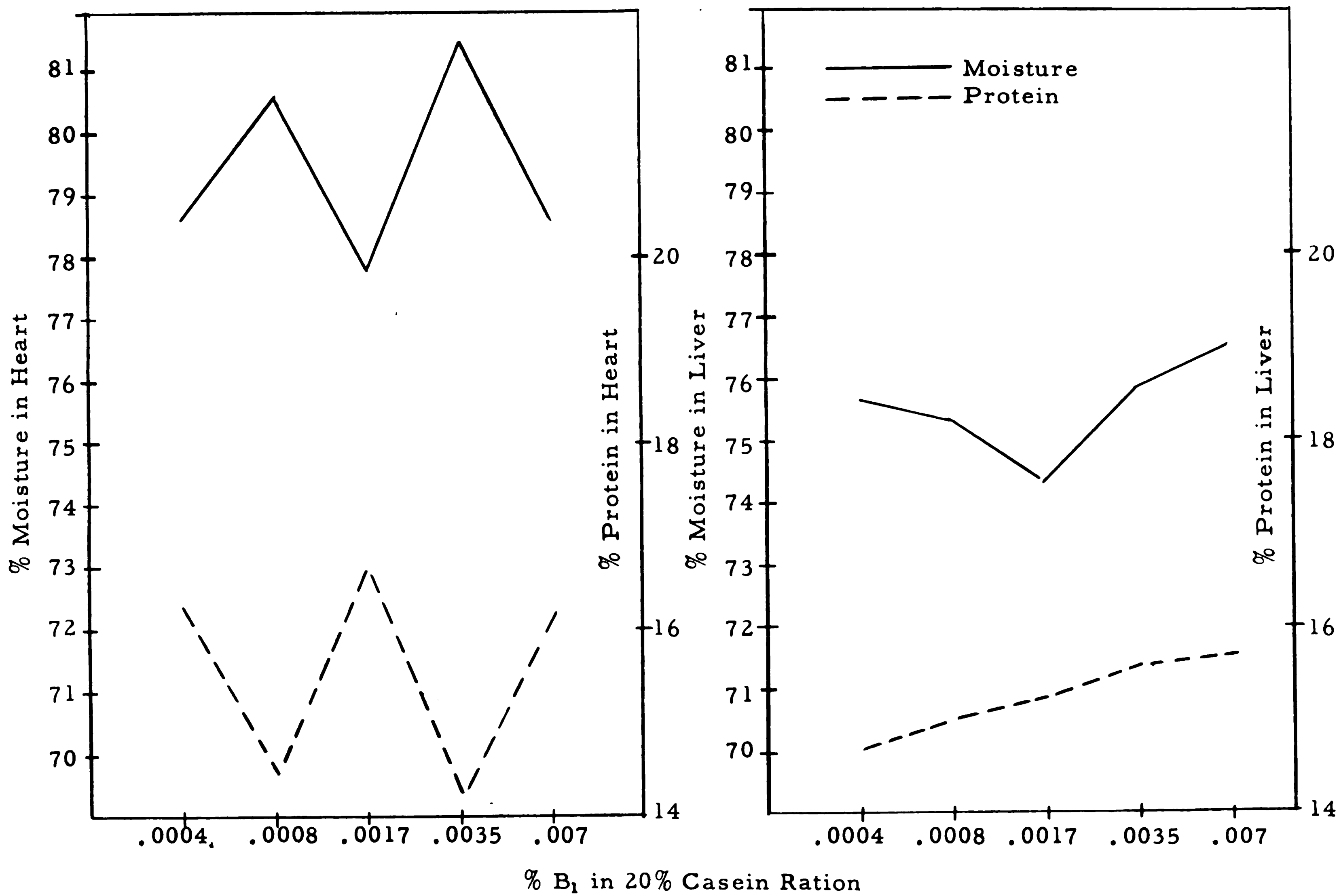
Table 17. **Composition of Tissues from Rats Fed Rations Containing 20% Casein with Supplements of Thiamine. The Carbohydrate in All Rations was Sucrose. All Values Expressed on the Basis of Fresh Tissue. (Glycogen was Calculated as the Difference Between the Sum of the Other Percentages and 100.)**

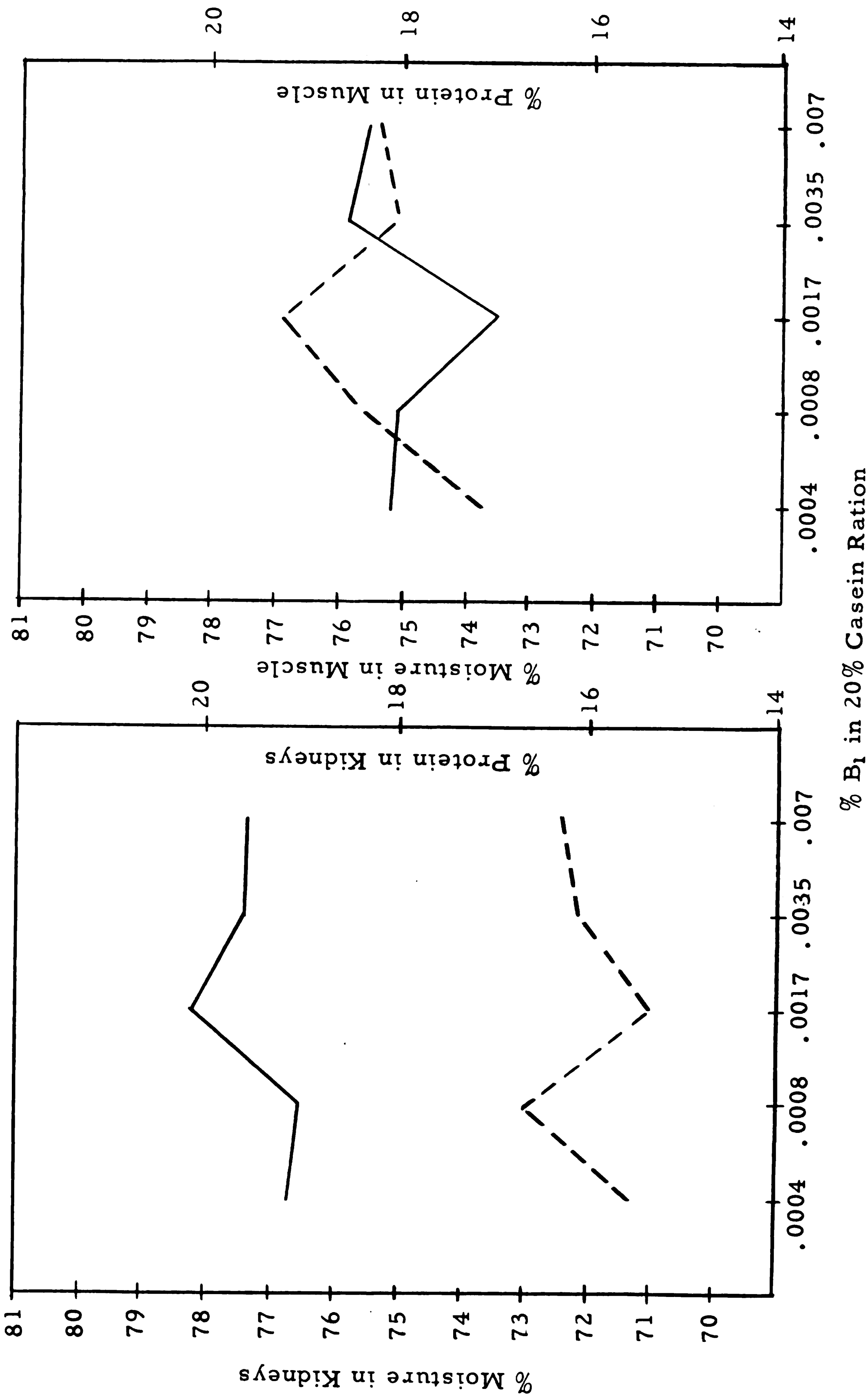
Tissue	No. of Animals	Diet No.	Diet Description	Moisture %	Protein %	Moisture/Protein	Fat %	Ash %	Glycogen %
Heart	5	21	Basal, 20% casein	78.79	16.38	4.81	1.34	1.44	2.05
	5	30	Basal + 0.0008% B_1	80.66	14.88	5.42	1.46	1.34	1.66
	5	29	Basal + 0.0017% B_1	77.79	16.75	4.64	1.58	2.52	1.36
	5	28	Basal + 0.0035% B_1	81.50	14.19	5.74	1.60	0.91	1.80
	5	27	Basal + 0.007% B_1	78.59	16.31	4.82	1.52	1.52	2.06
Kidney	5	21		76.93	15.69	4.90	3.36	1.17	2.85
	5	30		76.61	16.81	4.56	3.25	1.42	1.91
	5	29		78.28	15.50	5.05	3.75	1.31	1.16
	5	28		77.43	16.31	4.75	3.00	1.57	1.69
	5	27		77.41	16.38	4.73	2.90	1.80	1.51
Liver	5	21		70.01*1	18.48*1	3.79	2.41*1	1.64	7.46
	5	30		70.55	18.26	3.86	2.23	1.56	7.40
	5	29		70.70	17.86	3.96	2.20	1.97	7.27
	5	28		71.32	18.58	3.84	2.14	1.66	6.30
	5	27		71.48	18.98	3.77	2.44	1.83	5.27
Muscle	5	21		75.22	17.39	4.33	4.78	1.87	0.74
	5	30		75.20	18.44	4.08	4.25	1.52	0.59
	5	29		73.55*2	19.44	3.78	4.98	1.82	0.21
	5	28		75.92	18.04	4.21	4.16	1.53	0.35
	5	27		75.60	18.23	4.15	4.29	1.64	0.24

*1 Average for Trial 3.

*2 5% Level of Significance with Diet Nos. 21, 27, 28, 30.

Figure 12. Percent moisture and percent protein in heart, kidneys, liver and muscle for rats fed 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.




% Protein in Muscle
% Moisture in Muscle
% Protein in Kidneys
% Moisture in Kidneys
% B_1 in 20% Casein Ration
.0004
.0008
.0017
.0035
.007
81
80
79
78
77
76
75
74
73
72
71
70
20
18
16
14

Very high levels of thiamine in the rations for four weeks had no effect on the composition of the tissues as measured by the tests used in this research. None of the slight differences observed for any components were found to be significant. For this reason the tissues from the intermediate groups of rats were not analyzed. Analyses were carried out on all groups receiving an adequate protein intake since it was felt that such animals would be more nearly comparable to the mass of people in the United States who may consume excessive amounts of thiamine.

The most interesting data can be observed in Tables 13 and 14. The percentages appeared to be about the same within groups for moisture, protein and fat content. However, when Diets 1 vs. 6, 2 vs. 7, 3 vs. 8, 4 vs. 9, 5 vs. 10 were compared for moisture to protein ratio (Table 13) those for sucrose are all lower than those for dextrin. Yet as discussed previously dextrin seems to contain some component which contributes to higher gains in weight the first week following weaning and also to a lowered incidence of fat deposition in the liver. Evidently in some way the carbohydrate also influences the moisture-protein ratio of tissues other than the liver.

The data in Table 14 indicates that as the protein content of the ration increased, the moisture to protein ratio of the liver decreased. This was apparent when the animals fed the 12, 20, and 30% casein basal rations were compared: Diet 11 = 4.15; 21 = 3.95; 16 = 3.79. This was also true for the rats receiving 0.2% excess thiamine: Diet 12 = 4.63; 17 = 3.82; 0.4% excess thiamine: Diet 13 = 4.45 and 18 = 3.83; 0.5% excess thiamine: Diet 14 = 4.41 and 19 = 3.94; and 1% excess thiamine: Diet 15 = 4.33 and 20 = 3.98. The supplementary thiamine levels evidently had no influence on these ratios.

Omitting choline from the ration did not change the moisture to protein ratio, Diets 21 vs. 22 (Table 14). Even the addition of 0.1%

thiamine to a ration from which choline was omitted (Diet 24) or the addition of both choline and 0.1% thiamine to a ration (Diet 23) did not alter the moisture to protein ratio significantly. The writer does not fully understand the significance of these data though it probably has something to do with the lipotropic action of protein or the interaction of the various dietary components in the rations.

