INTERACTIONS BETWEEN THIAMINE, CORTISONE, ALLOXAN AND INSULIN ON CARBOHYDRATE AND PROTEIN METABOLISM IN RATS

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This is to certify that the

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Joseph Meites
Major professor

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By

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A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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DEDICATION

To Odette F. Wilwerth

INTERACTIONS BETWEEN THIAMINE, CORTISONE, ALLOXAN AND INSULIN ON CARBOHYDRATE AND PROTEIN METABOLISM IN RATS

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AN ABSTRACT

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ABSTRACT

1. When young rats were maintained on a thiamine-free diet, symptoms of thiamine deficiency developed within 15 to 20 days. Supplementation of the diet with 2 mg. of thiamine per kilo of diet increased appetite and body weight gains, slightly increased blood sugar and greatly increased glucose tolerance.

2. When 4 mg. of cortisone acetate daily were injected into thiamine-deficient rats, there was a slight increase in the excretion of urinary nitrogen, a slight or no increase in blood glucose, decreased glucose tolerance, reduction in body weight gains and reduced appetite. When 2 mg. of thiamine per kilo of diet (or higher levels of thiamine) were fed to cortisone-treated rats and they were allowed to eat ad libitum, urinary nitrogen increased greatly, blood glucose increased moderately, glucose tolerance was partially improved and body weight was maintained at the same initial level or was slightly increased.

3. Thiamine at high levels, fed to rats on a limited-food intake, largely prevented the development of thiamine-deficiency symptoms but was unable to increase the blood glucose of cortisone-treated rats. It slightly increased urinary nitrogen excretion. It is concluded that large doses of thiamine, greater than normal requirements for growing rats, can partially counteract the protein catabolic action of cortisone by increasing food consumption and increasing the availability and utilization of carbohydrate by the organism.

4. Cortisone partially interferred with the favorable action of large doses of thiamine on the efficiency of food utilization for body growth. Hyperglycemia, glucosuria, increased nitrogen excretion and increased insulin resistance were noted, and therefore less carbohydrate was available to exert a "sparing action" on protein for transformation into body weight gains.

5. (a) When young rats were fed a thiamine-free diet, the weight of the kidneys, heart and adrenals were increased and the weight of the thymus and seminal vesicles were greatly decreased. When 1 mg. daily of cortisone acetate was injected into thiamine-deficient rats, a still greater increase in the weight of the kidneys and heart was noted, and a slight increase was found in the weight of the testes and adrenals. The low thymus weight was not decreased further by cortisone treatment, while the seminal vesicles weighed twice as much as those of thiamine-deficient rats.

(b) When thiamine was fed to cortisone-injected rats, the kidneys, heart and testes showed a slight increase in weight. The thymus showed less involution, the adrenals were reduced in weight and the seminal vesicles were slightly but not significantly increased in size. The increases in thymus and seminal vesicles weights apparently were not due to thiamine per se but to the concomittant increase in food intake.

6. Alloxan-diabetes did not further reduce the efficiency of food utilization of thiamine-deficient rats or rats on a limited-food intake, but slightly reduced the efficiency of food utilization of thiamine-adequate rats. In the latter there was a consistent increase in blood

glucose and urinary nitrogen, while in the thiamine-deficient rats there was neither an increase in blood glucose nor of urinary nitrogen. Rats on a limited food intake but fed thiamine showed a consistent increase in both blood glucose and urinary nitrogen which decreased progressively as chronic inanition developed. When the treatment of the thiamine-deficient rats was reversed, by administering large amounts of thiamine, there was a pronounced hyperglycemia and an increase in urinary nitrogen excretion. It is concluded that thiamine, by increasing food intake, permits hyperglycemia to develop in alloxan-diabetes.

7. When guinea pigs were maintained on a thiamine-free diet, symptoms of thiamine-deficiency developed within 25 days. Supplementation of their diet with 16 mg. of thiamine per kilo of diet increased appetite and body weight gains. Injections of 5 or 10 mg. of cortisone acetate daily did not appear to reduce body weight significantly in thiamine-deficient guinea pigs in contrast to rats. When cortisone was injected into thiamine-deficient guinea pigs there was no increase in urinary nitrogen or blood glucose, as in rats. When 16 mg. of thiamine or more per kilo of diet were fed to cortisone-treated guinea pigs, only a slight increase in blood glucose was observed with 5 mg. of cortisone injected daily and a consistent increase with 10 mg. of cortisone daily. Cortisone did not increase blood glucose of thiamine-deficient guinea pigs at any level.

8. Insulin was much more effective in reducing blood glucose in normal and alloxanized rats than in cortisone-treated rats maintained

on either a thiamine-deficient or adequate diet. A thiamine-deficient diet reduced the hypoglycemic action of insulin, indicating that thiamine is essential for the maximum action of insulin. The greatest resistance to insulin was found in cortisone-treated rats, confirming the observation that cortisone increases insulin resistance.

9. It is suggested that the over-all effect of large doses of cortisone in young rats, by virtue of its ability to interfere with carbohydrate utilization but at the same time increase the secretion of insulin, is to increase the need for thiamine. The beneficial action of a large intake of thiamine in cortisone-treated rats is believed to be brought about by its ability to increase carbohydrate intake and utilization in the presence of hyperinsulinism.

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INTRODUCTION

Large doses of cortisone has been shown to stimulate pancreatic islet function in the rat (Baker et al. 1952) and guinea pigs (Hausberger et al. 1953), but at the same time to interfere with the action of insulin on carbohydrate utilization (Ingle et al. 1945). Thiamine has been demonstrated to be necessary for the full effects of insulin on carbohydrate metabolism (Samuels, 1948). Long (1954) suggested that the influence of insulin on carbohydrate metabolism is accomplished through three main metabolic pathways: (a) an increase in the amount of glucose or glycogen which is oxidized to CO_2 and water (b) polymerization of glucose into glycogen, both in the liver and muscle and (c) conversion of glucose to fatty acids, both in the liver and adipose tissues. This suggests that thiamine, cortisone and insulin may be interdependent insofar as their actions on carbohydrate and protein metabolism are concerned.

In previous reports from this laboratory, Meites (1951, 1952, 1953) observed that large doses of vitamin B_{12} partially counteracted the inhibitory effects of large doses of cortisone on body, hair and thymus growth in young rats. He demonstrated that large doses of vitamin B_{12} exerted a beneficial effect by increasing food intake and permitting a greater utilization of carbohydrate. Feng (1954) reported that injections of 2 to 4 mg. daily of cortisone increased the urinary excretion of radioactive vitamin B_{12}, in rats fed a vitamin B_{12}-deficient diet.

She also reported that on a diet meeting just the normal requirements for vitamin B_{12} (20 mcg. per kilo of diet), cortisone did not increase urinary vitamin B_{12} excretion unless at least 4 mg. of cortisone were injected daily. It was concluded that the amount of vitamin B_{12} excreted in the urine bore a direct relationship to the dose of cortisone given and to the amount of vitamin B_{12} fed in the diet. The increased excretion of vitamin B_{12} was explained on the basis of the ability of cortisone to interfere with insulin action and carbohydrate utilization, and hence to reduce the body need for vitamins concerned with carbohydrate metabolism. The beneficial effects of large doses of vitamin B_{12} was explained on the basis of its ability to enhance insulin action and thus counteract the inhibitory effects of cortisone on carbohydrate utilization.

It was the purpose of this thesis to determine the nature of and the possible relationships between the actions of thiamine, cortisone and insulin on protein and carbohydrate metabolism and on body growth. Specifically, this thesis will deal with the following questions:

1. What are the effects of large doses of cortisone on thiamine-deficient, thiamine-adequate and thiamine-abundant rats and guinea pigs? Do large doses of cortisone increase or decrease the requirements for thiamine?
2. Through what means does a large intake of thiamine partially counteract some of the catabolic actions of large doses of cortisone?

3. Why do not cortisone-treated rats and guinea pigs fed a thiamine-deficient diet develop hyperglycemia? Is this due to reduced food intake or to lack of thiamine?
4. To what extent does alloxan-diabetes or insulin alter the body need for thiamine?
5. To what extent is thiamine essential for glucose utilization in normal, alloxanized and cortisone-treated rats?

LITERATURE REVIEW

Introduction

Since this thesis deals with the interactions between thiamine, cortisone and insulin as related to carbohydrate and protein metabolism it is pertinent to briefly review some of the salient actions of the former on the latter. The writer felt it necessary to choose from a vast literature, and for the most part the articles reviewed here were selected because of their direct bearing on the thesis problem.

THIAMINE

Requirements for thiamine under different conditions.

The requirement for thiamine by the rat is a function of several factors. The composition of the diet is very important. It was shown by Wainio (1942) that rats receiving a diet containing 62 percent sucrose and 20 percent casein required 33 mcg. of thiamine per day, whereas only 20 mcg. per day was required in a diet containing 64 percent casein and 19.6 percent sucrose. Dann (1945) was able to maintain rats for more than a year on a thiamine-free synthetic diet containing 80 percent purified casein. Increasing the carbohydrate intake resulted in diminution of urinary output of thiamine (Reinhold et al. 1944), indicating a greater use for this vitamin in metabolism. When fat is the sole source of non-protein energy much less vitamin B is necessary

for growth than when sucrose is the only source of such energy (Evans et al. 1929). However, when thiamine is fed at high levels, a sucrose diet can hardly be considered disadvantageous.

When rats ingest dextrinized cornstarch as the sole carbohydrate, bacterial synthesis of thiamine and riboflavin is capable of lowering the dietary requirement for these vitamins (Guerrant et al. 1934). This contrasts with the poor synthesis induced by feeding commercial cornstarch, sucrose, glucose or lactose.

Temperature is another factor which influences the requirement for thiamine. Mills et al. (1946) showed that 80 mcg. of thiamine per 100 gm. of food was mildly inadequate in the cold and markedly so in the heat for weanling rats, and that rats 12 months old required 120 mcg. in the cold and 200 mcg. per 100 gm. of food in the heat.

The requirements for thiamine ranges from 80 to 200 mcg. per 100 gm. of food (Brown et al. 1949) according to different conditions to which the rat is submitted. An animal receiving a thiamine-deficient diet shows a loss of weight and food intake is reduced to a low level. After adding a small fraction of a milligram of thiamine to the diet however, appetite is restored and weight increases considerably (Jansen, 1949). This led to the supposition that thiamine was involved in cell metabolism. The work of Kinnersley and Peters (1929) on polyneuritic-pigeons showed that an increased amount of lactic acid in the brain was the only abnormality found. Kinnersley and Peters (1929) also showed that thiamine plays an important role in carbohydrate metabolism,

especially in pyruvic acid metabolism. They demonstrated that the oxygen uptake by the brain of polyneuritic-pigeons was less than that of the brain of normal pigeons. The addition of a solution of thiamine to minced brain of polyneuritic-pigeons suspended in pyruvic acid solution increased the oxygen uptake of this mixture. This effect was shown to be due not to thiamine itself but to a compound synthesized from the free vitamin which decarboxylates pyruvic acid to acetaldehyde.

Effects of thiamine on carbohydrate, fat and protein metabolism.

It has been long known that thiamine exerts a preponderant role in carbohydrate metabolism. Neuberg (1911) demonstrated that yeast can cause the decarboxylation of pyruvic acid to acetaldehyde and carbon-dioxide. He termed this enzyme carboxylase. Simola (1932) showed that tissues of rats maintained on a thiamine-deficient diet had a greatly reduced content of carboxylase. It was also shown that washed yeast cells lost the capacity to perform this reaction, and it was established that a diffusible cofactor was required for decarboxylation. Since the enzyme which catalyzes this reaction was termed carboxylase, the cofactor was named cocarboxylase.

Lohmann et al. (1937) succeeded in isolating this cocarboxylase, and showed it to be thiamine pyrophosphate. The phosphorylation of thiamine to give cocarboxylase may be effected either chemically or enzymatically with ATP as the phosphorylating agent. Thiamine pyro-phosphate (TPP) functions not only as the prosthetic group of the yeast carboxylase but in other reactions representing the principal pathways

of pyruvic acid metabolism (Stotz 1945). Thus, TPP is essential for the simple decarboxylation of pyruvic acid to acetaldehyde and CO_2, and also for the oxidative decarboxylation of pyruvate to form acetyl-CoA. TPP likewise appears to be essential for the oxidative decarboxylation of alpha-keto glutarate to succinic acid, through succinyl-CoA. Green et al. (1941) assumed that the enzymatic decarboxylation of keto-acids other than pyruvic acid also appears to involve cocarboxylase as a cofactor.

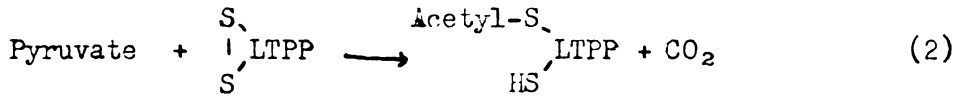
Lipoic acid is also involved in this system. Thiamine pyrophosphate is conjugated to lipoic acid (amide linkage) giving rise to lipothiamide pyrophosphate (LTPP). This seems to be the active form of cocarboxylase. Since only catalytic amounts of LTPP are required to generate acetyl-CoA from pyruvate, the reduced LTPP produced in this transportation must be converted to oxidized LTPP for the reaction to continue over and over again. Oxidation of reduced LTPP is accomplished by DPN. It was shown that TPN does not replace DPN in this reaction (Reed, 1953).

The generation of active acetate (acetyl-CoA.) from pyruvate by purified pyruvate oxidase preparations from bacterial and animal sources has been postulated by Reed et al. (1953), as shown in the following reactions:

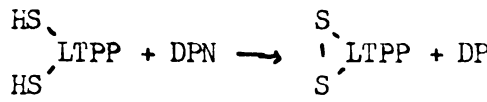
$$\text{Pyruvate} + \text{DPN} + \text{CoA.} \longrightarrow \text{Acetyl-CoA.} + \text{DPNH} + CO_2 + H^+ \quad (1)$$

Reed et al. (1953) studied soluble pyruvate apooxidase preparations from E. coli mutant which cannot synthesize LTPP and revealed that reaction (1) did not proceed in the absence of S-LTPP. This reaction

comprises two steps:



(2)



(3)

Lactate reacts in a similar manner to produce acetyl-CoA. but it requires lactic dehydrogenase and pyruvate apooxidase for the reaction to be performed.

In thiamine deficiency the amount of free thiamine and of TPP in the tissues fall markedly and there is an increase in the level of pyruvate and lactate in the blood and urine. Accumulation of lactic acid appears to be due to the fact that the action of lactic dehydrogenase is inhibited by high concentration of pyruvate (Sherman et al. 1936). The role of coenzyme A and DPN in the oxidative decarboxylation of pyruvate and in the transfer of the acetyl group to the Krebs cycle has been studied by several workers (Korkes et al. 1951; Littlefield et al. 1952; Sanadi et al. 1953). They all showed that thiamine pyrophosphate is involved in the reaction.

Sure and Smith (1929) showed that hypoglycemia in cases of avitaminosis-B was progressive and not just a premortal state. Since

the hypoglycemia was quite evident during a period of prolonged vitamin B depletion without appreciable changes in body weight, they concluded that B vitamins had a determining influence in carbohydrate metabolism. Long before, Magne and Simonnet (1922) had stated that an injection of glucose failed to raise the R.Q. of rats with beriberi but succeeded in cases of inanition. Styron et al. (1942) showed that in diabetic rats fed a thiamine-deficient diet, glucosuria decreased toward the end of the deprivation period. They also showed that tolerance for glucose was impaired by the end of the depletion period and that the administration of 4 to 8 times the maintainance dosage of thiamine appeared to improve consistently the tolerance for glucose. Lowry et al. (1945) found, after a depletion period of 16 days, a drop in urinary glucose of alloxan-diabetic rats. After the injection of 50, 100 and 200 mcg. of thiamine, all rats showed an alleviation of the deficiency symptoms and the excretion of glucose in urine increased consistently. On the other hand, it was claimed that in diabetes induced by alloxan in rats, thiamine requirements were not increased (Styron et al. 1942; Lowry et al. 1945).

Thiamine appears not to interfere in fat metabolism. Boxer et al. (1944) could not find any difference in the deposition of newly formed fat in rats that were fed on a complete diet compared to rats on a thiamine free diet. They concluded that thiamine was necessary but not the sole condition for the formation of fats from carbohydrate. It was shown that fats exert a "sparing" action on thiamine. Evans et al. (1929) showed that when a diet containing 50 percent lard but no

anti-neuritic vitamin was fed, symptoms of deficiency were not observed. This finding was later confirmed by other workers (Lecoq, 1932; Banerji, 1940; MacDonald et al. 1940).

It seems that thiamine is not involved in protein metabolism. It was shown that diets containing a high percentage of protein decreased the requirements for thiamine (Wainio, 1942) and the survival of rats maintained on a thiamine-free diet containing 80 percent casein was very long (Dann, 1945).

Effects of thiamine on organ weights.

It is well known that thiamine-deficiency induces anatomical and histological changes in certain organs. Stoerck and Zucker (1944) reported that thiamine-deficient rats showed a decrease in thymus weight greater than in riboflavin or pantothenic acid deficient fed rats, but less than in pyridoxine-deficient rats. Even in partial thiamine deficiency the pattern was the same; otherwise the weight losses were not so accentuated.

Severe deficiency of thiamine induces weight changes in the adrenals and thymus gland (Deane et al. 1947). These changes were due to a stimulation of the adrenal cortex by ACTH resulting in increased adrenal weight and production of corticosteroid hormones which induced involution of the thymus. Deane et al. (1947) concluded that the stimulation of the adrenals occurred earlier in thiamine deficiency than during a comparable degree of inanition, and therefore thiamine deficiency was a stronger stressor than starvation alone. Goodsell (1941) found

increases in the weight and in the steroid concentration of the adrenals of dogs in acute vitamin B_1 deficiency. Similar findings were reported by Skelton (1950) in rats, who found in addition to adrenal hypertrophy and thymus involution, a renal hypertrophy which was considered to be due to the prolonged negative nitrogen balance, and failure of development of the sex organs due to unbalanced production of pituitary gonadotrophic hormones.

During starvation or chronic undernutrition, the vital organs usually lose weight together with loss of body weight. In pair-fed rats it was observed that starvation produced similar organ/body weight ratios to ad libitum fed controls, while thiamine-deficient rats showed enlargement of the adrenals and kidneys and little change in heart size (Pecora, 1952). It was concluded that the kidneys and the adrenals were enlarged because of lack of thiamine and not inanition, and that in thiamine-deficient rats starvation per se exerted little or no effect on organ size. Later, Pecora et al. (1953) reported data on the organ/body weight ratios of the heart, kidneys, testes and pituitary in thiamine-deficient, pair-fed and positive-control rats. They showed that the values for both the thiamine-deficient and the pair-fed groups were the same, except for the kidneys, indicating that changes in organ sizes were not due to the effect of thiamine deficiency but to food restriction. The values for the kidney/body weight ratio were considerably greater in the thiamine-deficient group than in the pair-fed rats, due to the effect of thiamine deficiency superimposed on

food restriction. The absolute organ weights of thiamine-deficient and pair-fed rats showed equal weight losses, except for the kidneys of the thiamine-deficient group which showed little weight loss. Dunn et al. (1947) observed higher organ/body weight ratios for the heart and brain of thiamine-deficient mice, although actual enlargement of the organs was not found.

ADRENAL CORTICAL HORMONES

Effects of ACTH and cortisone on carbohydrate and protein metabolism, and body growth.

a) Carbohydrate metabolism

The possible interaction between adrenal cortical hormones and carbohydrate metabolism was first demonstrated by Britton (1932) and Britton et al. (1937). Long, Katzin and Fry (1940) and Ingle (1940) demonstrated that large doses of ACTH or cortisone produced transitory hyperglycemia and glucosuria and a negative nitrogen balance. This suggested that blood glucose might come at least in part from gluconeogenesis from protein. Ingle (1941) and Ingle et al. (1945, 1946, 1951) reported that ACTH or cortisone were able to induce hyperglycemia and glucosuria in force-fed rats.

Long et al. (1940) showed that cortisone or other 11-oxysteroids could intensify the glucosuria of partially depancreatized rats. This finding was confirmed later by Ingle (1940) and Ingle et al. (1941). Glucosuria induced in normal force-fed rats by the administration of large doses of cortisone acetate daily was not sustained throughout the treatment with the steroid (Lazarow et al. 1950). Diabetic-rats (alloxan-treated or partially depancreatized) were more sensitive to the diabetogenic effects of ACTH or cortisone (Long et al. 1940; Kendall, 1942).

ACTH or cortisone can cause alteration of the beta cells in the islets of Langerhans. Kobernick et al. (1950) observed the development of a diabetic state associated with hydropic degeneration in the islet cells in the pancreas of rabbits given 20 mg. of cortisone daily. This observation was confirmed later by Franckson et al. (1953) who showed a similar degeneration in the rat after prolonged treatment with cortisone. Baker et al. (1952) reported that in the rat ACTH induced degranulation, hypertrophy and increase in number of the beta cells in the islet of Langerhans.

Steroid diabetes has been reported to increase resistance to insulin (Ingle, 1945; Sprague, 1951; Franckson, 1953). Swingle et al. (1953) reported the induction of diabetes insipidus in adrenalectomized dogs, with accentuated polyuria and polydipsia, when cortisone was given in high doses. This finding confirmed the work of Sirek et al. (1952) who reported that a syndrome closely resembling diabetes insipidus could be produced in dogs by daily injections of cortisone in doses of 50 to 300 mg. daily.

b) Protein metabolism and body growth

It has been demonstrated that large doses of ACTH and cortisone induced very striking effects on the nitrogenous constituents of the body. Cortisone has been shown to be a potent growth inhibitor in normal young rats (Ingle et al. 1940, 1941; Wells, 1940; Kuizenga et al. 1943; Winter et al. 1950). A single injection of 0.25 mg. of

cortisone given to baby rats at 24 hours of age resulted in a failure to gain weight normally for several days (Parmes et al. 1951). They also found that five daily injections of 0.1 mg. of cortisone beginning at 24 hours of age resulted in marked inhibition of growth of the newborn rat and failure to regain body weight three months after the cessation of injections. Kuizenga et al. (1943) demonstrated that when immature adrenalectomized rats were treated with cortisone in doses of 0.25 to 1.0 mg. daily, they were able to survive and grow, but at a subnormal rate.

Food intake has been shown to be reduced in cortisone treated rats (Winter et al. 1950; Meites 1950, 1951, 1952), and this can account at least in part for the retardation of growth. Injection of ACTH or cortisone in relatively high doses inhibits growth due to failure in the synthesis of protein and protein catabolism. The growth inhibiting potency of ACTH and cortisone parallels the magnitude of the negative nitrogen balance (Ingle 1946). It was demonstrated by Ingle (1941) and Ingle et al. (1945, 1946) that the temporary diabetes in rats due to the administration of ACTH or cortisone was accompanied by loss in body weight and a pronounced increase in urinary nitrogen. It was also observed that the organism had some ability to adapt itself to the catabolic effects of these hormones. The peak of nitrogen excretion was not sustained during the administration of ACTH or cortisone. It had already been demonstrated (Long et al. 1940; Ingle, 1940) that large doses of either ACTH or cortisone produced negative nitrogen

balance in laboratory animals. Bennet et al. (1948) showed that ACTH induced nitrogen losses in rats with alloxan-induced diabetes and Bennet (1948) also showed that ACTH increased both urinary glucose and nitrogen excretion of hypophysectomized-diabetic rats. Engel et al. (1949) reported an increase in urea formation beginning three hours after the subcutaneous administration of adrenal cortical extracts (ACE) to fasted nephrectomized rats. This increase was prevented by intraperitoneal injections of glucose three hours after ACE. He stated that the action of ACE in nitrogen metabolism was on whole protein rather than on amino acids. He suggested that the amount of glucose or of glycogen precursors available might be determining factors in whether protein catabolism would be stimulated by ACE.

This view was supported by Ingle et al. (1950) who found that cortisone accelerated the rise of amino acids in the blood of liverless rats. It was reported by Goodman et al. (1951) that ACTH induced proteinuria in normal rats and aggravated the proteinuria of renin-treated rats. Clark (1953), employing isotopic glycine, studied the effects of cortisone on protein metabolism in the rat and reported a decreased protein synthesis in cortisone-treated animals as compared to control rats. Inhibition of incorporation of isotopic glycine into protein by adrenalectomized and by adrenalectomized-thyroidectomized-parathyroidectomized rats due to the administration of cortisone was demonstrated by Hoberman (1950). These observations supported the view of Albright (1943) that the effects of cortisone-like steroids are antianabolic rather than catabolic.

c) Hair and thymus growth

Hair growth has been shown to be inhibited by percutaneous application of cortisone or by injection of ACTH (Whitaker et al. 1948; Baker et al. 1948; Winter et al. 1950), probably due to induction of atrophic changes in accessory structures of the skin such are known to occur in Cushing's disease. Meites (1952) demonstrated that when large doses of cortisone were injected into rats on a vitamin B_{12}-deficient diet, the deficiency symptoms became aggravated as indicated by inhibition of body and hair growth. This was counteracted in part by feeding 20 times the normal requirements for vitamin B_{12}. The involution of the thymus which follows cortisone administration (Antopol, 1950; Winter et al. 1950) was partially prevented by vitamin B_{12} (Meites, 1952) but the adrenal atrophy was not prevented. This finding was confirmed recently by Venkatarman et al. (1954).

d) Organ weights

The adrenal glands increase in weight when an animal is submitted to any type of stress (Selye, 1937; Ingle, 1938, 1939), and it is believed that this response is an aspect of the functional adaptation of the adrenals to increased requirements for cortical hormones by the organism. Ingle (1938) showed that during work, rats show adrenal hypertrophy which does not appear if the animal is hypophysectomized. This hypertophic condition of the adrenals in stressing situations has been confirmed by many authors. On the other hand, large doses of ACE may induce atrophy of the adrenal cortices of rats (Ingle and

Kendall, 1937). Later, Ingle et al. (1938) demonstrated atrophy of the adrenal cortices when pellets of cortisone were implanted. No atrophy was observed in the absence of the pituitary gland, showing that the mechanism which controls the size and function of the adrenal in stressing conditions lies in the pituitary gland. Sayers et al. (1949) found that release of ACTH was inhibited by the administration of cortisone given prior to subjecting rats to stress. Selye and Dorne (1942) showed adrenal atrophy in animals submitted to any stress but treated with significantly high doses of adrenal cortical hormones. However, when rats under a stress (cold) were given ascorbic acid, hypertrophy of the adrenals was prevented (Dugal et al. 1949).

Antopol (1950) observed the effects of administering large doses of cortisone to mice: a striking lymphopenia, loss in body weight, atrophy of the thymus and spleen and reduction in size of the adrenals. Testes, seminal vesicles and prostate were smaller than in the controls and the ovaries also appeared smaller. These findings were confirmed later by Ingle et al. (1952). Administration of cortisone or ACTH in high doses to rats or mice causes atrophy of the thymus, spleen and lymph nodes, and a decrease in the number of lymphocytes in the blood (Sprague, 1951). Heart and kidney enlargement, adrenal atrophy and failure of body growth were reported by Hall et al. (1952) following injection of adrenal cortical hormones. These findings are in disagreement with Winter et al. (1950), who did not find any effect of cortisone on the size of the kidneys and heart.