The excess niacin (Diet 25) appeared to depress the protein level in the liver and had some effect on the fat level (statistically significant at the 5% level with Diet 21 and Diet 23). Handler _et al._ ('42) found that the choline requirement increased when niacin was increased in the ration. None of the differences which can be observed between Diets 22 and 25 are significant.

The greater part of the data in the literature on chemical composition has been for rat carcasses; more recent reports include values for the liver and occasionally for other tissues. Hatai, whose data are on the dry weight basis, Moulton, whose data are on the basis of a fat-free carcass, and Chanutin and Dunn, whose data are also on carcass, give these figures for animals 42 days old (rats in this research were around 45-49 days old when sacrificed): moisture, 75%, protein 15-18%, and ash 3.7-3.8%. Chanutin and Dunn give a lower moisture content, 61-67% and a high fat content, 12-14%. None of these workers calculated the glycogen content of the carcass.

The moisture values for the tissues examined ranged between 70 and 81%; most of the ash data were between 1-2%; fat between 1.7 and 4.4; the protein data were comparable to the data presented above for carcasses. However, the fact that individual tissues were examined in this study and not the entire carcass must not be lost sight of when comparing results with that of others.

Kosterlitz reported the following liver analysis: moisture, between 69.75 and 70.3%; protein, 13.85-17.8%; fat, 1.67-4.18%; glycogen, f

s

c

r

p

T

s

th

n

A

s

le

s

f

a

a

d

a

T

gi

ea

gl

2.98-5.71% depending upon the protein content of the ration. Ash, by difference would range between 3.45 and 3.87%.

Serum Protein Composition

A limited study was made of the protein components in the blood serum of rats fed rations containing 6% casein with various supplements of thiamine, Table 18. Electrophoretic analysis of the serum from the rats showed that the level of thiamine in the diet had no influence on the percentage of the various protein fractions. The information in this Table represents data from only one trial of the several 6% casein-sucrose dietary studies conducted.

All values for the protein fractions are expressed as a percent of the total proteins. Total protein could not be determined by conventional methods because all the serum was used for the electrophoresis analysis. Although the "t" values obtained in the statistical analysis indicated some significance for albumin, alpha one and beta fractions, because of the low standard errors the biological importance of these data is open to serious question. Differences of this magnitude in plasma protein fractions are frequently seen among animals fed the same diet.

Robertson ('12-'13) has reported total globulin, 26% and total albumin, 74% for normal rats; for fasted rats, a total globulin of 36% and a total albumin of 64%. Donaldson ('24) indicates for rats 45 to 50 days old a total of 56% for albumin and 44% for globulin.

Moore ('45) indicated that adult rat sera do not usually contain alpha-globulin, while gamma globulin in many rats was extremely low. The sera of young rats (21-29 days old) contained a definite alpha-globulin and a hardly measurable amount of gamma-globulin. In an earlier study Moore *et al.* ('44) stated that the ratio of albumin to globulin depended partly upon the pH of the buffer used. At a pH of 7.4

Table 18. Serum Protein Fractions as Determined by Electrophoresis of Rats Fed 6% Casein Diets With Supple-

Table 18. Serum Protein Fractions as Determined by Electrophoresis of Rats Fed 6% Casein Diets With Supplements of Thiamine. In All Rations the Carbohydrate was Sucrose. All Values for the Protein Fractions are Expressed as a Percent of the Total Proteins.

No. of Animals	Diet No.	Diet Description	Electrophoresis Data: Albumin %	Globulin %: Alpha One %	Globulin %: Alpha Two %	Globulin %: Beta %	Globulin %: Gamma %	Total Globulin %
20	1	Basal, 6% casein	44.0±1.0	15.0±0.5	14.0±0.5	18.0±0.6	9.0±0.6	56.0%
20	2	Basal + 0.2% B_1	47.0±1.1	15.0±0.5	14.0±0.5	16.0±0.5	8.0±0.7	53.0%
20	4	Basal + 0.4% B_1	46.0±1.3	16.0±0.6	14.0±0.6	16.0±0.7	8.0±0.5	54.0%
20	26	Basal + 1.0% B_1	48.0±1.2	14.0±0.4	14.0±0.4	16.0±0.4	8.0±0.9	52.0%

Level of Statistical Significance of Differences Between Groups

	Albumin		Globulin: Alpha One		Alpha Two		Beta		Gamma	
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
Groups Compared	0	1 with 26	4 with 26	0	0	0	1 with 2, 26	1 with 4	0	0

the percent of albumin was about 70%; that for globulin, 30%; in a buffer with a higher pH, 8.5, albumin was 56% and globulin, 44%. These data were just the opposite of the latter and also of Donaldson's (buffer this research, pH, 8.6). It may be that the techniques used in the experimental procedure or in calculation may explain the differences.

Concentration of Thiamine in Tissues

It is generally assumed that the body is unable to store thiamine for any length of time. Thiamine depletion of an individual occurs most rapidly from the muscles and most slowly from the brain, nervous system, heart and liver (Bicknell and Prescott, '53). The thiamine in skeletal muscle is intracellular and extracellular, mostly the former. The latter is freely diffusible and in equilibrium with that of the plasma (Carleen, '44). According to Holt ('44) when the thiamine intake is increased a point of saturation is reached, and the tissues do not store any further thiamine in spite of an increased intake. The largest part of what is stored is stored in the muscles, according to Holt. If more thiamine is ingested than is needed, the excess is metabolized in the body or excreted in the urine.

Ferrebee ('42) indicated that the total amount of thiamine in the body of a well-nourished person is about 25 mg. The richest tissue is heart muscle (2-3 gamma per gram) followed by brain, kidney, and liver (1 gamma per gram), and skeletal muscle (0.5 gamma per gram). Ferrebee did not analyze the testes. A Texas publication of "Studies on the Vitamin Content of Tissues" ('41) stated that the thiamine content in tissues depends on the sex of the rat: liver is the richest tissue of the male, 7.8 gamma per gram; heart is the richest tissue of the female, 7.6 gamma per gram; the next richest tissue of the male is brain, 4.8 gamma per gram, but for the female, the kidneys, 4.4 gamma per gram.

Muscle is the least rich tissue in thiamine--1.2 to 1.3 gamma per gram of moist tissue.

The blood level range of thiamine is 4 to 10 gamma per 100 ml. whole blood (Carleen, '44). Using different methods of estimation a range of 2 to 17 gamma has been reported (Sinclair, '39; Meiklejohn, '37; Rowlands and Wilkinson, '38; Ritsert, '39; Goodhart, '40, '41; Schultz and Knott, '41; Waismann et al., '51). Thiamine is present in the red and white cells mainly as cocarboxylase (3 to 12 gamma per 100 ml. with an average of 7 gamma); free thiamine is present mainly in the plasma (0.5 gamma to 2 gamma per 100 ml.). The blood thiamine varies widely at a given intake even in the same individual and is not related to the rate of excretion (Friedemann et al., '48). The concentration in the tissues (Bicknell and Prescott do not state which ones) is about twenty times that in the blood.

Table 19, Figures 13, 14, 15, and 16 summarizes the thiamine concentration data for brain, heart, kidneys, liver, muscle, testes, and blood of rats fed various casein diets with supplements of thiamine. The concentration was greatest in the liver with the heart, kidneys, and testes next and approximately equal to each other. Least thiamine was found in the thigh muscle and none in the adrenals.

A significant observation was the inverse relation between the protein level in the ration and the thiamine concentration in many of the tissues. This was most obvious for the tissues from the rats fed the basal rations which contained 4 mg. of thiamine/kg. The liver showed the most dramatic response in this respect (Figure 14) with the rats fed the protein-free ration having 10.3 gamma of thiamine per gram of fresh liver. This decreased to 5.6 gamma per gram in the rats fed the 6% casein ration and to 2.2 gamma per gram in the rats fed the 20% casein ration. An equally dramatic inverse relation for these two parameters occurs in the thigh muscles from these rats (Figure 15).

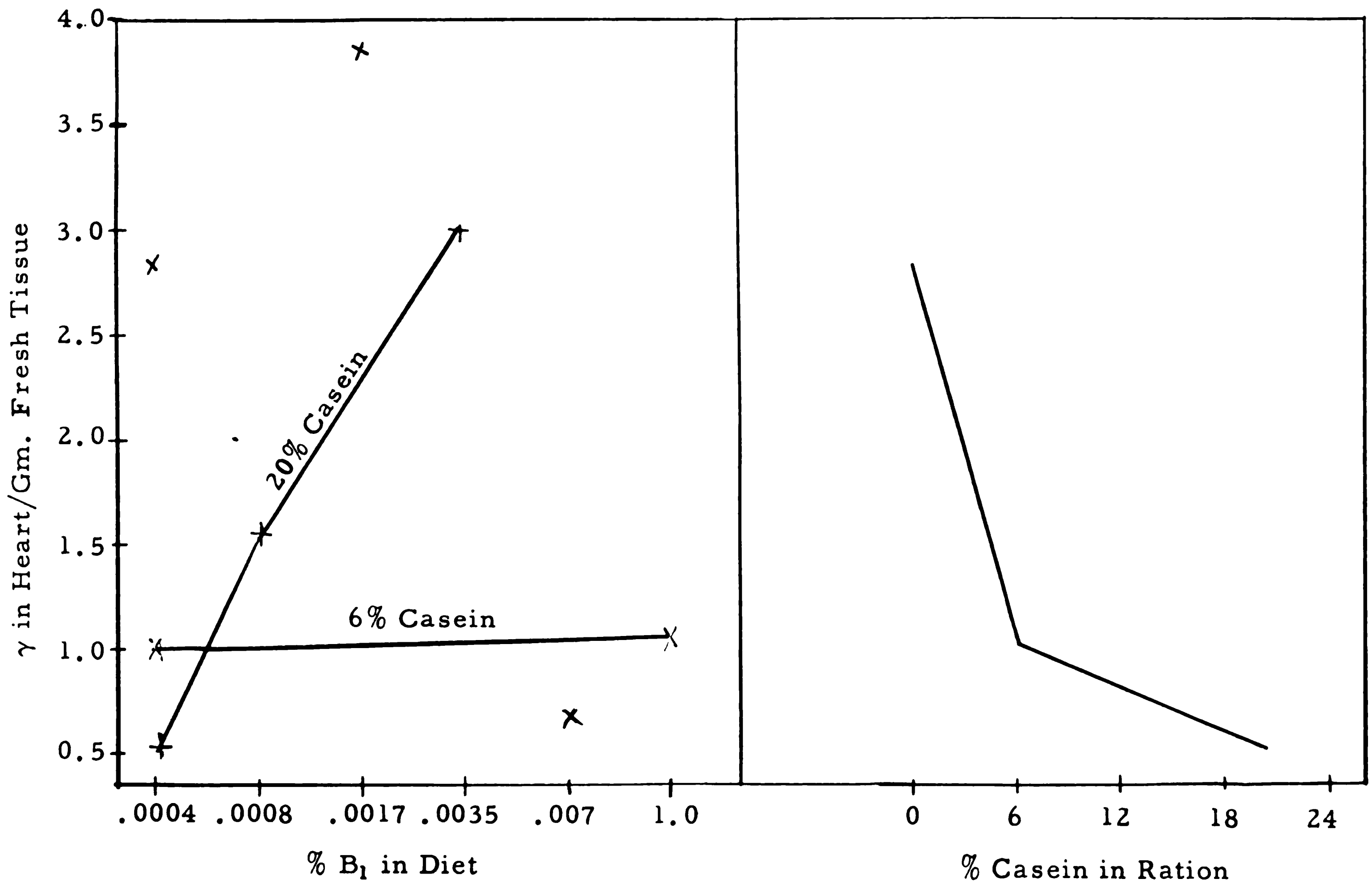
Table 19. Average Thiamine Concentration (in gamma per gm. fresh tissue) for Tissues of Rats Fed Rations Containing Different Levels of Casein, with Supplements of Thiamine. All Rations Contained Sucrose as the Carbohydrate.

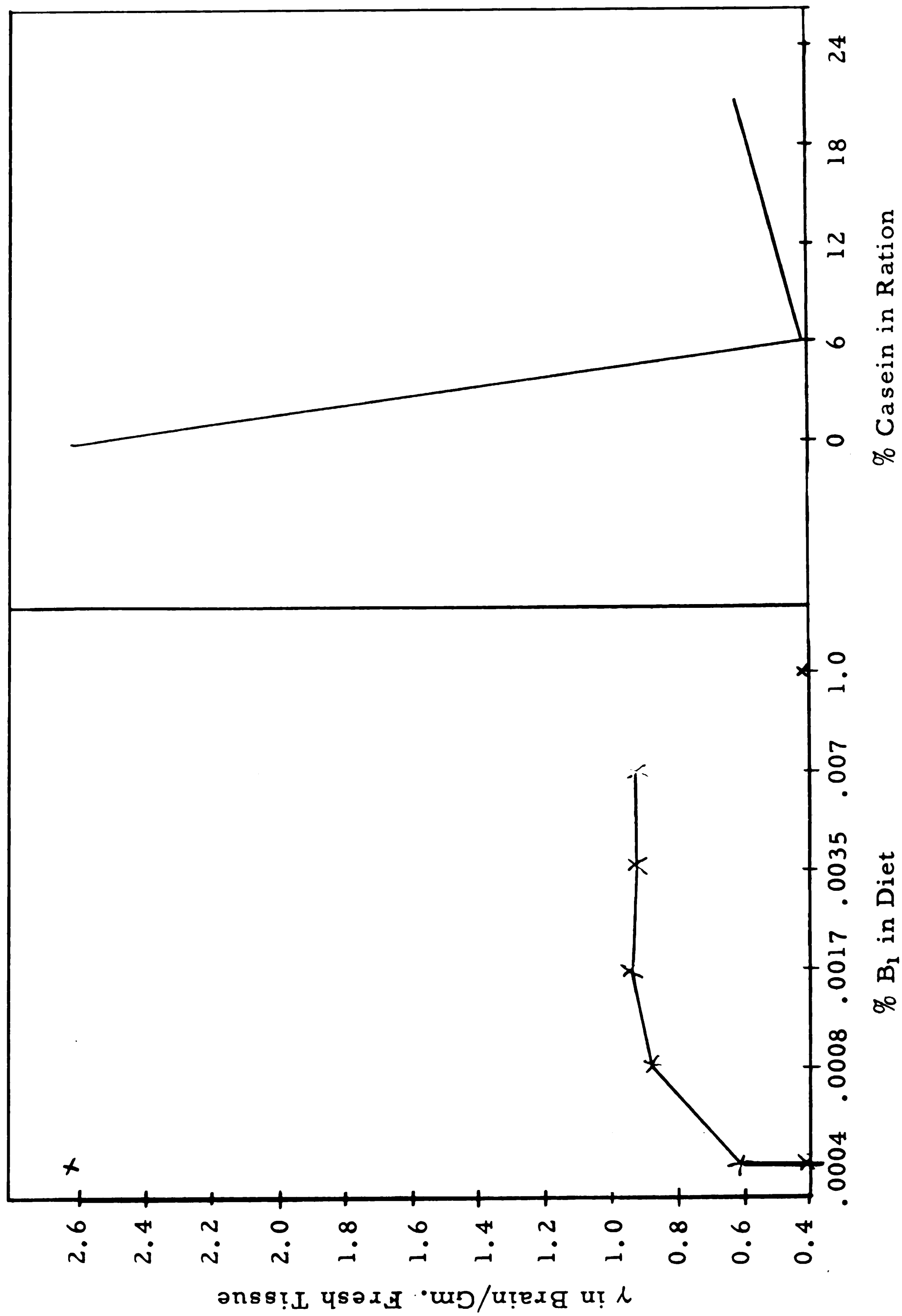
No. of Animals	Diet No.	Diet Description	Tissue: Brain	Heart	Kidneys	Liver	Muscle	Testes	Blood
5	21	Basal, 20% casein	0.61±0.1	0.47	0.78±0.0	2.17±0.1	0.09±0.0	3.34±0.2	0.08
5	30	Basal + 0.0008% B_1	0.88±0.1	1.46±0.0	2.09±0.4	4.53±0.1	0.66±0.1	2.33±0.3	0.09
5	29	Basal + 0.0017% B_1	0.95±0.1	3.79±0.5	1.80±0.3	2.20±0.2	0.15±0.0	2.71±0.1	0.12
5	28	Basal + 0.0035% B_1	0.92±0.2	3.12±0.8	1.95±0.4	6.06±0.6	0.25±0.1	2.43±0.5	0.14
5	27	Basal + 0.007% B_1	0.94±0.2	0.70	1.96±0.4	3.75±0.2	0.22±0.0	2.19±0.3	0.13
5	1	Basal, 6% casein	0.40±0.1	1.06±0.1	0.57±0.1	5.62±0.9	0.86±0.1	3.68±0.5	0.05
5	26	Basal + 1% B_1	0.40±0.1	1.02±0.2	0.46±0.0	2.73±0.1	1.18±0.1	4.10±0.8	0.20
5	31	No protein	2.66±0.1	2.82±0.1	3.51±0.0	10.30±0.6	1.32±0.2	—	0.01

Level of Statistical Significance of Differences Between Groups

	Tissue: Brain 1%	Brain 5%	Heart 1%	Heart 5%	Kidneys 1%	Kidneys 5%	Liver 1%	Liver 5%	Muscle 1%	Muscle 5%	Testes 1%	Testes 5%
Groups Compared	31 with 1, 21	0	1 with 31	29 with 26, 30	1 with 31	26 with 28, 29, 30	1 with 21, 31	26 with 1	0	21 with 1, 31	0	0
					29 with 30		28 with 21, 26, 29	27 with 21, 28, 29		26 with 21, 27, 28, 29		
							30 with 21, 26, 29					

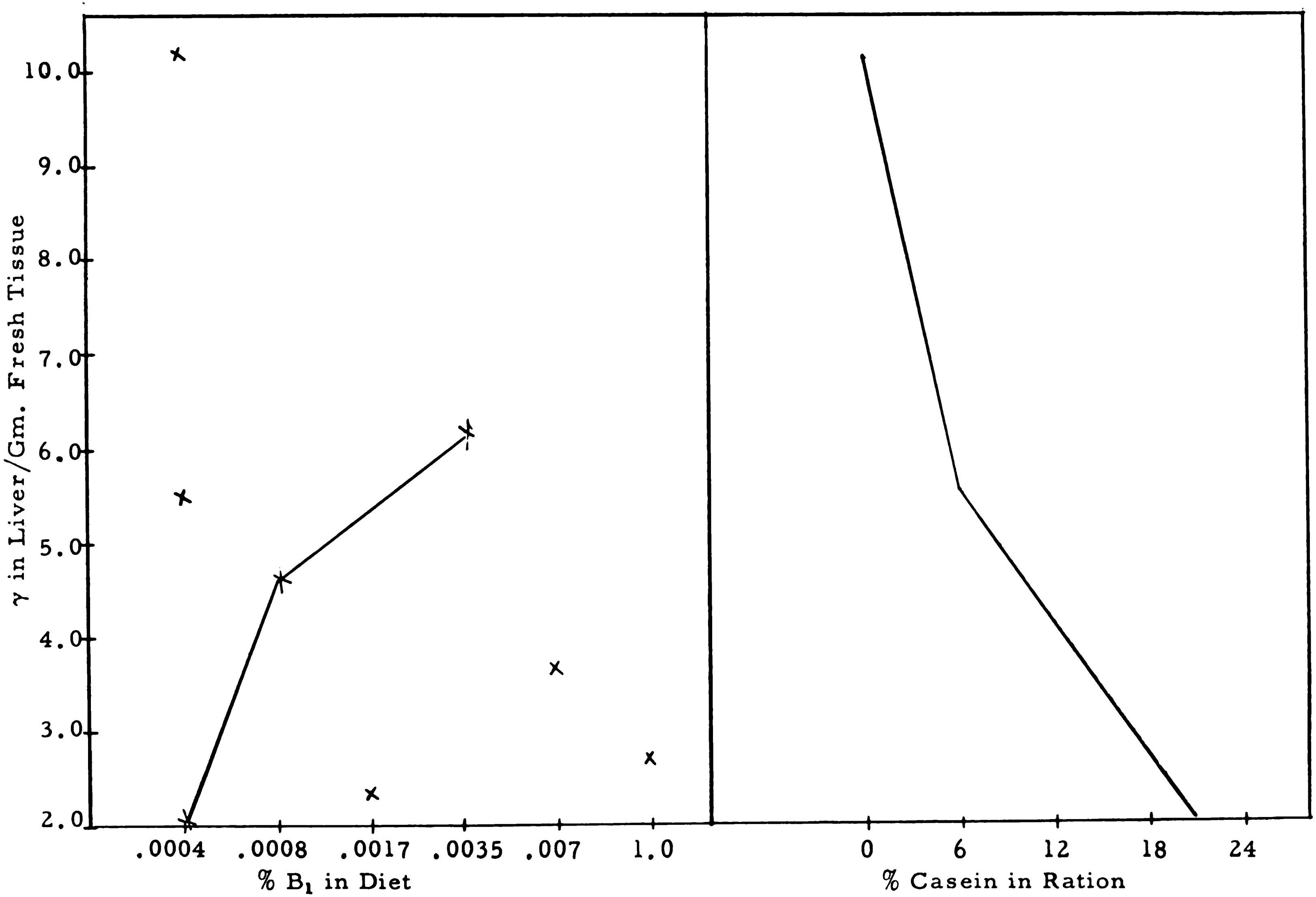
Figure 13. Concentration of thiamine in heart and brain per gm. fresh tissue for rats fed 0%, 6% and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.





γ in Brain/Gm. Fresh Tissue
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
.0004
.0008
.0017
.0035
.007
1.0
% B_1 in Diet
0
6
12
18
24
% Casein in Ration

Figure 14. Concentration of thiamine in liver and kidneys per gm. fresh tissue for rats fed 0%, 6%, and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.