Selye (1952) showed that the inhibitory effect of cortisone on body growth could be counteracted by growth hormone (STH), and that the two hormones are also antagonistic with regard to their effects upon a variety of other target organs: the involution of the thymus and adrenal cortices were counteracted by simultaneous STH-cortisone treatment. Testosterone also has been shown to counteract the catabolic effects of cortisone. Albright (1943) reported that testosterone propionate induced anabolic response in patients with Cushing's syndrome and that this therapy produced some improvement in these patients. Methyl testosterone also, when administered simultaneously with ACTH prevented the development of a negative nitrogen balance (Bartter et al. 1949). These effects of cortisone and ACTH on body organs are transitory and reversible and generally disappear within 10 to 17 days after discontinuation of injections (Winter et al. 1950).

ALLOXAN-DIABETES AND INSULIN

Effects of alloxan

Jacobs (1937) found that the intravenous injection of alloxan produced a transient period of hyperglycemia in rabbits, followed by a severe and fatal hypoglycemia with convulsions and death in 7-10 hours. Intravenous or intraperitoneal injection of glucose could save these animals. Later, Dunn et al. (1943) found that the pancreas of alloxan-treated rabbits showed selective necrosis of the islets of Langerhans. This fact led to the discovery that alloxan could be used to induce permanent diabetes in animals.

Bailey et al. (1943), Goldner et al. (1943) and Dunn et al. (1943) demonstrated the possibility of inducing diabetes mellitus in the rabbit, dog and rat, by injecting alloxan. They could protect the animals' life by injecting glucose during the hypoglycemic phase which usually followed alloxan administration. This discovery was very important for the study of experimental diabetes, since it offered a technique for destroying the insulin-producing tissue of the pancreas without requiring pancreatectomy, a technique not feasible in some species of animals.

With the exception of the guinea pig which appears to be resistant to the diabetogenic effect of alloxan, other laboratory animals are susceptible to it. The required dose of alloxan varies with the species and the mode of administration (Bailey, 1949), the rate of injection

(Pincus et al. 1954), the pH of the solution (Klebanoff et al. 1954) and sex (Beach et al. 1951).

The diabetogenic action of alloxan is due to its selective necrotic destruction of the beta cells in the islets of Langerhans of the pancreas. The pancreatic islet lesions produced by a single injection of alloxan show degenerative changes of all or nearly all of the beta cells within 24 hours. Duff et al. (1947) found hydropic degeneration of the islet of Langerhans in alloxan-treated rabbits. This confirmed the findings of Bailey et al. (1944), who also found hydropic degeneration of the beta cells in rabbits two months after the development of alloxan diabetes.

Goldner et al. (1943), Dragsted et al. (1943) and Thorogood et al. (1945) reported a high insulin tolerance by alloxan-diabetic dogs. On the other hand, Thorogood et al. (1945) demonstrated that pancreatectomy of alloxan-diabetic dogs resulted in a significant reduction of their insulin requirement. The withdrawal of insulin after pancreatectomy led the animals rapidly into ketosis and coma, while alloxan-diabetic dogs could survive for a long period of time without insulin. This confirms the findings of Young (1939) who found that dogs made permanently diabetic by injections of anterior pituitary extracts required more insulin than depancreatized animals on the same diet. Dragsted (1943) demonstrated that dogs with partial pancreatectomy required more insulin than after complete pancreatectomy.

Effects of insulin on carbohydrate metabolism

Long (1954) has proposed that the influence of insulin on the rate of glucose utilization is through three major metabolic pathways: (1) increase in the amount of glucose or glycogen which is oxidized to carbon dioxide and water; (2) polymerization of glucose to glycogen both in the liver and muscle; and (3) conversion to fatty acids, both in the liver and adipose tissue.

Experiments with excised diaphragm muscle of rats have shown that extremely small amounts of insulin caused a measurable increase in the uptake of glucose by this tissue. This stimulation of glucose uptake was accompanied by an increased rate of glycogenesis (Stadie et al. 1947). Insulin also stimulated glucose consumption and glycogen synthesis in the muscle. This was demonstrated by Bouckaert et al. (1947) and Wick et al. (1951) in the intact and eviscerated animal, and they concluded that the primary physiological effect of insulin in lowering blood glucose was its increase in the utilization of glucose in the organs and tissues of the body and decreasing the net production of glucose by the liver.

Bouckaert and DeDuve (1947) measured quantitatively the amount of glucose which disappeared in the liver and in the peripheral tissues under the action of insulin. This was done by determining the amount of glucose needed to maintain the blood sugar at a constant concentration after insulin. By comparing normal and hepatectomized animals, it was found that the liver accounted for a large fraction of total glucose

utilization. Insulin promotes the net uptake of glucose by the liver, since hepatectomy greatly reduced the amount of glucose necessary to maintain the blood sugar level after a large dose of insulin.

Marks et al. (1939) showed that upon the administration of glucose to fasted dogs the R.Q. was promptly increased, denoting an increased utilization of carbohydrate. However, in the fasted diabetic animal the R.Q. was not raised. More direct evidence for an impairment of glucose oxidation in diabetics has been provided by isotopic experiments in which C^{14}-labeled glucose was administered to depancreatized dogs (Feller et al. 1951) or to alloxan-diabetic rats (Stetten et al. 1951). In each instance a markedly decreased capacity to convert the administered glucose to $C^{14}O_2$ was observed. In depancreatized dogs given insulin the rate of glucose oxidation returned to the values observed for normal animals.

These observations are in accordance with those of Wick et al. (1951) who demonstrated that the oxidation of radioactive glucose to CO_2 in the eviscerated rabbit was increased by insulin. Villee and Hasting (1949) who studied the effect of insulin on carbohydrate metabolism of isolated tissues found that in the rat diaphragm, insulin increased the utilization of glucose and the formation of glycogen and carbon dioxide from labeled glucose. These reactions proceeded at lower rate in the diaphragm from alloxan-diabetic rats.

The energy provided from these oxidative reactions is not released in the form of heat, but is used to form substances possessing very

high energy which can subsequently be liberated on their breakdown. This energy is in the form of phosphate bonds and the important known "high energy phosphate bond substances" are ATP and creatine phosphate. The former is probably the immediate source for mechanical energy such as is required for the performance of work in muscle contraction, growth and reproduction. The latter is perhaps only a storage form of phosphate bond energy.

Effects of insulin on fat metabolism

In addition to the effects of insulin in promoting glucose oxidation and glycogenesis, it also induces lipogenesis from carbohydrate. This was demonstrated by Chernick et al. (1950) with the use of isotopes, by comparing fat synthesis by liver slices from normal and alloxan-diabetic rats, with or without insulin. Brady et al. (1950) also demonstrated that the synthesis of higher fatty acids from acetate in vitro was accelerated by insulin. The process of fat synthesis from two-carbon fragments requires much energy and must be coupled to energy-yielding reactions. It appears likely that any appreciable decrease in the normal rate of glucose utilization will lead to a corresponding decrease in the energy available for lipogenesis. This view was supported by the observations of Baker et al. (1952) who noted that feeding of fructose to diabetic rats induced formation of fat from acetate.

In diabetic animals the conversion of glucose to fatty acids is impaired, possibly due to a deficiency in the energy required for fat synthesis from two-carbon fragments. Consequently the tissues largely

oxidize fatty acids and lead to the formation of ketone bodies and ketonuria, and acidosis develops. The decreased ability of liver slices of diabetic rats to synthesize fat from acetate is a secondary effect related to impairment of glucose metabolism (Stadie et al. 1940). Bloch et al. (1948) and Brady et al. (1950, 1951) measured the incorporation of labeled acetate into fatty acids in surviving rat liver slices. They found that insulin produced a significant increase in the synthesis of fat from acetate by normal liver slices, while liver slices from alloxan-diabetic rats or depancreatized cats almost lost their ability to synthesize long chain fatty acids.

Chernick et al. (1950) also demonstrated a decreased formation of fat from labeled glucose as well as a decreased oxidation of glucose to CO_2 in liver slices in alloxan-diabetic rats. Pretreatment of the diabetic rats with insulin repaired this inability to utilize carbohydrate for lipogenesis. Stetten and Boxer (1944) claimed that the major metabolic defect in the diabetic organism is its inability to synthesize fat from carbohydrate.

Effects of insulin on protein metabolism

A further consequence of the decreased utilization of carbohydrate by diabetic animal is an accelerated rate of breakdown of tissue protein. An increased excretion of urinary nitrogen, leading the animal to a negative nitrogen balance, is observed in diabetes. It appears possible that protein formation is favored by insulin in the same way as for lipogenesis. Since the fasted diabetic animal continues to excrete

glucose, it appears that gluconeogenesis still proceeds in the absence of insulin. In diabetes large amounts of nitrogen are excreted in the urine (Duncan, 1942). Bach et al. (1937) showed that insulin inhibited the deamination of amino acids by liver slices and concluded that insulin inhibited protein catabolism and consequently gluconeogenesis.

This nitrogen sparing action of insulin was further demonstrated by Gaebler et al. (1942). They showed that whereas anterior pituitary extracts administered to normal animals resulted in nitrogen retention, the same treatment in diabetic animals induced increased nitrogen excretion. Lotspeich (1949) observed that insulin accelerated the disappearance of amino acids from the blood stream and its appearance in muscle protein, and he claimed that insulin could synthesize protein in vivo.

Further evidence for the anabolic action of insulin on protein was given by Milman et al. (1951). They found that administering growth hormone to hypophysectomized-depancreatized cats caused no retention of nitrogen but increased glucosuria. In depancreatized cats given a constant supply of food and insulin, the administration of growth hormone resulted in nitrogen storage. This storage is correlated to the amount of insulin given. The authors concluded that insulin was essential for the protein-anabolic effect of growth hormone, and probably an increased secretion of insulin follows the administration of growth hormone to normal animals. More recently Best (1952) showed that it was possible to induce the hypophysectomized rat to grow by treatment with 1-6 units of insulin per day.

Mechanism of action of insulin

1) The hexokinase theory of insulin action

The most specific action which has been given to insulin is in the hexokinase reaction suggested by Cori (1945-46) and Price et al. (1945). The first step in the utilization of glucose for degradation or for glycogen synthesis is the formation of glucose-6-phosphate. The enzyme involved in this reaction is hexokinase (glucokinase) and phosphate is supplied by ATP:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6 phosphate} + \text{ADP}$$

Price et al. (1945) showed that tissue from alloxanized rats or rats which received anterior pituitary extracts had subnormal hexokinase activity. In vitro hexokinase reaction is retarded when anterior pituitary extract is added to the constituents of the reaction. Addition of insulin was shown to abolish this inhibition (Price et al. 1945). Adrenal cortical extract also was shown to depress hexokinase activity of muscle preparation taken from alloxan-diabetic animals. This inhibition of adrenal cortical extracts also could be released by addition of insulin (Colowick et al. 1947).

It is well known that the anterior pituitary and the adrenal cortex act opposite to insulin with respect to blood sugar, and the work of Price et al. (1945) helps to explain at least in part, the Houssay animal and Long's double operated cats.

This assumption of Price et al. (1945) of the action of insulin was not confirmed by Smith (1949) and Stadie et al. (1950).

Nevertheless, the idea that insulin acts in the initial phosphorylation of glucose seems to have considerable merit. The increased glucose uptake of the rat diaphragm by insulin demonstrated by Stadie et al. (1947), can only be explained by assuming that the rate of hexokinase reaction was increased. Further support for the hexokinase theory was supplied by Baker et al. (1952) who showed that fructose, but not glucose feeding improved the ability of liver slices of diabetic rats to synthesize fat from acetate.

2) The permeability theory of insulin action

The rate of transfer of metabolites such as glucose across cell membrane is thought to be influenced by insulin. Levine et al. (1949) attempted to show that insulin accelerated the entrance of a metabolite into the cell. It was found that if galactose were administered to the eviscerated, nephrectomized dog the blood level decreased rapidly and finally became stationary. However, if insulin were added the final concentration was much lower than in the absence of insulin, and it was assumed that insulin caused a transfer of galactose from the blood to the tissues. They concluded that insulin acts upon the cell membranes of certain tissues in such a manner that the transfer of hexoses and perhaps other substances from the extracellular fluid into the cell is facilitated.

On the other hand, Wick and Drury (1951) studied the action of insulin on the permeability of cells to sorbitol by determining the distribution of C^{14}-labeled sorbitol in the body fluids of nephrectomized,

eviscerated rabbits. They observed that the distribution was not increased with insulin. They concluded that the entrance of sorbitol into the cell is dependent on an enzyme mechanism and not a physical one like permeability. The Levine theory still requires confirmation.

Control of insulin secretion

The elaboration of insulin by the pancreas appears to depend on the blood sugar level. Zunz et al. (1927) found in cross-circulation experiments (in which the pancreatic-duodenal vein of a dog was connected to the jugular vein of a second dog), that when the blood glucose of the donor dog was elevated, blood glucose concentration fell in the recipient animal. This was interpreted to be the result of increased insulin secretion in response to the stimulus of hyperglycemia.

More recently Anderson et al. (1947) demonstrated that the secretion of insulin by the isolated rat pancreas was increased during perfusion for one hour with hyperglycemic fluid. Administration of large amount of glucose for a long period can also induce diabetes in some species. Dohan et al. (1946) reported hydropic degeneration of the beta cells and a diabetic state following glucose administration for long periods of time. Lukens (1944) showed that this occurred when large doses of anterior pituitary diabetogenic extracts were given to partially depancreatized cats.

Relation of insulin to nutrition

The work of Best, Haist and Ridout (1939) established the importance of the diet in regulating the production of insulin by the pancreas.

A high fat diet, fasting or insulin administration lowers the insulin content of the pancreas below that on a high carbohydrate diet alone. On the other hand, high protein diets give intermediate values. It appears probable that the carbohydrate in the diet determines the production of insulin. If insulin production is stimulated at high levels, due either to too high intake of carbohydrate or administration of diabetogenics hormones such as adrenocorticotrophic, growth or adrenocortic hormones (Lukens, 1944), the islet cells may be exhausted and permanent diabetes may develop.

The relation between the amount of insulin and the carbohydrate which can be utilized appears to be the principal problem in controlling diabetic patients. The decreased utilization of carbohydrate in the insulin-deficient individual is followed by a decreased need for the accessory factors involved in carbohydrate metabolism. Thiamine, niacin and perhaps riboflavin and pantothenic acid are known to be involved in carbohydrate oxidation systems as coenzymes. The need for these vitamins is reduced in diabetes, according to Samuels (1948), and is also reduced when animals are kept on a high fat, low carbohydrate diet (Evans et al. 1929).

Styron et al. (1942) found no significant difference in the length of time required by diabetic and non-diabetic rats on a thiamine-free diet to develop signs of thiamine deficiency. The urinary output of thiamine has been demonstrated to decrease when the intake of carbohydrate increases (Reinhold et al. 1944). The requirements of B vitamins

increase following the administration of insulin, due to the increased utilization of carbohydrate. The effectiveness of insulin appears to depend on the presence of these vitamins. Martin (1937) found that depancreatized dogs on a vitamin-B-deficient diet became resistant to insulin. Insulin resistance and poor glucose tolerance tests were also shown by Burke et al. (1938) and Lepkovsky et al. (1930) in vitamin B-deficient animals.

A progressive decrease in the response to insulin in a woman as a result of a deficiency of B-vitamins was reported by Elsom et al. (1940). Upon administration of thiamine and riboflavin the subject became abnormally sensitive to insulin. Vitamin therapy was found by Biskind (1945) to be effective in decreasing the hormone requirement of insulin-resistant diabetes. Feng (1954) found that vitamin B_{12} was essential for maximum insulin action. Single injections of insulin, 2.0 units per rat, were more effective in reducing blood glucose in normal, alloxan-diabetic and cortisone-treated rats on a vitamin B_{12}-adequate than on a vitamin B_{12}-deficient diet.

EXPERIMENTAL

Experiment I - The Effects of Cortisone on Thiamine-Requirements of Young Rats

Purpose

Large doses of cortisone may increase requirements for vitamin B_{12} in the young rat (Meites 1951, 1952a,b) and the baby pig (Wahlstrom et al. 1951) and for other B-vitamins such as pantothenic acid (Schultz et al. 1952), pyridoxine (Kiel, 1953) and riboflavin (Wilwerth et al. 1953). It was the purpose of the present study to determine the effects of large doses of cortisone on the requirements for thiamine in the young rat.

Methods

Fifty-three male Carworth rats weighing 65 gm. were fed a semi-synthetic diet from which thiamine was omitted for a period of 10 days, when body growth stopped and began to decrease. The composition of this semi-synthetic diet is presented in detail in the appendix. After the depletion period the rats were divided into five uniform groups on the basis of body weight, and were kept in metal cages with raised screen bottom at a mean room temperature of $76^{\circ}\pm1^{\circ}$ F. Artificial light was supplied from 7.00 a.m. to 6.00 p.m. daily; water and food were available at all times.

At the end of this period, the following treatment was inaugurated:

Group 1 - positive controls, 2 mg. of thiamine per kilo of diet
Group 2 - negative controls, no thiamine
Group 3 - no thiamine, 1 mg. of cortisone daily
Group 4 - 2 mg. of thiamine per kilo of diet, 1 mg. cortisone daily
Group 5 - 10 mg. of thiamine per kilo of diet, 1 mg. cortisone daily

Body weight and food consumption were measured every two days. Cortisone acetate (Cortone, Merck) was given subcutaneously in daily injections. At the end of 18 days the rats were killed and the organs were removed and weighed on a Koller-Smith balance to the nearest milligram. In this and in all subsequent experiments, the standard error of the mean was determined by the following formula:

$$S.E. = \sqrt{\frac{d^2}{n\,(n-1)}}$$

Significant differences between groups were determined as follows:

$$S.D. = \frac{m_1 - m_2}{\sqrt{E_1^2 + E_2^2}}$$

Results

1. Effects on body weight (Table I and Figure 1).

At the beginning of the depletion period the rats averaged 65.0 gm. each and at the end of the depletion period the rats averaged 103.2 gm. each in body weight, at which time the diets were changed according to the schedule already outlined. At the end of the experiment, the rats which were fed 2 mg. of thiamine per kilo of diet (group 1) showed an increase in average body weight of from 101.7 ± 2.3 gm. to 151.3 ± 4.1 gm.,

or a gain of 49.6 gm. The group of rats maintained on the thiamine-free diet (group 2) lost an average of 25.0 gm. in body weight and was far below the average weight of the positive controls (group 1), showing a final average body weight of 80.8±3.1 gm. (Figure 1).

An even greater loss in body weight occurred in the thiamine-deficient rats which received 1 mg. of cortisone daily (group 3). This group which started at an average of 105.2±2.3 gm. in body weight at the beginning of the experiment finished with an average weight of 64.8±2.9 gm., showing a loss of 44.4 gm. by the end of the 18 days of cortisone treatment. On the other hand, when thiamine (2 mg. per kilo of diet) was added to the ration and cortisone was given daily (group 4), body weight was maintained about the same throughout the experiment. These rats started at an average body weight of 102.0±2.7 gm. and by the end of the experiment the average weight was 110.8±5.1 gm., representing a gain of 8.8 gm.

When the amount of thiamine in the diet was increased five-fold (group 5), these rats were able to almost completely counteract the growth-inhibiting effect of cortisone and the growth curve of this group followed quite closely that of the positive controls (group 1). The initial average body weight of these rats (group 5) was 101.8±2.8 gm. and at the end of the experiment was 137.6±4.9 gm., representing an average body weight gain of 35.8 gm. The daily average gain was much greater in the first five days following the addition of thiamine to the diet, as compared to the gain of the positive controls (group 1) during the same period.

In addition to the body weight loss and extreme emaciation the thiamine-deficient rats exhibited typical symptoms of deficiency such as hunched posture, paralysis and spinning when picked up by the tail (groups 2 and 3). These symptoms were more pronounced in the thiamine-deficient, cortisone-injected rats (group 3). Eight of ten rats in this group showed pronounced priapism, a phenomenon not seen in any of the other rats. The addition of 2 mg. of thiamine per kilo of diet enabled cortisone-treated rats (group 4) to overcome partially the depression of body growth and eliminated all the other gross symptoms of thiamine deficiency.

2. Effects on food intake (Table I and Figure 1).

Food intake was affected primarily by the deficiency of thiamine in the diet and later by the administration of cortisone. During the 10-day depletion period all rats showed a progressive decrease in appetite, dropping from a daily average food intake of 9,2 gm. at the beginning to 6.1 gm. by the end of the depletion period. When thiamine was added to the diets of groups 1, 4 and 5, appetite was restored. The rats in group 1 ate an average of 153.0 gm. each and group 4 consumed 135.0 gm. each, while the rats in group 5 with a higher level of thiamine consumed an average of 170.0 gm. each, as shown in Table I.

The groups which were maintained on the thiamine-free diet (groups 2 and 3) ate less every day. By the end of the experiment the total food intake was about 65.0 gm. for the thiamine-deficient controls (group 2) and 62.0 gm. for the thiamine-deficient, cortisone-treated rats (group 3).

3. Effects on organ weights (Table II)

Since a deficiency of thiamine has been shown to influence the weights of certain vital organs (Skelton, 1950; Pecora, 1952; Pecora et al. 1953), and on the other hand cortisone has been demonstrated to reduce the size of the thymus and adrenals and increase the weight of the kidneys (Ingle, 1938, 1939; Ingle et al. 1952; Selye, 1937, 1952; Antopol, 1950; Meites, 1951, 1952), it was of interest to determine the effects of both thiamine-deficiency and cortisone administration on the weights of the adrenals, thymus, kidneys, seminal vesicles, testes and heart.

The results are shown in Table II. Both the actual weights of the organs and weight of organ per 100 gm. of body weight are presented. The adrenals were greatly increased in size in the rats which were maintained on the thiamine-deficient diet (group 2) or on the thiamine-deficient diet and cortisone (group 3). Adrenal weights averaged 32 and 26 mg. per 100 gm. of body weight, respectively. When 2 or 10 mg. of thiamine per kilo of diet were fed to the cortisone-treated rats (groups 4 and 5) the weight of the adrenals averaged the same on a body weight basis as in the positive controls (group 1).

The average weight of the kidneys are also increased in both the thiamine-deficient rats (group 2) and cortisone-treated, thiamine-deficient rats (group 3). These two groups showed an average kidney weight of 1550 mg. per 100 gm. of body weight when compared to the positive control rats (group 1), which averaged only 940 mg. per 100 gm.

of body weight. The cortisone-treated rats maintained on an adequate diet (group 4) and on 10 mg. of thiamine per kilo of diet (group 5), had an average kidney weight of 1219 and 1090 mg. per 100 gm. of body weight, respectively.

The thymus gland showed a very accentuated involution on the thiamine-deficient diet (group 2), while the rats treated with cortisone on the thiamine-deficient diet (group 3), showed an average of 33 mg. per 100 gm. of body weight. The thymus of the cortisone-treated rats fed 2 mg. (group 4) or 10 mg. of thiamine per kilo of diet (group 5) weighed 2 to 3 times as much as in group 3, although the average weights were far below that of the positive controls (group 1).

The seminal vesicles were very much decreased in the thiamine-deficient rats (group 2) and in the cortisone-treated, thiamine-deficient rats (group 3), averaging 34 and 73 mg. per 100 gm. of body weight, respectively. The average weight of the seminal vesicles of the cortisone-treated rats maintained on an adequate diet (group 4) and on 10 mg. of thiamine per kilo of diet (group 5) were above that of the positive control rats (group 1). Group 4 showed an average of 202 mg. and group 5 had 211 mg. per 100 gm. of body weight, while the positive controls (group 1) averaged 171 mg. per 100 gm. of body weight.

The heart appeared to be slightly larger in both the thiamine-deficient and cortisone-treated rats. The most striking increase was shown by the cortisone-treated, thiamine-deficient rats (group 3) which had an average weight of 600 mg. per 100 gm. of body weight. No consistent difference was observed in the other rats (groups 2, 4 and 5)

which averaged about 500 mg. per 100 gm. of body weight, although this average was above that of the positive controls (group 1) which showed only 426 mg. per 100 gm. of body weight.

The weight of the testes was not consistently altered in this experiment. With the exception of the cortisone-treated, thiamine-deficient rats (group 3), which showed an average testes weight of 2334 mg. per 100 gm. of body weight, the other groups averaged about 1600 mg. per 100 gm. of body weight.

Conclusions

1. Thiamine-deficiency in young rats induced a decrease in food intake and body weight, as demonstrated in previous experiments by other workers. Cortisone also decreased appetite and caused loss of body weight, and aggravated the symptoms of thiamine deficiency when given to thiamine-deficient rats. It was concluded that normal, and particularly excessive amounts of thiamine (10 mg. per kilo of diet) partially counteracted the depressant effects of cortisone on appetite and body weight gains, but the catabolic effects were not completely overcome.

2. Insofar as organ weights are concerned, it was concluded that thiamine deficiency induced atrophy of the thymus and seminal vesicles and enlargement of the adrenals, kidney and heart, and cortisone-treatment depressed the thymus and adrenals and slightly increased the size of the kidney, heart and seminal vesicles.