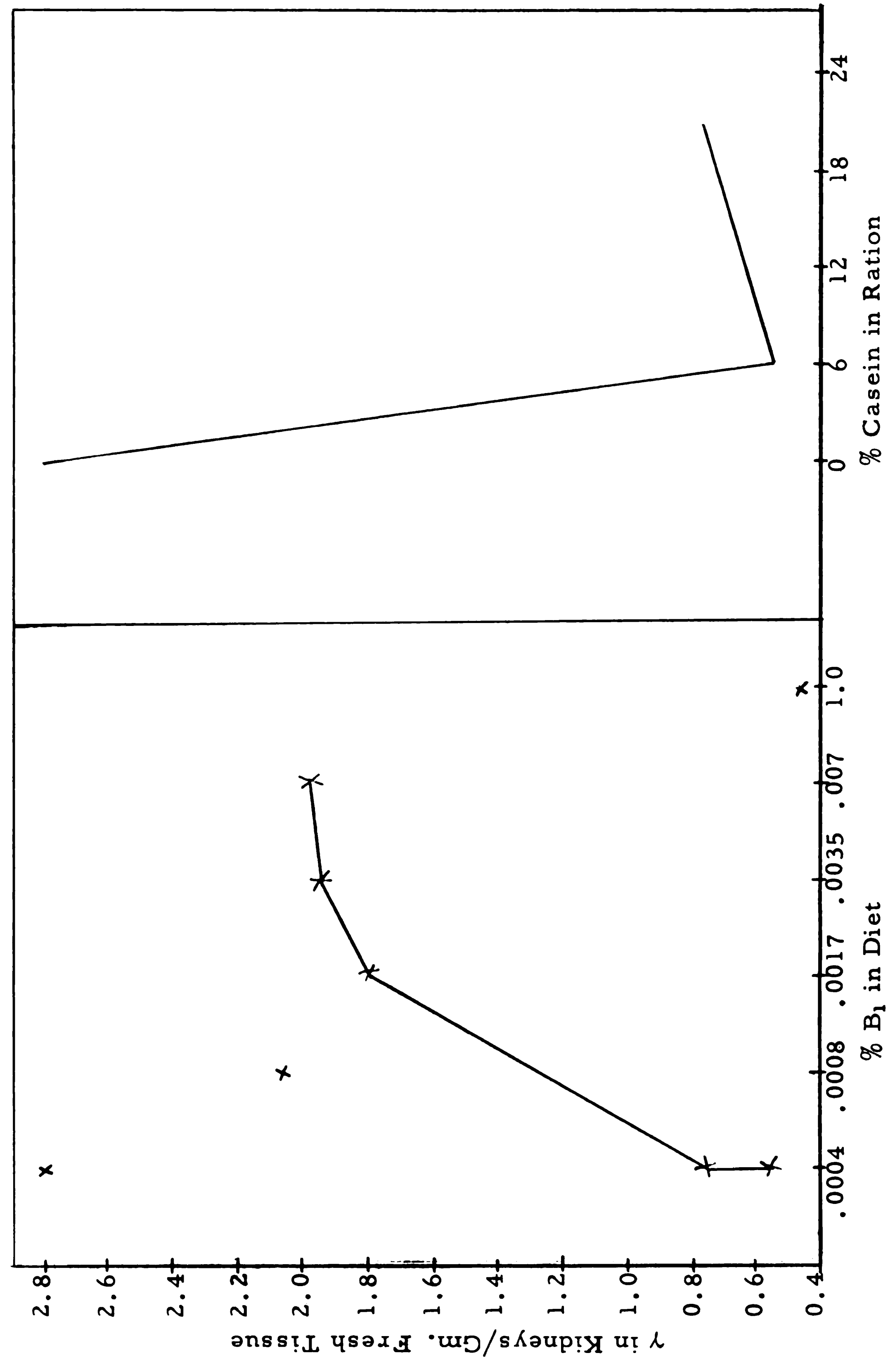
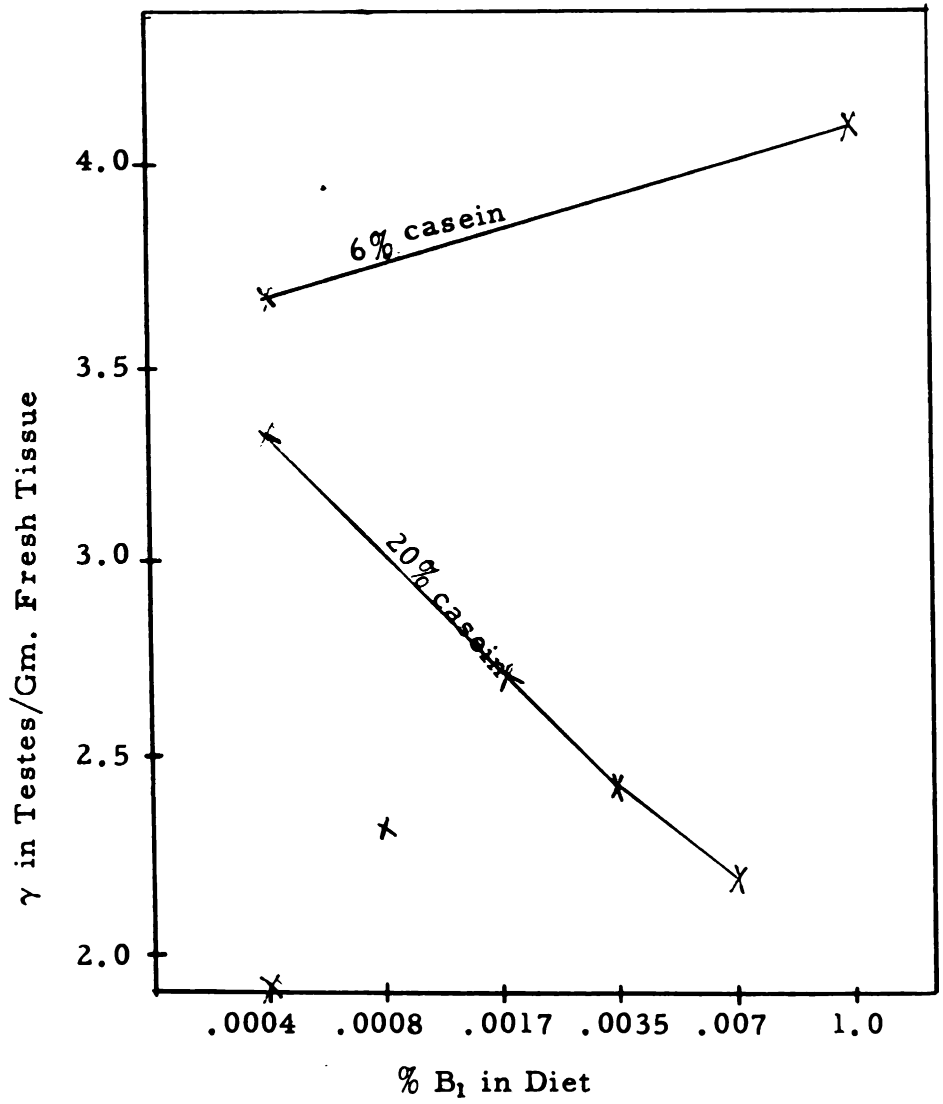

γ in Kidneys/Gm. Fresh Tissue
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
% B_1 in Diet
.0004
.0008
.0017
.0035
.007
1.0
% Casein in Ration
0
6
12
18
24

Figure 15. Concentration of thiamine in testes and muscle per gm. fresh tissue for rats fed 0%, 6%, and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.



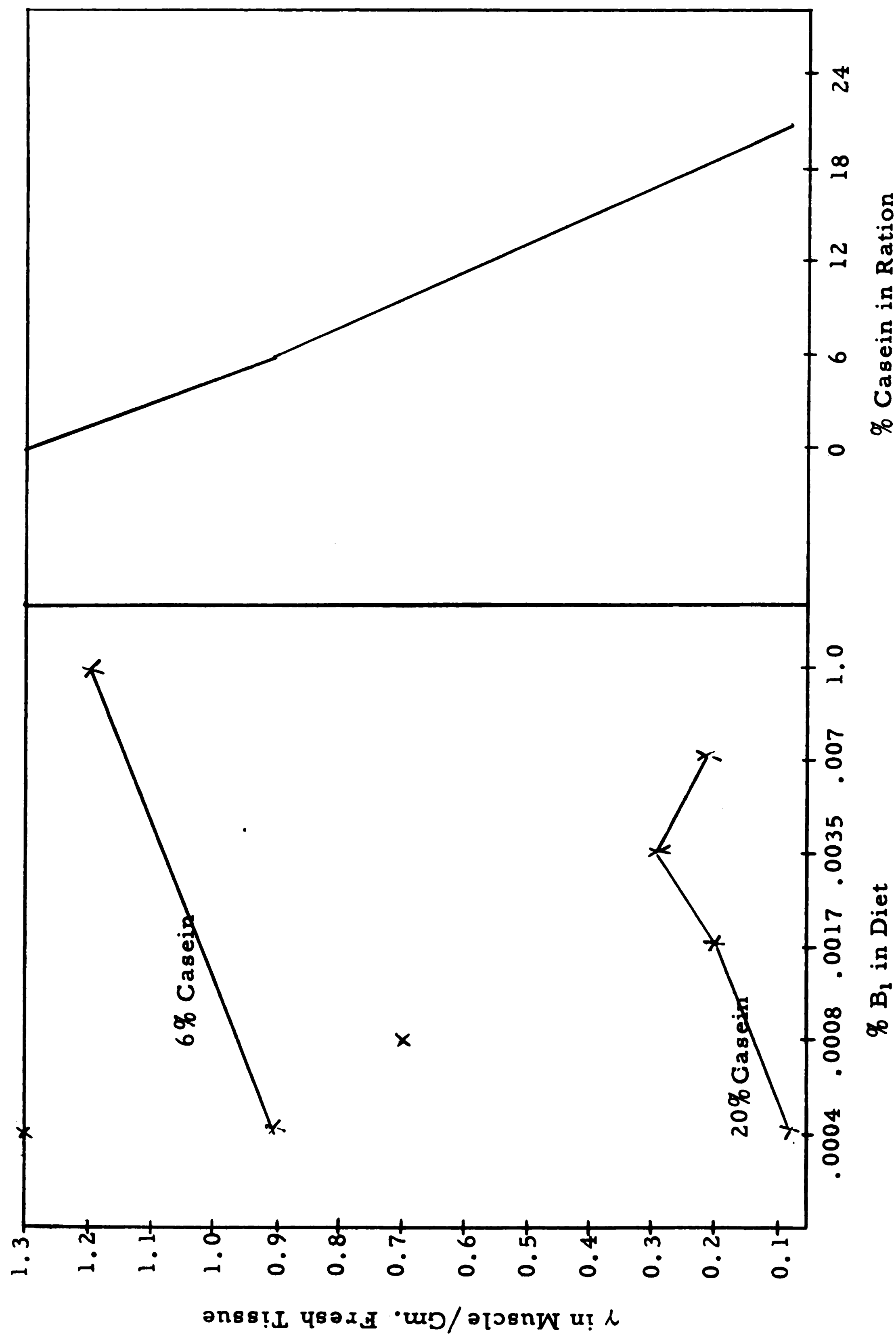
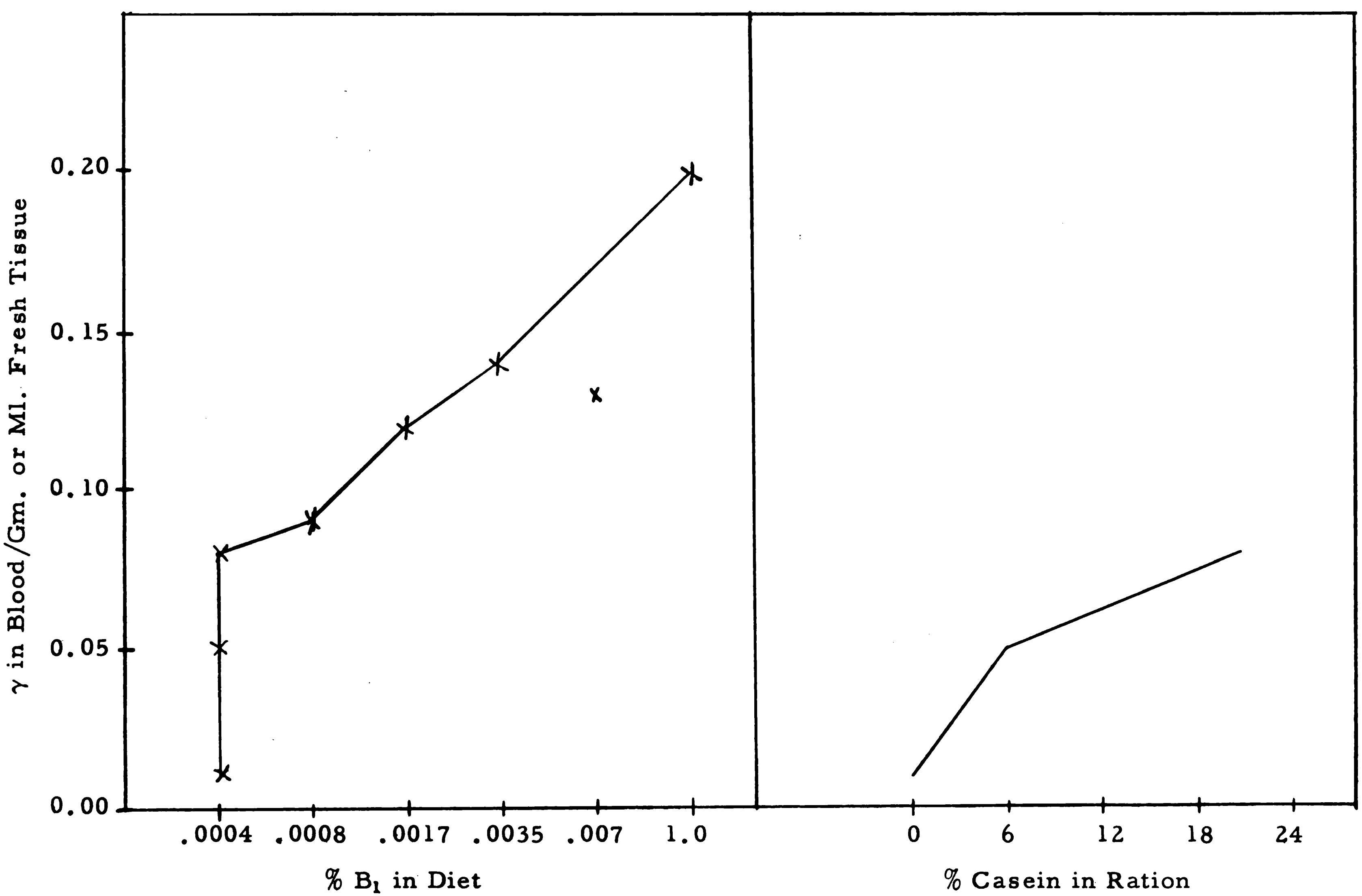

γ in Muscle /Gm. Fresh Tissue
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
6% Casein
20% Casein
.0004
.0008
.0017
.0035
.007
1.0
% B_1 in Diet
0
6
12
18
24
% Casein in Ration

Figure 16. Concentration of thiamine in blood per ml. for rats fed 0%, 6%, and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.