TABLE I

EFFECTS OF THIAMINE AND CORTISONE ON BODY WEIGHT, FOOD INTAKE AND EFFICIENCY OF FOOD UTILIZATION

Group and Number of Rats	Treatment	Initial* Body Weight	Final Body Weight	Avg. Food Intake Total	Avg. Food Intake Per gm. Gain Body Weight
		gm.	gm.	gm.	gm.
1 (13)	2 mg. thiamine	101.7 ± 2.3**	151.5 ± 4.1**	153	3.0
2 (10)	No thiamine	105.6 ± 2.07	80.8 ± 3.1	65	-
3 (10)	No thiamine+ cortisone	105.2 ± 2.3	64.8 ± 2.9	62	-
4 (10)	2 mg. thiamine+ cortisone	102.0 ± 2.7	110.8 ± 5.1	135	15.3
5 (10)	10 mg. thiamine+ cortisone	101.8 ± 2.8	137.6 ± 4.9	170	4.7

* Represents average body weight after thiamine-depletion period.
** Standard error of the mean.
(from Wilwerth et al. 1953)

I - 2 mg. thiamine
II - no thiamine
III - no thiamine, cortisone
IV - 2 mg. thiamine, cortisone
V - 10 mg. thiamine, cortisone

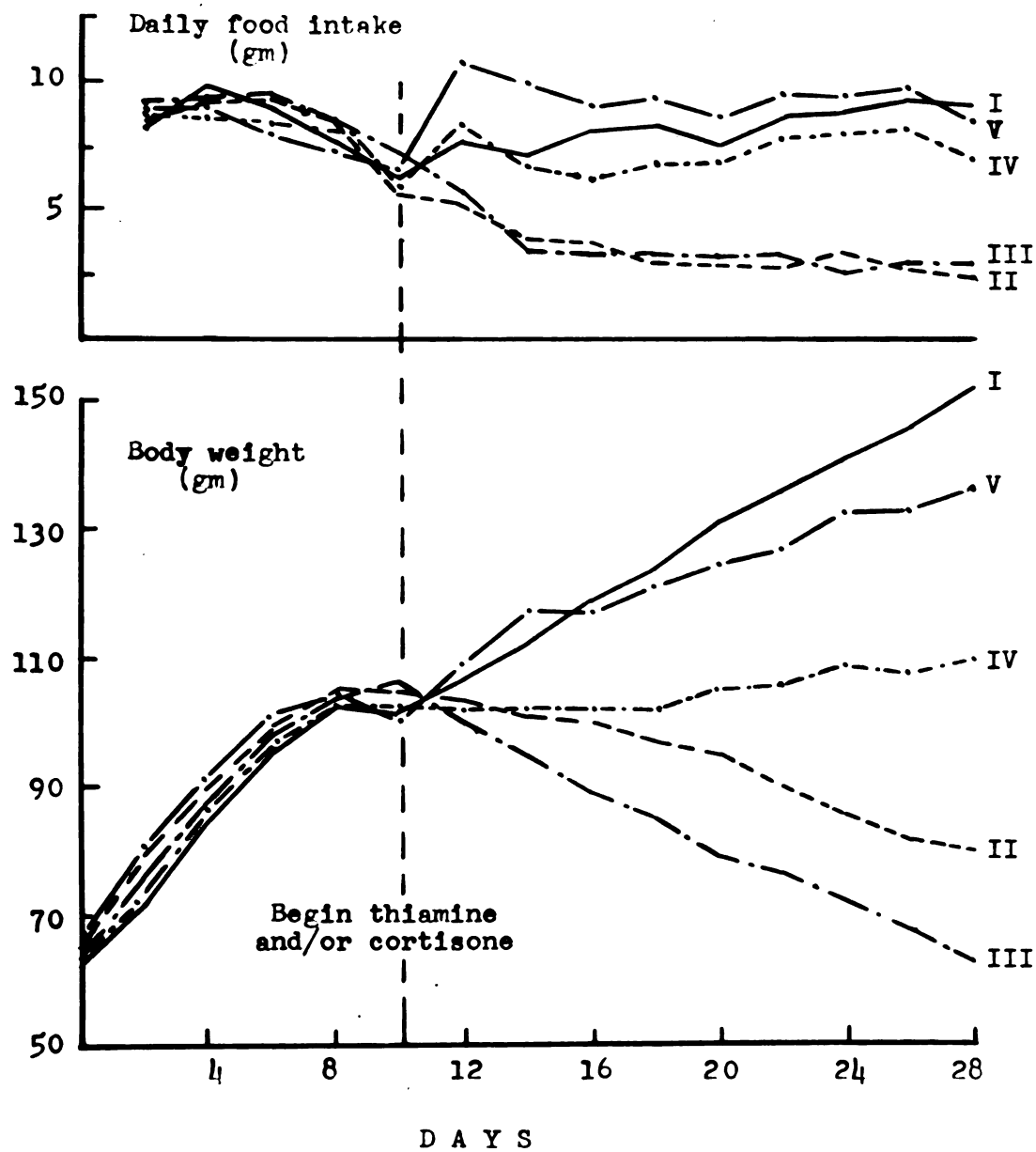


Fig. 1 - Effects of thiamine and cortisone on food intake and body weight.

TABLE II

EFFECTS OF THIAMINE AND CORTISONE ON ORGAN WEIGHT*

Group	Treatment	Final Body Weight gm.	Testes mg.	Seminal Vesicles mg.	Adrenals mg.	Thymus mg.	Heart mg.	Kidneys mg.
1	2 mg. thiamine	151.5	2299±77.8**	259±21.1	27±1.1	289±17.8	646±19.4	1425±59.4
			1523±52.3	171±14.1	18±0.8	191±13.8	426± 5.5	939±25.3
2	No thiamine	80.8	1434±77.8	28±4.7	26±1.4	27±2.8	390±15.6	1223±45.4
			1757±99.8	34±5.1	32±1.7	32±3.4	484±16.3	1525±59.8
3	No thiamine, cortisone	64.8	1504±80.5	48±1.5	17±1.8	21±2.6	386±18.9	1019±37.5
			2334±98.7	73±3.1	26±3.2	34±5.2	600±29.3	1590±57.8
4	2 mg. thiamine, cortisone	110.8	1990±132.2	230±22.6	20±1.5	78±7.7	563±15.9	1341±32.4
			1828± 62.8	202±38.1	17±1.1	69±4.7	514±19.2	1219±33.1
5	10 mg. thiamine, cortisone	137.6	2123±32.3	290±16.3	23±3.8	126±18.7	656±30.5	1500±68.8
			1559±81.3	211±11.8	16±1.3	89± 9.6	472±16.4	1091±40.6

* Actual organ weights on top and weight per 100 gm. of body weight on bottom.

** Standard error of the mean.

Experiment II - The effects of cortisone on thiamine-requirements of young rats.

Purpose

In this experiment an attempt was made to confirm the findings of Experiment I and in addition to find out whether thiamine doses larger than five times the normal requirement for growing rats would produce an increased counteraction of the depressing effects of cortisone on appetite and body growth. The effects of large amounts of thiamine on cortisone-treated rats whose food intake was limited were also observed.

Methods

Seventy-five male Carworth rats were divided into eight groups by body weight and fed a thiamine-free ration for 18 days. The initial body weight of all rats averaged 46.7±0.8 gm. and at the end of the depletion period averaged 73.8±1.6 gm. As in the previous experiment, the rats were housed in metal cages at a mean temperature of 76°±1°F and had artificial light from 7:00 a.m. to 6:00 p.m., daily. With the exception of group 4 whose food intake was limited, all other groups were permitted unlimited food at all times.

After the depletion period of 18 days, the following treatments were begun:

Group 1 - positive controls, 2 mg. of thiamine per kilo of diet

Group 2 - negative controls, no thiamine

Group 3 - no thiamine, 1 mg. of cortisone daily

Group 4 - limited food intake to group 3, 40 mg. of thiamine per kilo of diet, 1 mg. of cortisone daily

Group 5 - 2 mg. of thiamine per kilo of diet, 1 mg. of cortisone daily

Group 6 - 10 mg. of thiamine per kilo of diet, 1 mg. of cortisone daily

Group 7 - 20 mg. of thiamine per kilo of diet, 1 mg. of cortisone daily

Group 8 - 40 mg. of thiamine per kilo of diet, 1 mg. of cortisone daily.

Body weight and food intake were measured every two days. Cortisone was given daily in subcutaneous injections of 1 mg. At the end of the experiment, after killing the rats, the organs were removed and weighed.

Results

1. Effects on body weight (Table III and Figure 2).

The findings are summarized in Table III. At the end of the depletion period all rats averaged 73.8 gm. When thiamine was added in the amount of 2 mg. per kilo of diet (group 1), there was an average body weight gain of 30.0 gm. by the end of 10 days. The rats maintained on the thiamine-free diet (group 2) showed a loss in body weight of 12.8 gm. and were 45.3 gm. below the average body weight of group 1 at the end of the 10-day experiment. Greater loss of body weight was observed in the thiamine-free, cortisone-treated rats (group 3). This group showed a very pronounced cannibalism which resulted in the death of 8 out of 9 rats by the end of the 10 day experimental period. It was because of this group that the experiment was terminated at the end of 10 days.

Group 4, whose food intake was limited to that of group 3 but received 20 times the normal requirement for thiamine, together with cortisone, showed an average body weight loss of 10.8 gm. but all rats survived and showed no gross symptoms of thiamine deficiency. On the contrary, they all were in very good condition despite the loss of body weight.

When cortisone was given to the rats on the diet containing 2 mg. of thiamine per kilo of food (group 5), body weight was maintained at the same level throughout the experiment. On the other hand, when higher levels of thiamine were added to the diet (5, 10 and 20 times the normal requirement, groups 6, 7 and 8), there was a body weight gain in all these groups of about 24.0 gm. per rat. However, body weight did not reach the same level as in the positive control rats given 2 mg. of thiamine per kilo of food without cortisone (group 1).

2. Effects on food intake (Table III and Figure 2).

During the depletion period food intake dropped from 9.0 to 4.0 gm. by the end of the 18-day period. When thiamine was fed to groups 1, 5, 6, 7 and 8, recovery of appetite and increased food consumption was observed (Figure 2). The rats which were maintained on an adequate-thiamine diet (group 1) had an average total food intake of 82.0 gm. per rat, while in group 5 on a similar thiamine intake but also given cortisone, the total food intake averaged 61.0 gm. per rat.

The groups receiving higher levels of thiamine in their diets (5, 10 and 20 times the normal requirement, groups 6, 7 and 8) showed

a total food intake above that of group 1, with the greatest value in the group given the highest level of thiamine. In the rats maintained on a thiamine-deficient diet (group 2), total food intake was very low, averaging only about 30.0 gm. per rat during the experiment. When cortisone was given to the thiamine-deficient rats (group 3), appetite was very much depressed and a severe canibalism developed, resulting in the death of 8 rats in this group. The food intake in this group could not be measured.

3. Effects on organ weight (Table IV)

The effects of thiamine-deficiency and of cortisone were observed on the testes, seminal vesicles, adrenals, thymus, heart and kidneys. The results are shown in Table IV, as the absolute and relative weights. Data for group 3 could not be obtained because of the death of the animals.

The adrenals of the rats maintained on a thiamine-free diet (group 2) were significantly increased showing an average weight of 38.0 mg. per 100 gm. of body weight, while the positive control rats (group 1) averaged 25.0 mg. The rats which were partially starved and were treated with cortisone had an average adrenal weight of 24.0 mg. per 100 gm. of body weight. The cortisone-treated rats fed on adequate diet (group 5) and the other cortisone-treated rats (groups 6, 7 and 8) maintained on higher levels of thiamine showed a very consistent atrophy of the adrenals.

The weight of the thymus was very much reduced in the group maintained on the thiamine-free diet (group 2) and in the cortisone-treated, limited food intake rats (group 4) which had an average of 58.0 mg. per 100 gm. of body weight, while the positive control group (group 1) showed an average weight of 254.0 mg. The other cortisone-treated groups which received thiamine in different amounts (groups 5, 6, 7 and 8) showed significantly greater thymus weights than in groups 2 and 4.

The weight of the kidneys was increased in the thiamine-deficient rats (group 2) and in the limited food, cortisone-treated animals (group 4), as compared to the kidneys of the positive control rats (group 1). The cortisone-treated rats maintained on an adequate-thiamine diet (group 5) and those fed greater amounts of thiamine (groups 6, 7 and 8) showed progressively smaller kidney weights.

With the exception of the thiamine-deficient rats (group 2), which showed a significant decrease in the weight of the testes, all other groups had testes of about the same weight when compared on a body weight basis. The seminal vesicles were very much decreased in the thiamine-deficient rats (group 2), weighing only 21.0 mg., while the limited food, cortisone-treated rats (group 4) averaged 49.0 mg. Group 5, which was cortisone-treated and fed an adequate diet showed an average seminal vesicles weight of 77.0 mg. while the other cortisone-treated rats fed higher levels of thiamine (groups 6, 7 and 8) showed significant gains in seminal vesicles weight above that of the positive control rats (group 1).

Conclusions

1. The findings in this experiment, insofar as food intake, body weight and food efficiency are concerned, confirm the results of the first experiment.

2. The effects of thiamine-deficiency and cortisone on organ weight also followed much the same pattern as in Experiment I. A very pronounced decrease in the size of the thymus and seminal vesicles of the thiamine-deficient rats was observed, and also in the thymus and adrenals of the cortisone-treated rats. An increase in the size of the kidneys was found both in the thiamine-deficient and cortisone-treated rats. This confirms other reports of the effects of thiamine-deficiency on kidney weight (Pecora, 1952). The small increase in kidney weight effected by cortisone can perhaps be attributed to the salt and water retaining action of this hormone.

3. The increase in size of the seminal vesicles appears to be due mainly to increased thiamine and food intake, since cortisone did not increase the weight of the seminal vesicles in the rats given just an adequate thiamine intake (group 5). The increase in the weight of the thymus of the cortisone-treated rats given thiamine is believed to be primarily due to greater food intake.

TABLE III

EFFECTS OF THIAMINE AND CORTISONE ON BODY WEIGHT, FOOD INTAKE AND EFFICIENCY OF FOOD UTILIZATION

Group and Number of Rats	Treatment	Initial* Body Weight	Final Body Weight	Avg. Food Intake Total	Avg. Food Intake Per gm. Gain Body Weight
		gm.	gm.	gm.	gm.
1 (9)	2 mg. thiamine	77.3±1.1**	107.3±3.6**	82	2.73
2 (9)	No thiamine	74.8±1.2	62.0±2.4	30	-
3 (10)	No thiamine cortisone	73.0±1.2	-	-	-
4 (10)	40 mg. thiamine, limited food intake to 3, cortisone	73.8±1.7	63.0±1.4	30	-
5 (10)	2 mg. thiamine, cortisone	72.0±1.7	71.2±1.6	61.	-
6 (9)	10 mg. thiamine, cortisone	75.5±1.6	99.0±6.3	96	4.1
7 (9)	20 mg. thiamine, cortisone	73.1±2.03	94.0±3.6	93	4.4
8 (9)	40 mg. thiamine, cortisone	71.3±1.5	97.7±2.1	105	4.0

* Represents the average body weight after thiamine-depletion period.

** Standard error of the mean.

I - 2 mg. thiamine
II - no thiamine
III - no thiamine, cortisone
IV - 40 mg. thiamine, limited-food, cortisone
V - 2 mg. thiamine, cortisone
VI - 10 mg. thiamine, cortisone
VII - 20 mg. thiamine, cortisone
VIII - 40 mg. thiamine, cortisone

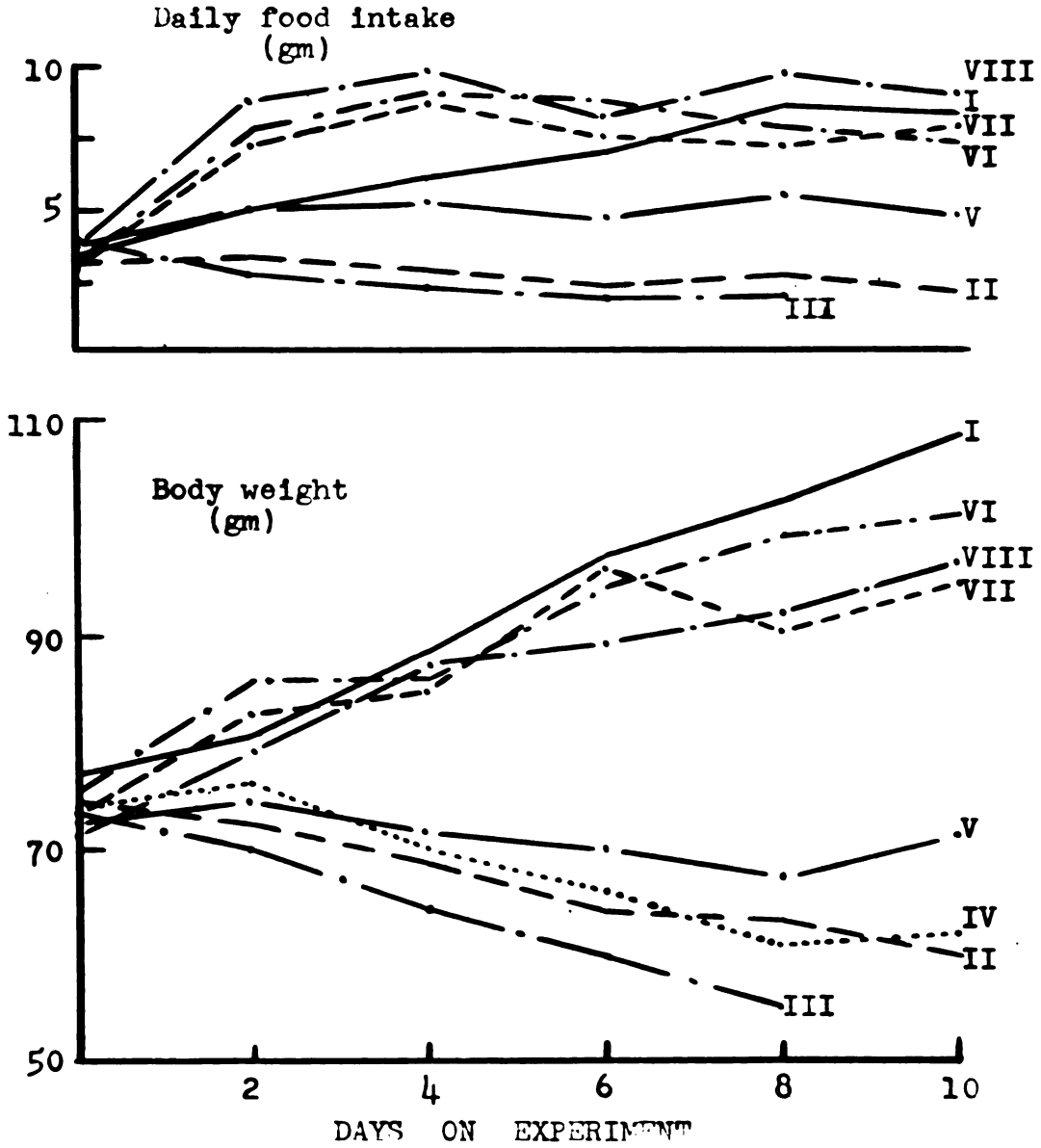


Fig. 2 - Effects of thiamine and cortisone on food intake and body weight.

TABLE IV

EFFECTS OF THIAMINE AND CORTISONE ON ORGAN WEIGHTS*

Group	Treatment	Final Body Weight gm.	Testes mg.	Seminal Vesicles mg.	Adrenals mg.	Thymus mg.	Heart mg.	Kidneys mg.
1	2 mg. thiamine	107.3	1757±101.1**	91±9.1	27±1.6	278±26.5	520±23.8	1131±44.5
			1637± 77.8	83±7.3	25±0.9	254±20.4	494±10.4	1030±29.9
2	No thiamine	62.0	730±114.6	14±1.8	24±1.4	37± 1.9	306±10.3	920±30.9
			1118±158.0	21±2.3	36±2.4	58± 2.1	479±17.0	1442±45.1
3	No thiamine, cortisone	-	-	-	-	-	-	-
4	40 mg. thiamine limited-food to 3, cortisone	63.0	1257±132.6	32±4.7	15±0.6	38±1.8	382±18.4	816±16.2
			1975±162.8	49±7.3	24±0.4	59±3.1	605±20.9	1318±26.3
5	2 mg. thiamine, cortisone	71.2	1214±104.6	56±7.8	13±1.3	76±6.8	432±11.2	886±32.7
			1669±170.0	77±9.8	18±1.8	105±11.5	597±16.3	1227±42.3
6	10 mg. thiamine, cortisone	99.0	1610±49.6	164±14.3	12±1.1	156±18.1	574±33.9	1113±65.4
			1761±129.8	179±20.6	13±1.9	174±30.9	599±57.4	1224±134.9
7	20 mg. thiamine, cortisone	94.0	1588±130.9	124±20.8	11±0.8	132± 8.3	514±20.3	1044±42.2
			1674±106.9	129±16.5	11±0.9	140±10.4	550±13.1	1113±18.8
8	40 mg. thiamine, cortisone	97.7	1733±72.4	177±26.0	14±1.1	115± 9.8	578±23.7	1077±27.7
			1775±71.9	178±24.3	14±1.1	116±10.2	591±19.4	1113±24.5

* Actual organ weights on top and weight per 100 gm. of body weight on bottom.
** Standard error of the mean.

Experiment III - Effects of thiamine and cortisone on body weight, carbohydrate and protein metabolism of guinea pigs.

Purpose

Since it had been demonstrated that large doses of cortisone induced transitory diabetes in guinea pigs (Hausberger et al. 1953) and slightly increased urinary nitrogen output, it was of interest to determine the effects of cortisone when given to normal and thiamine-deficient guinea pigs on body weight, urinary nitrogen and blood glucose.

Methods

Forty-three male guinea pigs of an average body weight of 354±1.22 gm. each were placed on a thiamine-depletion ration for a period of 30 days. At the end of this period they were divided into five groups, and housed in elevated, wide-meshed, wire-bottom galvanized iron cages, at a mean room temperature of $76^{\circ}\pm1^{\circ}F$. They were fed a standard semi-synthetic diet ad libitum and water was available at all times. The composition of the semi-synthetic diet (Reid et al. 1953) is given in the appendix. Artificial light was supplied from 7:30 a.m. to 9:30 p.m. daily.

The five groups were treated as follows:

Group 1 - positive controls, 16 mg. of thiamine per kilo of diet

Group 2 - negative controls, no thiamine

Group 3 - no thiamine, cortisone daily

Group 4 - 16 mg. of thiamine per kilo of diet, cortisone daily

Group 5 - 80.0 mg. of thiamine per kilo of diet, cortisone daily.

Groups 3, 4 and 5 received subcutaneous injections of cortisone at a daily dose level of 5 mg. for 10 days, after which the cortisone dose was increased to 10 mg. daily for 10 more days. This increase in dosage was established because the 5 mg. dose showed little effect in raising either blood glucose or urinary nitrogen. Body weight was measured at 4 to 5 day intervals throughout the experiment. Food intake could not be measured because of excess scattering of food by the guinea pigs.

Blood samples were collected after 10 days on the 5 mg. cortisone level and after 5 and 10 days on the 10 mg. level of cortisone treatment. Blood was collected by direct puncture of the heart with a 22 gauge needle and blood glucose was determined with 0.2 ml. of blood by the Hartmann, Shaffer and Somogy micro-method (Hawk et al. 1951). Twenty-four hour urine samples were also collected at the same time intervals as the blood collections. For urine collections, three guinea pigs from each group were placed in a single metabolism cage in which water was available at all times. To feed the guinea pigs, they were returned to their regular cages for a period of two hours, after being in the metabolism cages for 12-14 hours. After feeding, the guinea pigs were returned to the metabolism cages until the completion of a 24 hour period. Urine was collected in flasks containing citric acid as a

preservative. The samples were filtered and aliquots of each were placed under refrigeration. Nitrogen was determined by a standard micro-Kjeldahl method (Hawk et al. 1951). Both methods are described in the appendix.

Results

1. Effects on body weight (Table V and Figure 3)

The results obtained in this experiment on body weight are summarized in Table V and are shown more clearly in Figure 3. At the beginning of the experiment body weight averaged 354.0 gm. in all groups. The animals receiving 16.0 mg. of thiamine per kilo of diet (group 1) showed a normal growth curve and at the end of the experiment had an average body weight gain of 144.0 gm. Group 2, maintained on a thiamine-free diet, showed a body weight loss of 34.0 gm. and a final difference in average body weight of 181.0 gm. when compared to group 1. The cortisone-treated animals maintained on a thiamine-free diet (group 3) showed about the same loss in body weight as group 2. When cortisone was given to guinea pigs maintained on a diet containing 16.0 mg. or 80.0 mg. of thiamine per kilo of diet (groups 4 and 5), body weight was not significantly depressed and was about the same as the positive controls (group 1).

2. Effects on blood sugar (Table VI)

Blood glucose at the end of the depletion period averaged 94.4 mg. per 100 ml. of blood and did not show any significant variation for

the thiamine-adequate and thiamine-deficient guinea pigs (groups 1 and 2) during the experimental period. In group 3, which was treated with cortisone and maintained on a thiamine-free diet, blood glucose increased slightly from 92.0 to 112.0 mg. percent after daily injections of 5 mg. of cortisone for 10 days, and was slightly reduced to 106.0 and 84.0 mg. of glucose per 100 ml. of blood after daily injections of 10 mg. of cortisone for 5 and 10 days, respectively. Thus, no significant increase in blood glucose occurred in these cortisone-treated guinea pigs maintained on a thiamine-free diet.

Blood glucose rose from 104.0 to 124.0 mg. percent when 5 mg. of cortisone were given for 10 days to the guinea pigs fed 16.0 mg. of thiamine, and a still greater rise in blood glucose was observed after the dose of cortisone was increased to 10 mg. per day. Blood glucose increased to 145.0 mg. percent and after 10 days to 193.0 mg. percent. In the group maintained on the higher level of thiamine (group 5) the increase in blood glucose was greater after 5 days on 10 mg. of cortisone but did not increase further after 10 days on this dose of cortisone.

3. Effects on urinary nitrogen (Table VI)

The urinary nitrogen excretion per 100 gm. of body weight per 24 hours, after the end of the depletion period, averaged 74.0 mg. for the control guinea pigs (group 1). After the addition of thiamine to the diet the amount of nitrogen in urine varied from 72.0 to 78.0 mg. In the guinea pigs maintained on the thiamine-free diet (group 2) the nitrogen per 100 gm. of body weight was slightly but probably not

significantly higher than in the thiamine-fed guinea pigs (group 1). The guinea pigs treated with cortisone (groups 3, 4 and 5) tended to show slight increases in nitrogen per unit of body weight but these were of doubtful significance.

Conclusions

1. Thiamine-deficiency in guinea pigs, as in rats, produced a decrease in food intake and body growth. The guinea pigs were not as young as the rats and therefore the symptoms of thiamine-deficiency, such as anorexia, lassitude and loss of weight did not appear until after 25 days of thiamine deprivation and were not uniform in all guinea pigs. Some guinea pigs showed considerable resistance to thiamine-deficiency while others died. In general however, thiamine-deficiency was characterized by a slowing of body growth or loss of weight.

2. Cortisone did not appear to significantly affect body weight in the guinea pigs in contrast to the rats. The hormone also did not reduce body weight in the thiamine-deficient guinea pigs. This is in agreement with the work of Hausberger et al. (1953) who showed that large doses of cortisone did not depress body weight in guinea pigs.

3. In the thiamine-fed animals, blood glucose was not significantly elevated when 5 mg. of cortisone daily was given. However, when this dose was increased to 10 mg., a significant increase in blood glucose was observed at the end of 5 and 10 days of treatment. Neither dose of cortisone affected the blood glucose level of the guinea pigs maintained on the thiamine-free diet. This latter appears to be associated with

reduced food intake. In an attempt to test this idea, group 5 at the end of the experiment was put on a thiamine-free diet for a period of 35 days, and then 10 mg. of cortisone were injected daily for a period of 10 days. At the end of this time, blood sugar was determined and the results are shown in Table VII. These results show that 5 out of 9 guinea pigs, which had previously shown high blood glucose levels on the diet containing 80.0 mg. of thiamine per kilo of food, did not show any significant increase in blood glucose after being depleted of thiamine for 35 days. The other 4 guinea pigs were hyperglycemic and perhaps this can be accounted for by animal variability and the longer period of time required for an adult animal to exhibit symptoms of thiamine-deficiency.