Here there was a 10-fold difference in thiamine concentrations of the muscles removed from the rats fed the 0% and 20% casein rations. The hearts from these rats also showed a similar relation although not as pronounced as the liver and muscle (Figure 13). The brains and kidneys removed from the rats fed the 6% casein ration had very much less thiamine than the same tissues from the rats fed the protein-free ration (Figures 13 and 14). There was slightly more thiamine in both of these tissues from the rats fed the 20% casein rations than from those fed the 6% casein rations.

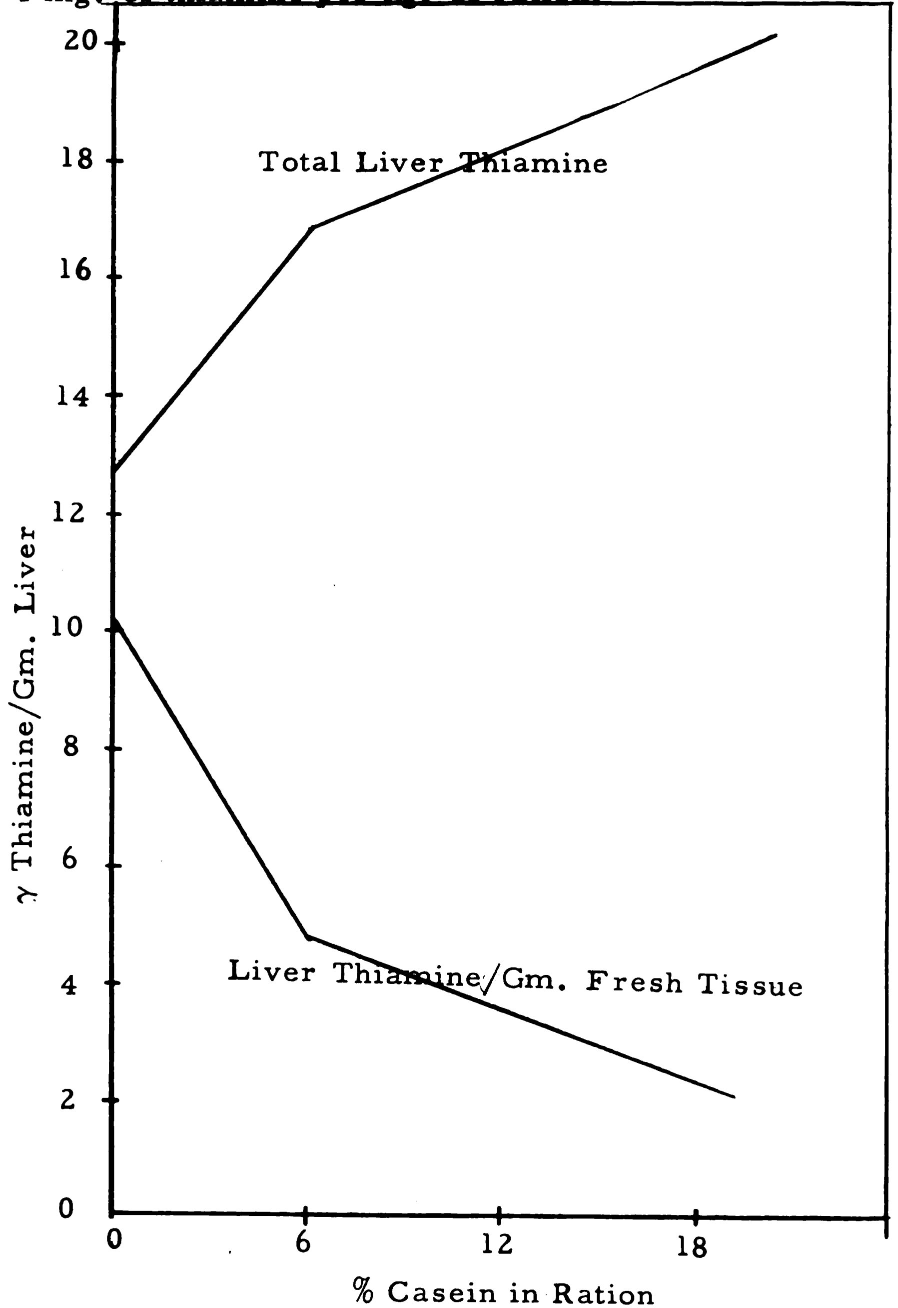
The astonishing features about these variations in thiamine content for the tissues from rats fed rations containing different levels of casein were: (1) the very much higher thiamine concentrations found in the organs from the rats fed the protein-free ration than from other rations supplemented with high doses of the vitamin. This would suggest that the mechanism involved in thiamine entrance into or retention by the cells of these organs is markedly altered by the protein-free diet. The alteration produced by the protein-free ration is so much greater than that which normally occurs by other types of dietary alterations (e.g. increasing the dietary thiamine by 1000 times). (2) Despite the inverse correlation between tissue concentration of thiamine and protein level in the rations, there was a direct correlation between the level of thiamine in the blood and the protein level in the ration (Figure 16). There was a progressive increase in the thiamine concentration in the blood which followed the increased intake of thiamine in the diet. Other tissues were not as responsive to dietary increases of thiamine. This was especially true of the testes (Figure 15). The testes of the rats fed no protein were not developed.

It might be suggested that the relation between dietary protein level and thiamine concentration is primarily a reflection of the poorer growth of the rats fed the low protein rations. According to such reasoning, the total thiamine in an organ such as the liver should show no change with dietary protein levels. The poorer growth should be associated with a reduced food intake and consequently the thiamine deposited in the tissues should be no greater on the basis of the entire organ or tissue than is the case for the animals eating more food and growing at a more rapid rate. To determine whether the data verified this statement the table below and Figure 17 present the amount of thiamine/gm. of liver, the average weights of the livers for each group, and the total amount of thiamine in those livers. Comparisons are made among the various protein diets, the 6% casein diets, and the 20% casein diets with various supplements of thiamine.

Dietary Protein	Dietary B-1 gamma/gm.	B-1 Intake/ 4 Weeks	Liver B-1 gamma/gm.	Liver Wgt. gm.	Total B-1 gamma
0	4	410.4gamma	10.30	1.31	13.49
6	4	862.4	5.62	3.14	17.65
20	4	1,400	2.17	9.43	20.46
6	4	862.4	5.62	3.14	17.65
6	10,004	2,031,812.4	2.73	2.99	8.16
20	4	1,400	2.17	9.43	20.46
20	12	4,027.2	4.53	8.08	36.60
20	21	7,337.4	2.20	8.67	19.07
20	39	14,043.9	6.06	9.27	56.18
20	74	26,218.2	3.75	10.13	37.99

Similar comparisons could be made for all the tissues analyzed for thiamine. The above information indicates that when the comparison was made on the basis of dietary protein an inverse relationship was found between the amount of thiamine retained in the liver per gram of tissue and the total amount of liver thiamine. This relationship was not evident when comparisons were made within dietary groups supplemented with various levels of thiamine. Poorer growth of the animals on the low protein rations was associated with a reduced food intake; on the other hand, the data show that there was greater deposition of thiamine in the tissues

Figure 17. Concentration of thiamine in liver per gm. fresh tissue and concentration of thiamine in total liver, fresh tissue, for rats fed 0%, 6%, and 20% casein rations containing 4 mg. of thiamine per kg. of ration.



of animals on the low protein rations. Because the number of animals in this study was small further research is needed to justify a conclusion.

Recovery of Thiamine in Urine and Feces

It has been recognized for many years that thiamine is excreted in the urine and feces. It has been further recognized that this excretion was determined primarily by the amount of thiamine ingested. When the intake is maintained constant and other factors, such as exercise and infection (Benson *et al.*, '42), which have been found to influence output, are eliminated as variables, the amount in the urine represented that portion of the intake which had not been lost through the gastrointestinal tract or decomposed or retained in the tissues or used in metabolic processes.

A limited study was made of combined urinary and fecal thiamine excretion in 24 hours. A combined percent recovery of thiamine was determined for rats fed 6% casein-sucrose rations (Table 20). With increasing levels of dietary thiamine both urine and fecal excretion increased but that for the feces increased faster than that for the urine. When the actual excretion of thiamine expressed as gamma per 24 hours was considered in both urine and feces, the level of statistical significance of differences between all groups was highly significant (1% level of probability). However, the percent of thiamine recovered (total excretion in the urine and feces as a fraction of the thiamine from the food consumed) indicated that the difference was significant only between Diet 1 and Diets 2, 4, and 26, but not between Diets 2, 4, and 26 (Table 20, 6% casein rations with supplements of thiamine). When rats were fed "normal" levels of thiamine in the ration (Diet 1), excretion in the urine was about 4.2 times that in the feces. As the thiamine level in the ration increased, the fecal excretion exceeded the urinary excretion by 1.5 to 2 times.

Table 20. Urinary Thiamine Excretion Data of Rats Fed Rations Containing Different Levels of Casein with Supplements of Thiamine. All Rations Contained Sucrose as the Carbohydrate.

			SUMMER 1961		
No. of Animals	Diet No.	Diet Description	Urinary Thiamine: Urine γ per 24 hr.	Urinary Thiamine: Feces γ per 24 hr.	% Recovery
20	1	Basal, 6% casein	1.49±0.2	0.36±0.0	5.8±0.8
20	2	Basal + 0.2% B_1	790.±50.	1492.±93.	14.1±0.4
20	4	Basal + 0.4% B_1	1979.±237	3823.±415.	17.8±1.3
20	26	Basal + 1% B_1	4976.±370	6396.±541.	15.0±0.8

Level of Statistical Significance of Differences Between Groups

	SUMMER 1961					
	Urine γ per 24 hr. 1%	Urine γ per 24 hr. 5%	Feces γ per 24 hr. 1%	Feces γ per 24 hr. 5%	% Recovery 1%	% Recovery 5%
Groups Compared	1 with 2, 4, 26	0	1 with 2, 4, 26	0	1 with 2, 4, 26	2 with 4
	2 with 4, 26		2 with 4, 26			
	4 with 26		4 with 26			

WINTER 1963				
No. of Animals	Diet No.	Diet Description	Urinary Thiamine γ per Ml.	Urinary Thiamine γ per 24 hr.
10	21	Basal, 20% casein	2.22±0.1	12.9
10	30	Basal +0.0008% B_1	4.81±2.3	17.8
10	29	Basal + 0.0017% B_1	20.42±0.4	132.7
10	28	Basal + 0.0035% B_1	30.82±0.4	163.3
10	27	Basal + 0.007% B_1	56.41±1.0	406.2
10	1	Basal, 6% casein	2.32±0.0	5.1
10	26	Basal + 1% B_1	218.92±34.5	678.7
10	31	No protein	1.35±0.5 1.53±0.8	1.4 0.8

WINTER 1963 Urine γ per Ml. 1%	5%
26 with All	0
27 with 21, 28, 1, 31, 29, 30	
28 with 1, 21, 29, 30, 31	
29 with 1, 21, 30, 31	

Because of the small percent of thiamine recovered in the urine and feces in this study (Diet 1, 6%; Diet 2, 14%; Diet 4, 18%; Diet 26, 15%), a second study was undertaken to measure the metabolites of thiamine which Pollack et al. ('41) considered as reflecting the body stores of thiamine more accurately, even at low levels of thiamine intake. It may have been that in the first study adequate time to establish equilibrium between the intake and excretion of thiamine was not allowed. Although the major adjustment occurs within the first ten days, Mickelsen et al. ('60) state that it may take as long as six weeks to establish equilibrium between thiamine intake and excretion of thiamine. Both the first and second studies, were only four weeks in length.