4. Urinary nitrogen did not show any pronounced alteration by any of the treatments. This is believed to reflect a greater resistance by guinea pigs to cortisone-induced gluconeogenesis from protein. It is probable that the increased blood glucose observed with the higher levels of cortisone was due to the well established ability of the hormone to increase insulin resistance and thus decrease glucose utilization (Ingle, 1945).

TABLE V

EFFECTS OF THIAMINE AND CORTISONE ON
BODY WEIGHT GAINS OF GUINEA PIGS

Group	Treatment	Initial Body Weight gm.	Final Body Weight gm.	Weight Change gm.
1	16 mg. thiamine	357.5 ± 21.5*	501.0 ± 90.3*	+ 143.5
2	No thiamine	354.0 ± 13.05	320.0 ± 50.02	- 34.0
3	No thiamine, cortisone	352.0 ± 19.5	300.0 ± 44.6	- 52.0
4	16 mg. thiamine, cortisone	355.5 ± 13.05	474.0 ± 25.5	+ 119.5
5	80 mg. thiamine, cortisone	351.0 ± 14.1	481.5 ± 25.7	+ 130.5

* Standard error of the mean.

I - 16 mg. thiamine
II - no thiamine
III - no thiamine, cortisone
IV - 16 mg. thiamine, cortisone
V - 80 mg. thiamine, cortisone

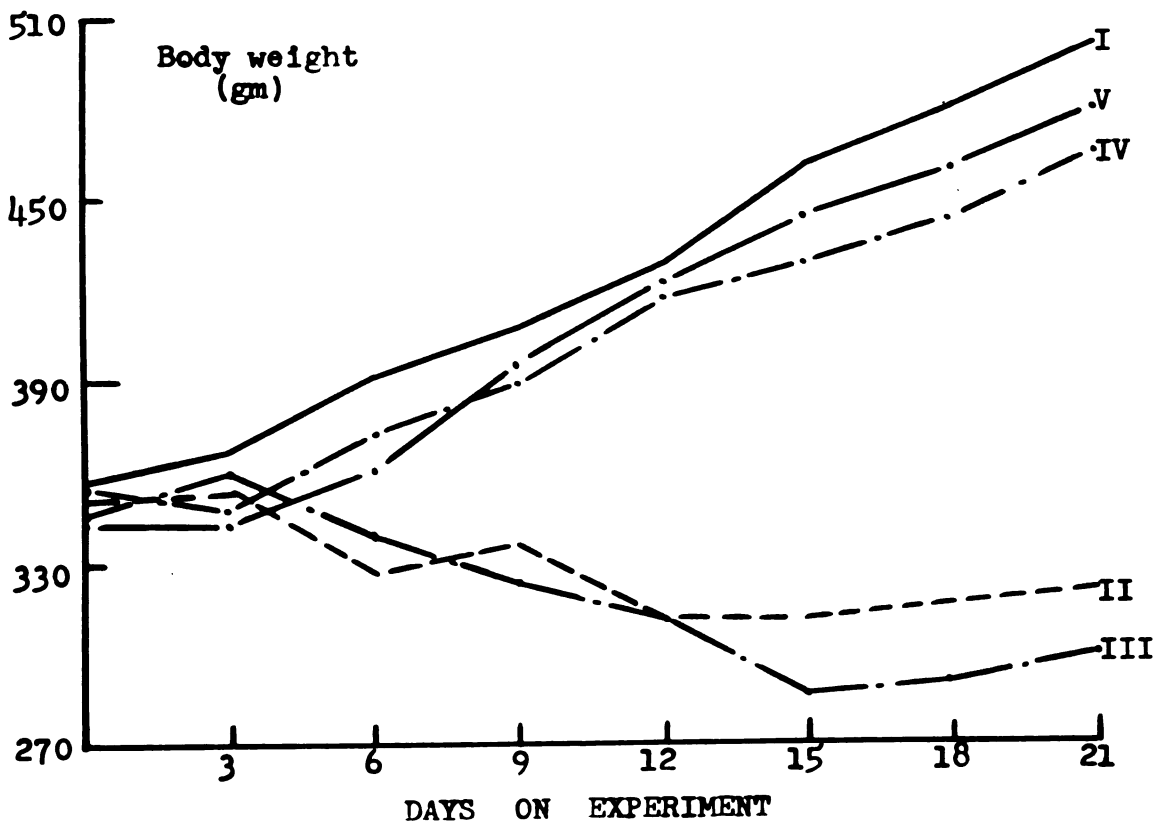


Fig. 3 - Effects of thiamine and cortisone on body weight.

TABLE VI

EFFECTS OF THIAMINE AND CORTISONE ON BLOOD GLUCOSE AND URINARY NITROGEN[1] IN GUINEA PIGS

		Group 1 16 mg. of Thiamine mg.	Group 2 No Thiamine mg.	Group 3 No Thiamine, Cortisone mg.	Group 4 16 mg. of Thiamine, Cortisone mg.	Group 5 80 mg. of Thiamine, Cortisone mg.
Controls[2]	Blood sugar[3]	96.0±3.8[4]	84.0±2.6	92.0±4.2	104.0±5.1	96.0±2.8
	N/guinea pig	204.2	297.4	288.6	284.0	258.1
	N/100 gm.	74.0	84.0	82.0	80.0	78.0
10 days on 5 mg. cortisone	Blood sugar	104.0±3.6	96.0±6.1	112.0±5.9	124.0±9.6	124.0±2.3
	N/guinea pig	270.0	253.5	260.3	331.8	313.2
	N/100 gm.	72.4	88.3	80.7	77.2	77.5
5 days on 10 mg. cortisone	Blood sugar	94.3±4.8	108.1±5.8	106.4±6.8	145.6±10.3	206.0±9.6
	N/guinea pig	286.1	240.0	252.2	348.9	328.2
	N/100 gm.	73.5	86.1	83.5	78.3	79.2
10 days on 10 mg. cortisone	Blood sugar	93.6±4.6	101.4±7.6	84.3±6.3	193.2±9.2	208.5±10.2
	N/guinea pig	394.2	248.6	320.8	417.0	412.2
	N/100 gm.	78.0	78.3	97.3	88.1	92.3

[1] All nitrogen values represent total nitrogen per 24 hours.
[2] At the end of the depletion period.
[3] Milligrams per 100 ml. of blood.
[4] Standard error of the mean.

TABLE VII

EFFECTS OF THIAMINE DEFICIENCY ON BLOOD SUGAR
BEFORE AND AFTER CORTISONE ADMINISTRATION

Guinea Pig Number	Before Cortisone mg. %	After Cortisone mg. %
1	84.0	116.0
2	92.0	304.0
3	84.0	84.0
4	84.0	88.0
5	92.0	94.0
6	144.0	260.0
7	104.0	172.0
8	84.0	104.0
9	104.0	220.0

Experiment IV - The effects of thiamine, cortisone and alloxan on body growth, blood sugar and urinary nitrogen in rats.

Purpose

Since it had been demonstrated that the administration of cortisone failed to induce hyperglycemia in most thiamine-deficient guinea pigs, even when given in doses of 5 and 10 mg. this experiment was undertaken to determine if this was due to deprivation of thiamine *per se* or merely to reduced food intake. Alloxan was also given to see whether thiamine would influence the action of this substance in producing diabetes.

Methods

Eighty male, adult Carworth rats were fed a semi-synthetic diet from which thiamine was omitted for a period of 20 days. At the end of this time the first symptoms of thiamine deficiency appeared, as indicated by anorexia, lassitude and body weight loss. Water and food were available at all times. Blood samples were collected every 5 days from the tip of the tail, and blood glucose was determined by the micro-method of Folin and Malmros (Hawk *et al.* 1951), using 0.1 ml. of blood. Twenty-four hour urine samples were collected every 5 days and the total urinary nitrogen was determined by the standard micro-Kjeldahl method (Hawk *et al.* 1951).

After the 20-day depletion period the rats were divided into eight groups on the basis of body weight, and were treated as follows for an additional period of 28 days:

Group 1 - positive controls, 2 mg. of thiamine per kilo of diet

Group 2 - negative controls, no thiamine

Group 3 - 2 mg. of thiamine per kilo of diet, alloxan

Group 4 - no thiamine, alloxan

Group 5 - 20 mg. of thiamine per kilo of diet, but limited in food intake to group 4, alloxan

Group 6 - 2 mg. of thiamine per kilo of diet, 4 mg. of cortisone daily

Group 7 - no thiamine, 4 mg. of cortisone daily

Group 8 - 20 mg. of thiamine per kilo of diet, but limited in food intake to group 7, 4 mg. of cortisone daily.

The limited-food groups (5 and 8) were fed a ration containing 20 mg. of thiamine per kilo of diet but their food intake was limited to that eaten by the thiamine-deficient rats. After a 48-hour fasting period, alloxan was injected subcutaneously in the rats of groups 3, 4 and 5 at a level of 16.0 mg. per 100 gm. of body weight from a 3 percent freshly prepared solution. Cortisone was given to groups 6, 7 and 8 in daily subcutaneous injections of 4 mg. per rat during the experimental period. Body weight and food intake were measured every two days, and urinary nitrogen and blood glucose were determined every five days.

For urine collection five rats were placed in a single metabolism cage in which food was present in non-scattering metal feeders and water was available at all times. The rats remained in the cages for 24 hours and urine was collected in flasks containing 1 gm. of citric acid as a preservative.

After 15 days, the treatments for the thiamine-deficient rats of groups 4 and 7 were reversed, i.e., an intraperitoneal injection of 10 mg. of thiamine was given and 2 mg. of thiamine per kilo of diet was added. The daily food intake of groups 5 and 8 was limited to that consumed daily by groups 4 and 7, respectively.

Results

1. Effects on food intake and body weight (Table VIII and Figure 4).

The results are summarized in Table VIII and Figure 4. As in previous experiments during the depletion period, appetite was markedly depressed and body weight was greatly reduced. When 2 mg. per kilo of diet were fed to groups 1, 3 and 6, appetite was restored and body weight increased steadily. No great difference was observed between the positive controls (group 1) and the alloxan treated rats on 2 mg. of thiamine per kilo of diet (group 3). On the other hand the rats on 2 mg. of thiamine per kilo of diet and cortisone (group 6), showed less increase in food intake and body weight was maintained at the same level during most of the experiment.

The rats maintained on the thiamine-free diet during the first part of the experiment (groups 2, 4 and 7) showed a constant decrease in body weight and their food intake became gradually reduced. The thiamine-deficient, cortisone-treated rats (group 7) showed a more pronounced loss of body weight than any other thiamine-deficient group. This harmful effect of cortisone on thiamine-deficient rats was previously demonstrated in Experiments 1 and 2. The two limited-food groups

(5 and 8), which received 20 mg. of thiamine in their ration, followed groups 4 and 7 very closely. Once more, the limited-food group which received cortisone (group 8) showed a more pronounced decrease in body weight than the alloxan-treated rats (group 5).

After an intraperitoneal injection of 10 mg. of thiamine per rat to the previously thiamine-deficient rats (groups 4 and 7), appetite and body weight showed consistent increases. However, the alloxan-treated rats (group 4) showed a greater increase in food intake and body weight than the cortisone-treated rats (group 7). When the limited-food groups (5 and 8) received larger amounts of food to correspond with the increased appetite in groups 4 and 7, group 5 (alloxan-treated) showed an increase in body weight similar to group 4, and group 8 (cortisone-treated) showed an increase similar to group 7.

2. Effects on blood sugar (Table IX)

The effects of alloxan and cortisone on blood glucose in adequate and thiamine-free diet fed rats varied according to the diet given. After the end of the deprivation period the average blood glucose values were about 93.0 mg. per 100 ml. of blood. As can be seen in Table IX, group 1, after the addition of thiamine to the ration, showed an increase in blood glucose which remained at 120.0 to 127.0 mg. percent throughout the experiment. The thiamine-deficient control rats (group 2) showed values varying from 91.0 to 96.0 mg. percent.

When alloxan was given to the rats on an adequate diet (group 3), blood glucose levels rose to an average of 260.0 mg. percent and the

rats showed polydipsia and polyuria. In the thiamine-deficient, alloxanized rats (group 4) only a slight and probably insignificant increase in blood glucose was observed. The amount of sugar excreted in the urine could not be determined because the diet contained 62 percent of glucose (Cerelose) and some of this food contaminated the urine samples. The limited-food rats which were fed thiamine showed a very consistent increase in blood glucose 5 days after alloxan treatment (244.0 mg. %).

The cortisone-treated rats (groups 6, 7 and 8) showed greater increases in blood glucose when thiamine was fed than when it was withheld. When group 4 (alloxan-treated) and group 7 (cortisone-treated were injected with 10 mg. of thiamine, a very consistent increase in blood glucose was shown by the alloxan-treated rats (group 4) and a lesser increase occurred in the cortisone-treated rats (group 7). The limited-food groups (5 and 8) closely followed groups 4 and 7, respectively, as shown in Table IX.

3. Effects on urinary nitrogen (Table IX)

The average daily excretion of total urinary nitrogen per 100 gm. of body weight was about 85.0 mg. by the end of the deprivation period in all groups. After the addition of thiamine to the diet of the positive control rats (group 1), daily nitrogen excretion increased slightly, while the thiamine-deficient rats (group 2) showed no change in nitrogen. When alloxan was given to rats on an adequate diet (group 3), the daily urinary nitrogen excretion rose gradually from

86.0 to 184.0 mg. per 100 gm. of body weight. On the other hand, no increase in nitrogen was found in the alloxan-treated rats kept on a thiamine-free diet (group 4). However, the alloxan-treated rats on a limited-food intake but given thiamine (group 5) showed a very consistent increase in urinary nitrogen. In all rats receiving cortisone (groups 6, 7 and 8) nitrogen increased almost at the same rate. When thiamine was injected into group 4(alloxan-treated) and group 7 (cortisone-treated) there was a pronounced increase in the excretion of urinary nitrogen. This was also true of the corresponding limited-food groups (5 and 8).