Urinary Pyrimidine, Thiazole, and Thiamine

The first study of the estimation of thiamine in the urine of albino rats yielded a small recovery of the amount fed in the diet. Therefore, a second study was undertaken to study the metabolites of thiamine. Urine was collected for three days from each rat in each dietary group during the fourth week (final week) of the experiment. Two collections were made for the animals on the 0 protein ration because after eight days on the diet it looked as if the animals might die. From each of these 10 animals, therefore, urine was collected for a total of $14\frac{1}{2}$ days instead of just 3. The collection bottles for the last three days were kept separately and analyzed separately from the earlier collection.

One ml. of concentrated HCl and a layer of toluene were added to each collection container before it was placed under the funnel-like collecting unit of the metabolism cage. Evaporation no doubt occurred from the large surface of the collecting unit as well as from the collection bottle. As little water as possible was used in washing the funnel down at the end of the collection period. The urine was then filtered into a container which was covered, placed in the refrigerator and held at 0° C. until analyzed for the pyrimidine and thiazole components of thiamine by the yeast resynthesis method (Ziporin et al., '62).

The table below shows the volume of urine of the rats fed rations containing different levels of protein. These values should be considered estimates, not true urine volumes, since allowances must be made for evaporation and water used to wash down the funnel of the metabolism cage.

Diet	DietaryB-1, gamma/gm.	Urine Vol./Day/Animal, ml.
0 Protein	4	1.0
6% Casein	4	2.2
20% Casein	4	5.8

As the casein level in the ration increased the urine volume increased. This is in line with the increased requirement of water to excrete the degradation products of protein metabolism in the urine.

Holt and Kajdi ('44) in studying the nutritional requirements in inanition examined the water intake of the animals. On a diet of protein alone they found the intake of water high. The animals fed carbohydrate consumed somewhat less water than those fed protein. For both groups of animals the water intake paralleled the food intake. The surplus water (Holt and Kajdi) required by different foodstuffs (water taken in excess of that taken by fasting animals) is 0.85 cc. per calorie of ingested protein food, 0.29 cc. per calorie of ingested carbohydrate and only 0.08 cc. per calorie of ingested fat.

Holt and Kajdi did not investigate the effect of the different foodstuffs on the urine volume of the rat. Very likely the volume would have been closely related to the dietary intake. The primary exception would have been during the laying down of body tissue for which water is necessary.

In this study, the rats fed the 20% casein ration were consuming more food and laying down more body tissue, evidenced by an increase in weight (growth). At the same time the rats were probably consuming more water, although water intake was not measured, if the rats followed the observations of Holt and Kajdi of the need for more water on a higher dietary protein ration.

The rats fed the low-protein ration and especially the no protein ration were consuming more sucrose in their rations. Gamble ('44) has shown that approximately 100 grams of glucose can be substituted for 100 grams of water at almost no cost to water exchange. This figure was calculated from measuring urine water and available body water during fasting, then following the administration of 100 grams of glucose. As a result of this intake of glucose providing a source of fuel for the body as energy for catabolism, there was a large saving of body fluids which reflected a conservation or sparing of protein.

As stated above the rats fed 0 protein and low-protein rations were primarily receiving sucrose in the diet since the amount of fat in the ration was so low that there would be negligible alteration of the metabolism of the animals from the fat. In this sense, therefore, the diets in this research can be compared to the work of Holt and Kajdi. It would seem that the smaller urine volume per day collected from the animals fed these two diets was further evidence that sucrose in the diet contributed to a decreased urinary volume. The diets contained a small amount of salt. When men are working the administration of salt is used as a means of saving body fluids. It is not known to what extent the salt in the 0 protein and low-protein rations contributed to the small urine output.

Another observation from Holt's and Kajdi's study was the average number of days animals survived on sucrose--29.3. When dextrose was the carbohydrate source it permitted longer survival, 32.3 days.

(Carbohydrates studied--dextrose, levulose, invert sugar, sucrose and maltose.) The animals fed the no protein ration (or essentially a sucrose ration) were still alive on the 28th day of the study, the last day of the experiment.

Diet No. and Description	Dietary B-1 gamma/gm.	Urine Vol./day/ An., Ml.
21 Basal 20% casein	4	5.8
30 " + 0.0008% B-1	12	3.7
29 " + 0.0017% B-1	21	6.5
28 " + 0.0035% B-1	39	5.3
27 " + 0.007% B-1	74	7.2

At first glance of the data above one might make the erroneous observation that the level of supplementary thiamine influenced the urine volume. However, the urine volumes are so varied from diet to diet that there is no close relationship between intake of thiamine and output of urine. The data may have been subjected to several sources of error which have altered the volume of urine:

(1) This experiment was conducted between the months of January and March. During the winter the relative humidity in the animal room is fairly low.

(2) In the calculations no correction was allowed for the 1 ml. of HCl, which represents only a fraction of the 3-day volume.

(3) To wash down all the thiamine left on the funnel approximately 5 ml. of water was employed. It is not known whether this amount of water represented that lost by evaporation under the conditions of our animal room.

Table 21 and Figure 18 indicate that there was a dietary level of thiamine (about 10 gamma/gm. ration) below which very little urinary thiamine was seen. This also held true for the pyrimidine and thiazole components. They, too, appeared in the urine in increasing amounts when the level of thiamine in the ration exceeded 10 gamma/gm. ration. The urinary excretion of thiamine, as well as the thiazole and pyrimidine components were directly related to the protein content of the ration (Table 22, Figure 19). This was true for the rats fed the basal thiamine

Table 21. Urinary Pyrimidine, Thiazole, and Thiamine Excretion Data of Rats Fed Rations Containing Different Levels of Casein with Supplements of Thiamine. All Rations Contained Sucrose as the Carbohydrate.

No. of Animals	Diet No.	Diet Description	Pyrimidine γ/ml.	γ per 24 hr.	Thiazole γ/ml.	γ per 24 hr.	Thiamine γ/ml.	γ per 24 hr.
10	21	Basal, 20% casein	2.25±0.3	13.1	3.59±0.4	20.8	2.22±0.1	12.9
10	30	Basal +0.0008% B_1	3.15±0.9	11.7	4.26±1.9	15.8	4.81±2.3	17.8
10	29	Basal +0.0017% B_1	21.40±0.2	139.1	22.76±0.2	147.9	20.42±0.4	132.7
10	28	Basal + 0.0035% B_1	40.93±0.4	216.9	48.39±0.5	256.5	30.82±0.4	163.3
10	27	Basal + 0.007% B_1	74.86±0.7	539.0	74.11±0.8	533.6	56.41±1.0	406.2
10	1	Basal, 6% casein	2.10±0.1	4.6	2.23±0.2	4.9	2.32±0.0	5.1
10	26	Basal + 1% B_1	184.38±27.3	571.6	112.85±59.6	349.8	218.92±34.5	678.7
10	31	No protein	0.92±0.3	0.9	1.55±0.3	1.6	1.35±0.5	1.4
			0.86±0.6	0.5	1.81±0.3	1.0	1.53±0.8	0.8

Level of Statistical Significance of Differences Between Groups

	Pyrimidine 1%	Pyrimidine 5%	Thiazole 1%	Thiazole 5%	Thiamine 1%	Thiamine 5%
Groups Compared	26 with 1, 21, 27, 28, 29, 30, 31	0	Ditto	21 with 31a	Ditto	0
	27 with 1, 21, 28, 29, 30, 31		Ditto		Ditto	
	28 with 1, 21, 29, 30, 31		Ditto		Ditto	
	29 with 1, 21, 30, 31		Ditto		Ditto	

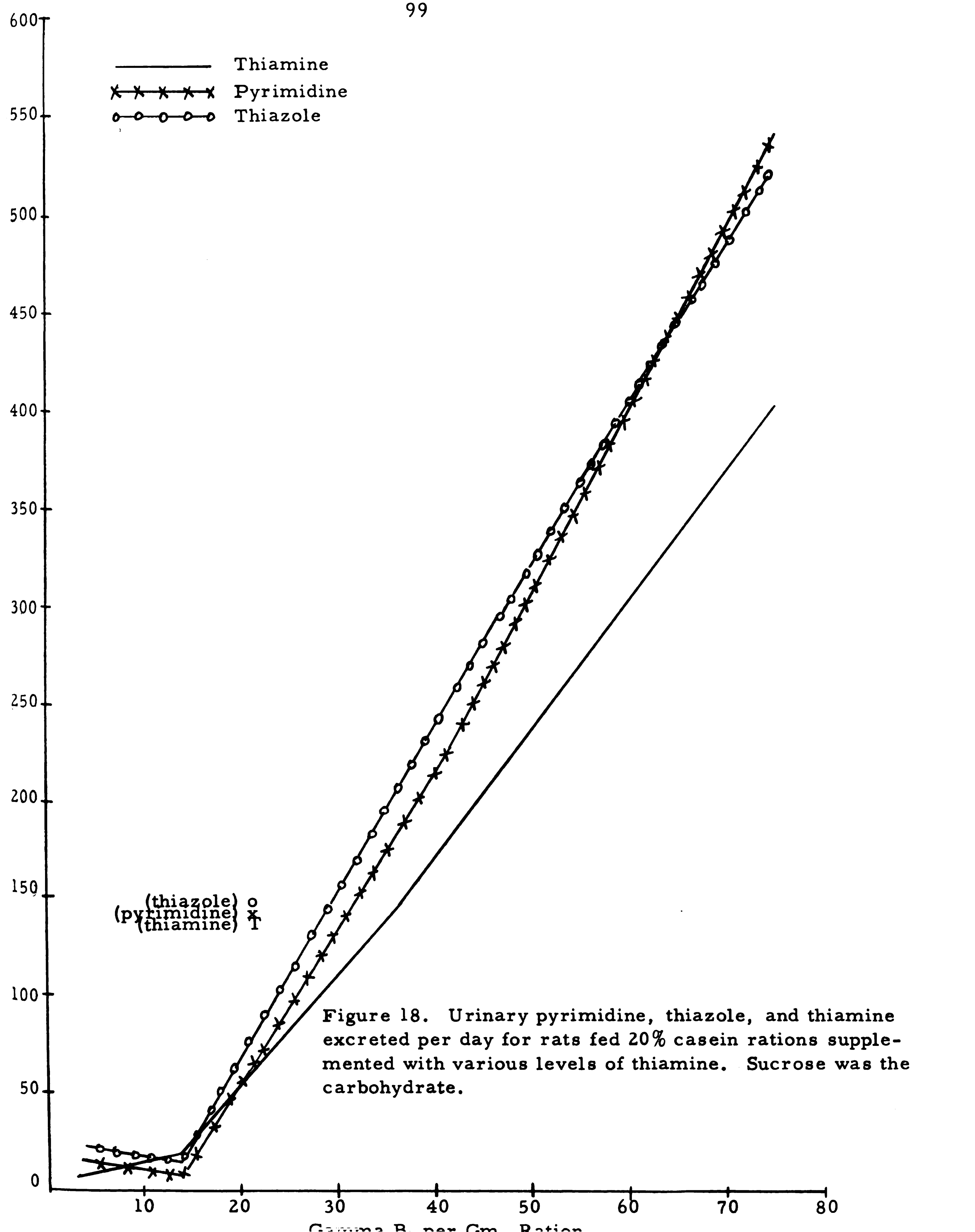


Figure 18. Urinary pyrimidine, thiazole, and thiamine excreted per day for rats fed 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.

Table 22. Recovery of dietary thiamine in urine of rats fed rations containing different levels of casein. All rations contained 4 mg. thiamine/kg. The carbohydrate was sucrose. There were 10 rats/group.

Dietary Casein	Intake/24 hrs.		Urinary B-1/24 hrs.	
%	Food (gm.)	Thiamine (γ)	γ	% of intake
0	3.7	14.8	1.4	9.4
6	7.7	30.8	5.1	13.3
20	12.5	50.0	12.8	25.6

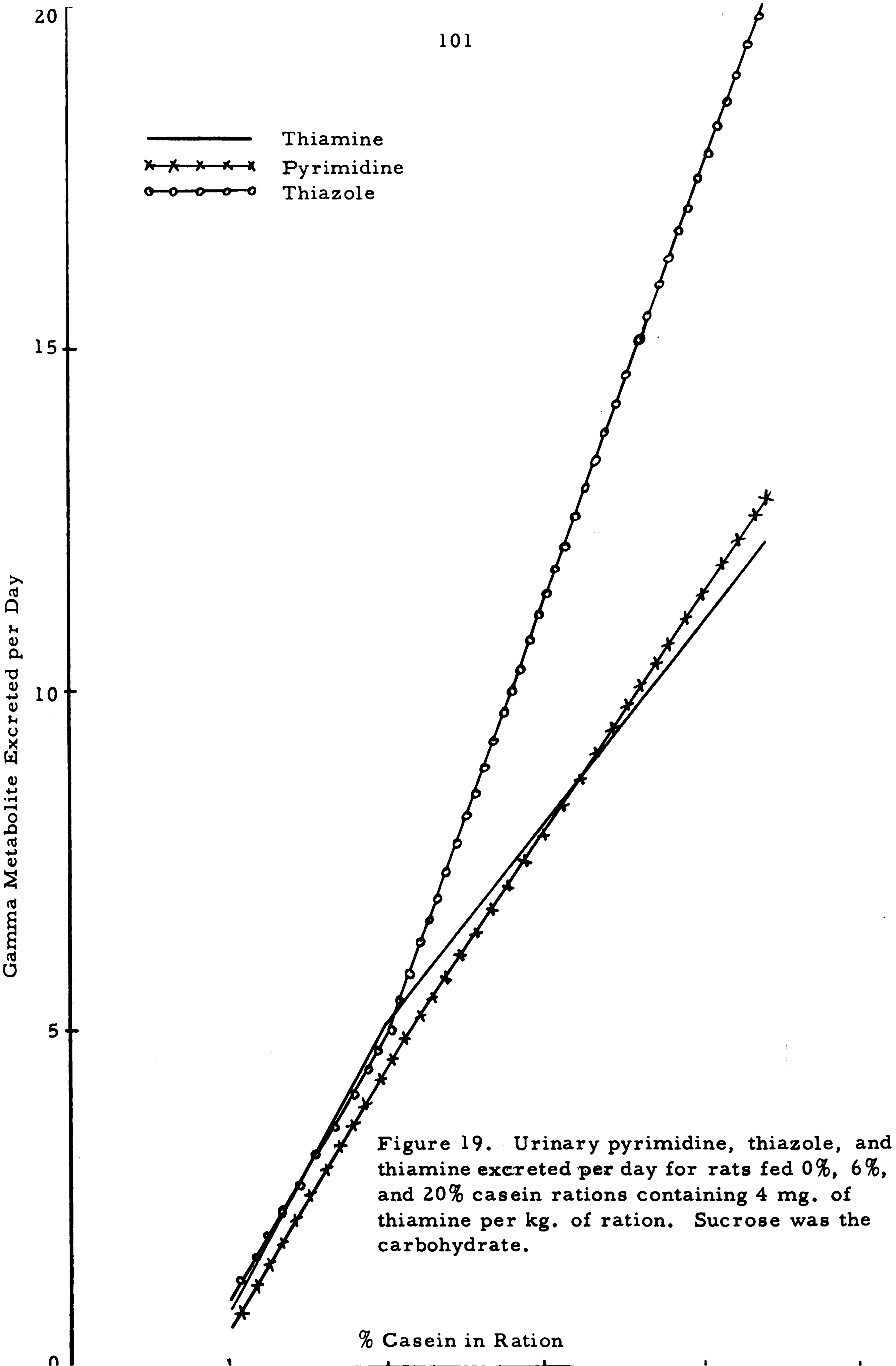


Figure 19. Urinary pyrimidine, thiazole, and thiamine excreted per day for rats fed 0%, 6%, and 20% casein rations containing 4 mg. of thiamine per kg. of ration. Sucrose was the carbohydrate.

ration (4 mg./kg.). The pyrimidine component followed the intact thiamine molecule in this relationship whereas the slope for the thiazole component was much steeper. This is just the reverse of the thiamine concentration level found in the livers of the experimental animals (Figure 15).

These observations suggest that the urinary excretion of thiamine and its degradation products may be related to the protein level in the ration. If a similar situation holds true for human beings, it may mean that the evaluation of urinary excretion data should be considered from the standpoint of dietary protein levels.

The data in Figure 19 suggested that the urinary excretion of thiamine when the dietary levels were the same varied by a factor of 10 or more as the protein level in the diet was increased from 0 to 20%. A similar situation existed insofar as the pyrimidine component was concerned. For the thiazole component, the factor for the increase in excretion between the 0 protein level in the ration and the 20% casein protein level was 20.

Figures 20 and 21 show that there is a kidney threshold for thiamine above which there is an increased thiamine excretion in the urine of the rat. When dietary thiamine intake was plotted against urinary thiamine excreted per ml. (Figure 21) and when blood thiamine concentration was plotted against urinary thiamine excreted per ml. (Figure 20) the curves suggested that very little thiamine was excreted until the level in the blood reached 0.09 gamma of thiamine/ml. which suggested a probable kidney threshold of about 0.075 gamma of thiamine/ml. The dietary intake at this threshold is approximately 4 gamma/gm., the level of thiamine suggested as the thiamine requirement for the albino rat.

A level below 4 gamma of thiamine/gm. should be studied before a definite value can be stated for the probable kidney threshold for

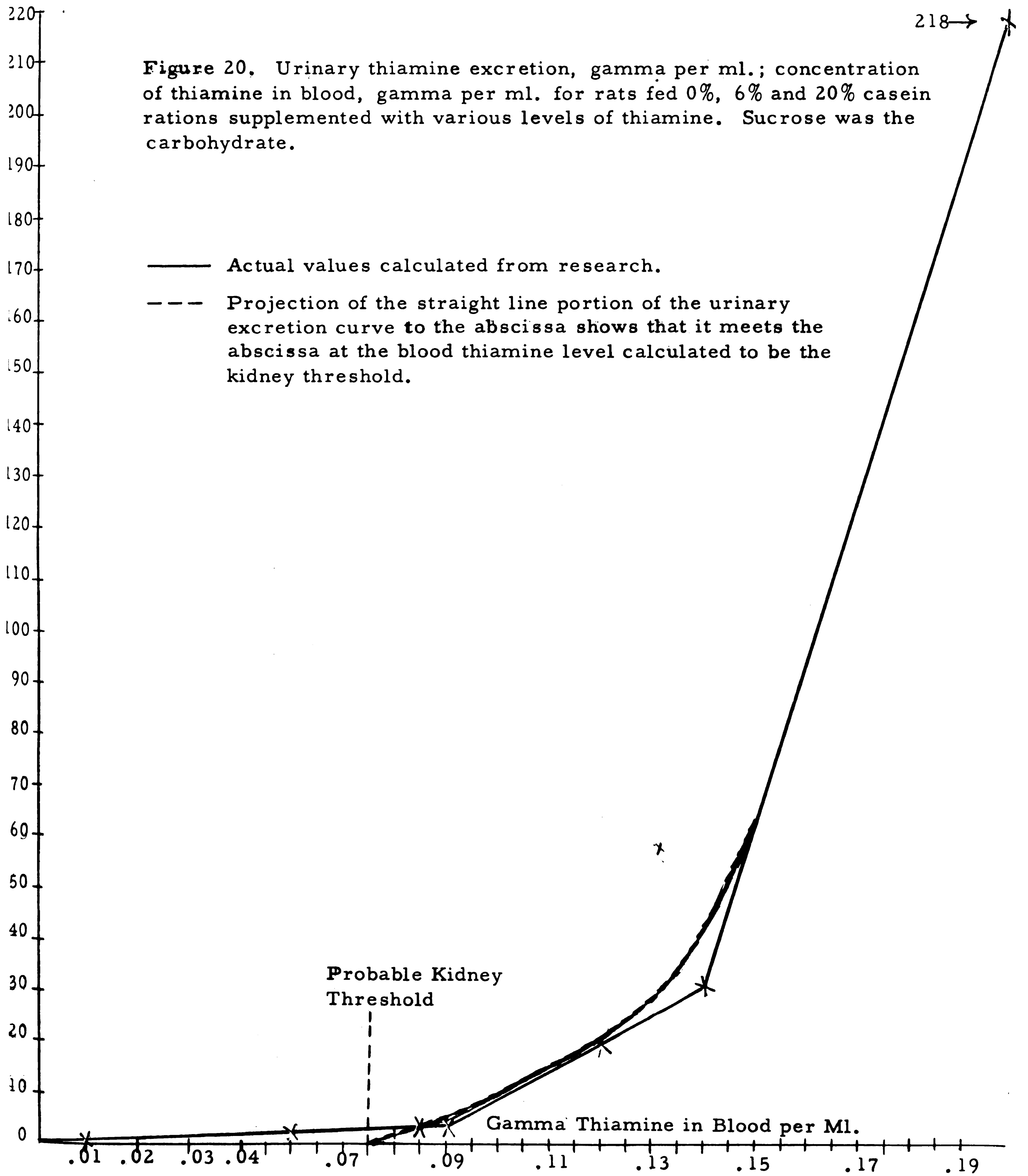
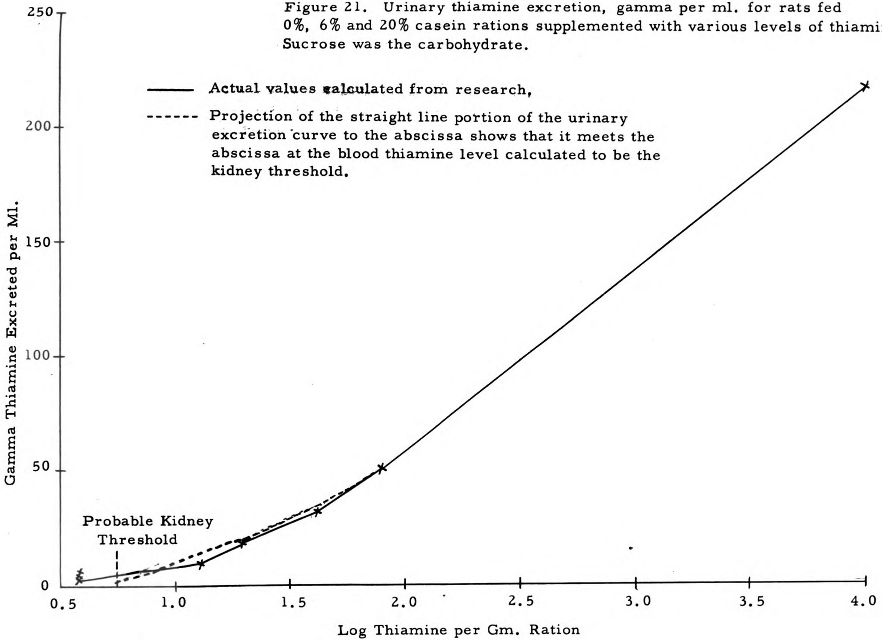


Figure 20. Urinary thiamine excretion, gamma per ml.; concentration of thiamine in blood, gamma per ml. for rats fed 0%, 6% and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.

Figure 21. Urinary thiamine excretion, gamma per ml. for rats fed
0%, 6% and 20% casein rations supplemented with various levels of thi[illegible]

Figure 21. Urinary thiamine excretion, gamma per ml. for rats fed 0%, 6% and 20% casein rations supplemented with various levels of thiamine. Sucrose was the carbohydrate.



thiamine. In addition, all thiamine levels fed in the 20% casein rations should be fed in the 6% casein rations. The relation of the dietary protein to the blood level of thiamine was not a phase of this research, but is an important factor which should be studied if dietary protein bears any relationship to the kidney threshold for thiamine.

Figure 22 shows that above a dose of 0.5 mg. of thiamine in man there is an increase of thiamine excreted in the urine. The ratio of urinary thiamine excretion to intake is five times greater for the rat compared to man.[1] It must be remembered that the rats were growing rats and most human studies on urinary thiamine excretion are on adult men, some on children.

These research data do not support that of Light et al. ('38) who showed a straight line relationship between intake and excretion of thiamine over a range of 15 to 500 gamma total thiamine intake. These data showed a straight line relationship after an intake level of 12 gamma/gm. Figures 18 and 21 indicate that between a dietary thiamine intake of 4-12 gamma/gm. there is no relative increase in the thiamine excreted in the urine.

These data also do not support the claim made by Hecht and Weese ('37) that thiamine was a diuretic. In fasting rabbits they showed a diuresis following the intake of 100 gm. of water by mouth after a subcutaneous injection of 0.1 gm./kg. of thiamine. They indicated a doubling effect in urine volume after the injection of thiamine. Hecht and Weese do not indicate whether any precautions were made to make sure the rabbit's bladder was empty before and after the experimental period. Rabbits are notorious in being able to retain urine in their bladders. Unless the animal were catherized, it would be impossible to

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[1] $$\frac{\text{Urinary B-1 Exc./24 hr.}}{\text{Food Intake/Day X gamma B-1/gm.}} = \text{Ratio urinary thiamine excretion to intake}$$

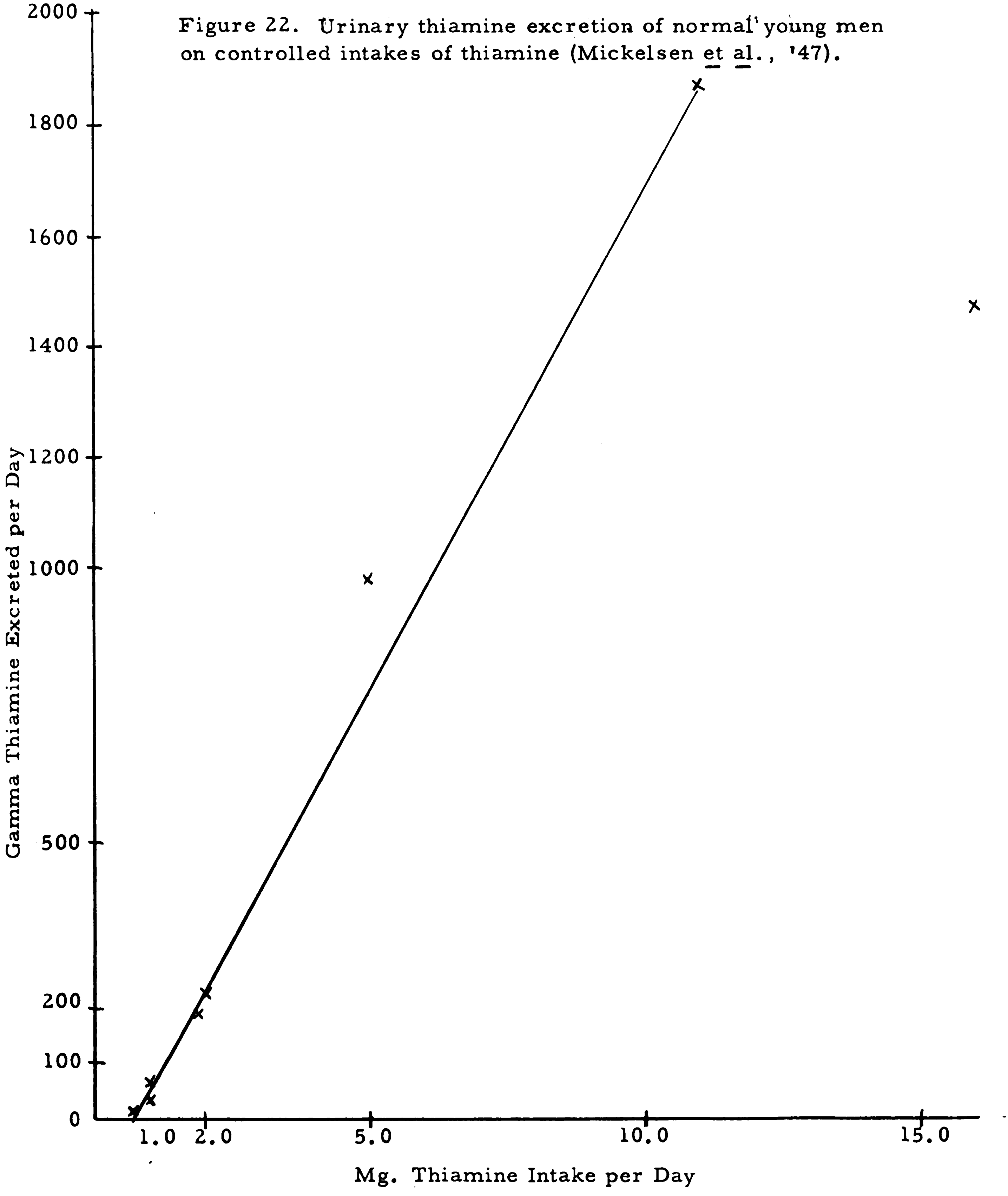

Figure 22. Urinary thiamine excretion of normal young men on controlled intakes of thiamine (Mickelsen *et al.*, '47).

determine exactly the urine volume within any period of time. Furthermore, it was not apparent whether Hecht and Weese used only one rabbit in their study.

As stated previously these data suggested a probable kidney threshold for thiamine at about 0.075 gamma of thiamine/ml. of blood. Wolf ('50) who reported work on dogs and rabbits as well as man classified thiamine as a "no-threshold" substance. According to Wolf this means that for thiamine there is neither a threshold of retention (the point at which concentrations of a given substance in the urine and in the plasma are identical) nor a threshold of appearance (that plasma concentration of a substance above which the substance appears frankly in the urine, and below which it does not appear in the urine in appreciable quantities).

Bicknell and Prescott ('53) claimed that the kidney concentrates thiamine from the plasma, perhaps twenty times or more although there was no direct relationship between urinary and blood levels. They did not state the species from which their data was derived. They went on to state that diuresis could affect thiamine excretion profoundly which suggested to these workers that thiamine was a non-threshold substance and renal clearance studies indicated that extensive tubular absorption of thiamine did not occur. The data from this study do not support these statements.

Mickelsen et al. ('47) showed that in man with increasing thiamine intakes the urinary excretion of thiamine increases but tends to reach a plateau at about 12% of intake. When the pyramin content of the urine was added to this, the total percent of the intake accounted for in the urine decreased as the intake increased. Even at the lower dietary intakes studied, a maximum of 42% of the intake was accounted for as the sum of thiamine and pyramin, whereas at levels of intake of 1 mg. only 35% was accounted for. There was no indication as to the fate of the remaining thiamine.

Data presented in Figures 18 and 19 indicated that the excretion of pyrimidine and thiazole in the urine generally followed that for thiamine. Figure 19 shows the same relationship between urinary excretion per day to dietary intake per day for the three metabolites. Figure 20 shows that the protein level had a pronounced effect on urinary thiamine excretion--almost twofold between 6 and 12% casein rations.

Based on the studies carried out in this research it would seem that the administration (intake) of high levels of thiamine above demonstratable physiological needs accrues no advantages to the rat. Very large doses did not effect growth over a four week period, did not stimulate the appetite especially when there was a dietary deficiency of a nutrient such as protein, did not effect the composition of tissues, had no influence on the percentage of various protein fractions in blood serum; the urinary and fecal thiamine levels increased with increased levels of dietary thiamine, and the metabolites of thiamine increased in the urine with increasing dietary levels of casein as well as increased dietary levels of thiamine.

In addition, caution needs to be used in consuming large amounts of thiamine because some people develop an allergic condition from such indiscriminate use. Economically, too, large intakes of thiamine do no good as evidenced from this research; they are expensive to buy; but most important of all the supply needed can be secured through the consumption of a good adequate diet of natural foods.

SUMMARY AND CONCLUSIONS

Approximately 600 male albino weanling rats, eight to thirty per group, have been fed rations in which choline, thiamine, carbohydrate and casein have been used as the principle variables. Animals from each group were sacrificed at different time periods during the several experiments. The following measurements were made on the rats:

Average gains in weight (in grams/week)

Average food intake in grams per week

Wet weight of liver, heart, kidney, thigh muscle
(Each of these tissues was analyzed for moisture, protein, fat, and ash and glycogen was calculated as the difference between the sum of the other percentages and 100.)

Average thiamine concentrations for eight tissues (gamma/gm.)

Average pyrimidine, thiazole, thiamine concentrations in urine (gamma/ml.)

Protein patterns in blood serum

The addition of very large amounts of thiamine to various protein rations had no deleterious nor beneficial effect on the growth of rats.

The large doses of thiamine had no appetite-stimulating activity.

High levels of thiamine did not contribute to the deposition of fat in the liver, nor did they alter the concentration of nitrogen and moisture in the liver. Very high levels of thiamine had no effect on the composition of tissues as measured by the tests used in this research.

The level of thiamine in the diet had no influence on the percentage of various protein fractions in blood serum.

Increased thiamine in the diet increased the thiamine level in all tissues examined except the adrenals. Greatest concentration of thiamine was found in the heart, kidneys, liver and testes.

With increased levels of dietary thiamine both urine and fecal thiamine excretion increased, but that for feces increased faster than that for urine. The excretion of pyrimidine, thiazole, and thiamine in the urine increased as the dietary level of thiamine increased.

There was a decrease in the growth of the rat the first two weeks following weaning when choline was omitted from the diet. The addition of high thiamine without choline did not improve the growth during these same two critical weeks.

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APPENDICES

APPENDIX 1

SUGGESTIONS FOR ADDITIONAL STUDIES BASED ON THE RESULTS OF THIS RESEARCH

I. Relationship between dietary protein levels and thiamine metabolism.

A. This research indicated a possible kidney threshold for thiamine. The following additional data would verify this point:
1. Study thiamine levels below 4 gamma of thiamine/gm. of ration.
2. Study thiamine levels in 0% and high protein diets; if significant effects noted explore other protein levels.
3. Concentration of thiamine in the blood should be studied as related to the protein level in the diet.
4. The same questions should be studied for the degradation products of thiamine as well as for thiamine.

B. Repeat the experiments with the two extremes of either protein or thiamine in the ration using various carbohydrates to determine the superiority of carbohydrates in terms of growth data, food intake data, deposition of fat in the tissues, etc. If significant effects noted explore other protein or thiamine levels. These studies should be at least six weeks in length.

C. Analysis of the chemical composition of the tissues from the animals above should be studied and the moisture-to-protein ratio. The present research indicated significant differences between sucrose and dextrin for these two parameters.

D. The thiamine content of the tissues should also be studied as related to the protein content of the diet.

E. Serum protein fractions should be determined for the above dietary levels of casein with supplementary thiamine.

F. Proteins other than casein, should be studied.

II. Additional checks on the safety of high thiamine intake.

A. Research is needed to determine whether the activity of the enzyme systems, of which thiamine is a cofactor, is altered in any way through the intake of excess thiamine or more specifically by the protein level in the ration when the thiamine in the diet is kept constant.

III. Miscellaneous

A. More research is needed to determine all the degradation products of thiamine--through the use possibly of isotopically labelled thiamine.

B. An exploration of the taste of the high thiamine supplemented diets would be interesting with continued study of the pH and thiamine content of these diets during various time periods.

APPENDIX 2

RAMIFICATIONS OF THIS RESEARCH FOR MAN

Everyone recognizes that animal research gives only clues. If danger signs are shown (for instance, to large intakes of "B" vitamins) then the use of those substances with human beings should be examined very carefully. If the use of the substance by animals appeared safe, it would not follow that the use of the substance would be safe for humans.

Clinical studies of thiamine have suggested that certain individuals may be allergic to large intakes of thiamine. The sites of toxic action seemed to be both central and peripheral producing immediate whealing at the site of injection. Other difficulties, such as headaches, hyperirritability, insomnia, rapid pulse, muscle tremor, weakness, and trembling, have been found both from injections of various types and from oral intake of thiamine.

This research has shown that the concentration of thiamine in the tissues of albino rats depended upon the protein level of the ration, but in the case of urinary thiamine excretion this relationship was reversed. If a similar situation holds true for man, this might mean that the evaluation of urinary excretion data should be considered from the standpoint of dietary protein levels when clinical analyses are conducted at the same time as dietary surveys.

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