Conclusions

1. Deprivation of thiamine depressed food intake and body weight, and reduced the efficiency of food utilization, as demonstrated in previous experiments. Alloxan administration slightly reduced and cortisone-treatment significantly decreased the efficiency of food utilization in the thiamine-adequate rats as compared to the positive control rats. Limitation of food intake with adequate thiamine intake produced no depressing effects on either body weight or food efficiency. Otherwise the rats showed a much better appearance.

2. When alloxan and cortisone were given to thiamine-adequate rats, hyperglycemia was produced. This was not evident when alloxan was given to thiamine-deficient rats. This contrasts with the findings of Feng (1954), who found that in vitamin B_{12}-deficiency, administration of alloxan or cortisone induced hyperglycemia and glucosuria. In fact

cortisone induced a greater hyperglycemia in the vitamin B_{12}-deficient than in the vitamin B_{12}-adequate rats. This may be due to the differences in the actions of thiamine and vitamin B_{12} on carbohydrate metabolism.

3. Urinary nitrogen excretion in cortisone-treated rats was increased at the same rate in all groups, irrespective of the amount of thiamine intake. However, hyperglycemia was not evident in either the thiamine-deficient rats or in the rats permitted only limited food intake. This suggests the possibility that the reduced food failed to provide sufficient carbohydrate to the organism or that gluconeogenesis from protein was at a low level or both.

Only the alloxan-treated rats on the thiamine-deficient diet did not show any increase in urinary nitrogen. The increase in blood glucose and urinary nitrogen in the limited-food, alloxanized rats given thiamine suggests that thiamine rather than increased caloric intake is primarily responsible for the manifestation of the diabetogenic action of alloxan. On the other hand, reduced food intake rather than a thiamine deficiency appears to be responsible for the absence of a pronounced diabetogenic effect in cortisone-treated rats.

TABLE VIII

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN ON BODY WEIGHT, FOOD INTAKE AND EFFICIENCY OF FOOD UTILIZATION

Group and Number of Rats	Treatment	Body Weight			Avg. Food Intake	
		Initial	Middle	Final	Total	Per gm. Gain Body Weight
		gm.[1]	gm.[2]	gm.	gm.	gm.
1 (10)	2 mg. thiamine	183.8 ±6.9[3]	220.0 ±7.6	242.1 ±10.1[3]	262.8	4.3
2 (10)	No thiamine	185.1 ±9.2	149.1 ±6.3	152.0 ±7.9	62.3	-
3 (10)	2 mg. thiamine, alloxan	182.0 ±10.1	194.3 ±8.2	229.3 ±14.1	239.5	4.9
4 (10)	No thiamine, alloxan	182.3 ±7.4	139.5 ±8.7	195.2 ±10.9	150.9	7.9
5 (11)	20 mg. thiamine, limited-food to 4, alloxan	183.2 ±6.5	146.3 ±8.2	200.4 ±12.3	150.9	6.3
6 (11)	2 mg. thiamine, cortisone	184.5 ±5.9	165.0 ±6.2	167.8 ±9.3	174.2	-
7 (11)	No thiamine, cortisone	185.4 ±5.9	124.4 ±4.7	151.8 ±5.5	112.5	-
8 (10)	20 mg. thiamine limited-food to 7, cortisone	185.8 ±4.6	119.0 ±3.2	147.5 ±8.1	112.5	-

1 Average body weight after thiamine-depletion period.
2 Average body weight at the date of thiamine injection and food treatment reversal.
3 Standard error of the mean.

I - 2 mg. of thiamine
II - no thiamine
III - 2 mg. thiamine, alloxan
IV - no thiamine, alloxan
V - 20 mg. thiamine, limited-food, alloxan
VI - 2 mg. thiamine, cortisone
VII - no thiamine, cortisone
VIII - 20 mg. thiamine, limited-food, cortisone

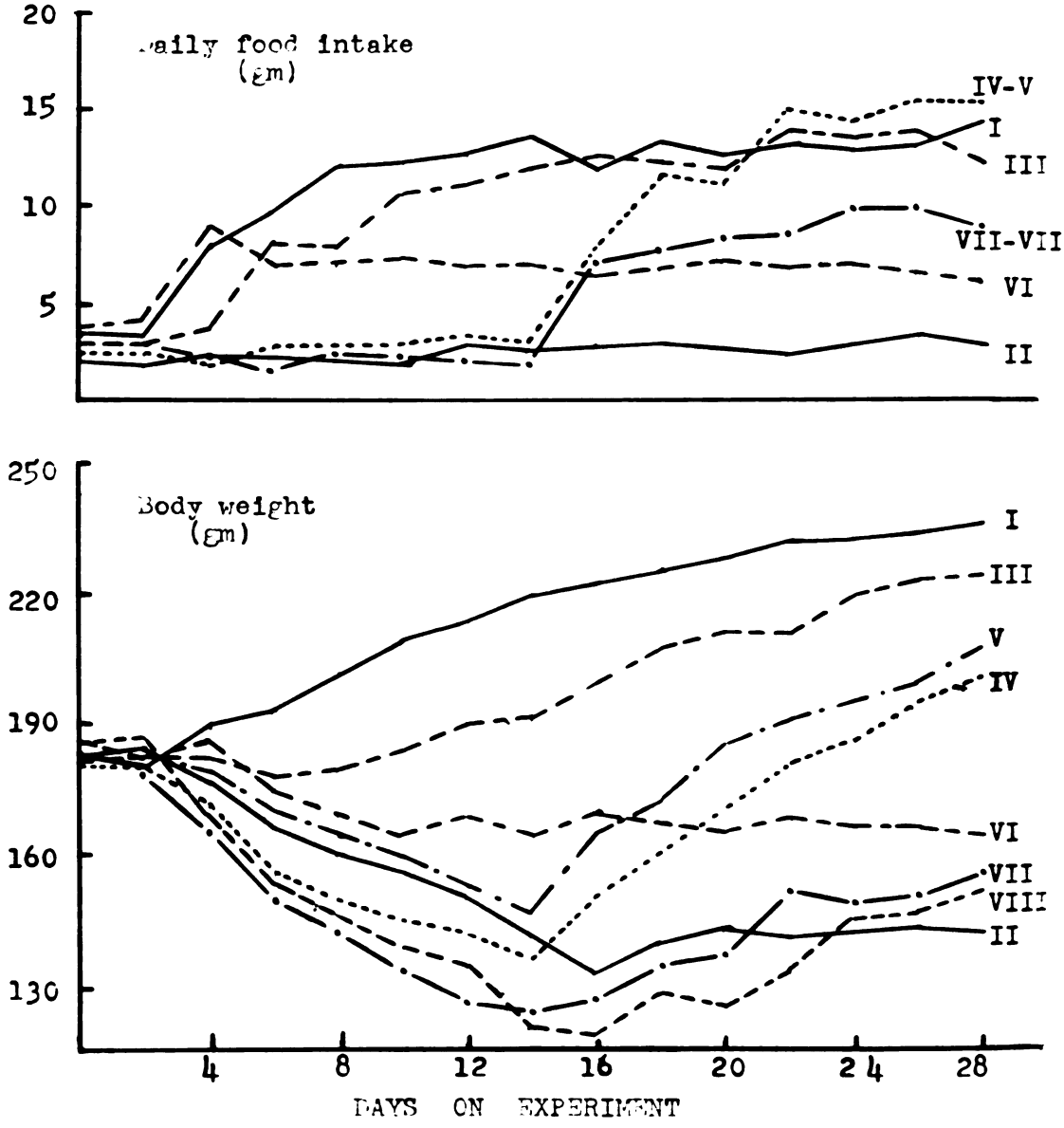


Fig. 4 - Effects of thiamine, cortisone and alloxan on food intake and body weight.

TABLE IX

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN ON BLOOD GLUCOSE AND URINARY NITROGEN IN RATS

B. G. - blood glucose (mg. per 100 ml. of blood)
N/rat - urinary nitrogen (mg. per rat/24 hours)
N/100 - urinary nitrogen (mg. per 100 gm. body weight/24 hours)

Days[1]		Group 1 Thiam.	Group 2 No thiam.	Group 3 Thiam. Alloxan	Group 4 No thiam. Alloxan	Group 5 Lim. food Thiam. Alloxan	Group 6 Thiam.	Group 7 No thiam. Cortis.	Group 8 Lim. food Thiam. Cortis.
0	B. G.	91.0 ±4.2[2]	93.7 ±5.5	98.8 ±5.1	94.4 ±4.7	84.4 ±3.8	95.2 ±4.9	92.3 ±4.05	94.2 ±3.1
	N/rat	156.8	165.7	169.4	163.8	170.2	163.0	181.6	163.0
	N/100	80.0	85.0	86.0	86.0	83.9	88.0	88.0	83.0
5	B. G.	117.7 ±4.7	93.9 ±3.4	260.5 ±25.5	108.2 ±5.7	244.0 ±10.2	155.0 ±6.8	103.4 ±2.2	121.9 ±3.1
	N/rat	231.1	162.9	220.2	179.1	288.8	255.7	172.8	253.4
	N/100	127.0	90.0	121.0	99.0	157.0	139.0	96.0	137.0
10	B. G.	120.2 ±4.1	92.6 ±2.9	262.0 ±21.1	117.2 ±3.6	190.0 ±8.6	167.2 ±5.2	109.0 ±4.0	105.7 ±2.6
	N/rat	251.5	156.9	226.5	141.1	225.4	257.2	208.2	224.7
	N/100	129.0	94.9	128.4	91.1	135.0	147.0	137.0	145.7
15	B. G.	127.5 ±4.6	96.3 ±2.4	245.0 ±18.5	118.3 ±5.8	162.7 ±9.5	175.0 ±8.6	115.2 ±3.2	129.2 ±2.4
	N/rat	237.5	152.5	305.9	126.3	191.7	219.3	175.1	176.2
	N/100	111.0	101.3	161.0	89.3	127.0	129.6	139.0	141.7
Reverse food treatment for groups 4 and 7									
20	B. G.	129.0 ±6.6	95.1 ±3.1	254.3 ±21.5	268.4 ±15.1	431.2 ±17.6	169.7 ±7.3	132.1 ±8.2	111.2 ±3.9
	N/rat	278.3	159.6	376.2	271.4	476.4	251.1	433.2	267.2
	N/100	121.6	114.8	175.4	174.0	287.0	146.5	303.0	219.2
25	B. G.	125.0 ±3.7	91.2 ±2.1	225.6 ±17.6	283.7 ±19.5	437.6 ±20.3	170.6 ±6.5	129.1 ±10.6	121.2 ±8.2
	N/rat	262.2	157.1	406.3	342.1	562.5	239.9	347.3	308.0
	N/100	109.6	105.5	179.3	189.2	293.0	142.5	227.3	220.2
28	B. G.	127.3 ±4.4	93.1 ±3.4	231.5 ±16.5	279.3 ±17.9	433.4 ±18.8	172.2 ±7.2	127.1 ±9.1	120.6 ±6.7
	N/rat	263.5	199.8	423.2	440.1	474.1	243.2	324.0	341.2
	N/100	108.3	105.6	184.3	219.8	229.5	152.3	207.1	223.7

1 Days after alloxan or cortisone treatment.
2 Standard error of the mean.

Experiment V - The effects of thiamine, cortisone, alloxan and insulin on body growth, blood glucose and urinary nitrogen in rats.

Purpose

In this experiment an attempt was made to confirm the results obtained in Experiment IV on blood glucose and urinary nitrogen as induced by the administration of cortisone and alloxan to rats maintained on adequate or thiamine-deficient diets. Insulin was given to determine the response of the rats previously treated with cortisone or alloxan and maintained on thiamine-adequate or deficient diets.

Methods

Sixty male, adult Carworth rats with an average body weight of 225.0 gm. each were placed on a semi-synthetic, thiamine-deficient diet for a period of 25 days until their average body weight decreased to 196.0 gm. The rats were then divided into six uniform groups on the basis of body weight and were housed in metal cages with raised screen bottom. Water and food were available at all times. After this period, the following treatment was established for each group:

Group 1 - positive controls, 2 mg. of thiamine per kilo of diet

Group 2 - negative controls, no thiamine

Group 3 - 2 mg. of thiamine per kilo of diet; 17.0 mg. of alloxan per 100 gm. of body weight given in one single subcutaneous injection

Group 4 - no thiamine; 17.0 mg. of alloxan per 100 gm. of body weight given in one single subcutaneous injection

Group 5 - 2 mg. of thiamine per kilo of diet; 4 mg. of cortisone daily

Group 6 - no thiamine; 4 mg. of cortisone daily.

Body weight and food intake were measured every two days during the 16-day period of the experiment. Blood samples were collected every 5 days for a 15-day period and blood analysis were made according to the Folin and Malmros micro-method (Hawk et al. 1951). Twenty-four hour urine samples were collected at the same time intervals as the blood collections, and nitrogen was determined by the standard micro-Kjeldahl method (Hawk et al. 1951). After the 15-day period, 2.0 units of insulin (Illetin, Lilly) were injected into the rats of groups 1, 3 and 5, and 0.2 units into the rats of the other groups. Blood samples were collected four hours later (Feng, 1954). Because of the weakness and low food intake of the rats of groups 2, 4 and 6 (thiamine-deficient rats), the insulin dose was less in these groups than in those on a thiamine-adequate diet.

Results

1. Effects on food intake and body weight (Table X and Figure 5)

The results are summarized in Table X. Deprivation of thiamine induced a markedly decrease in food intake and body weight. When 2 mg. of thiamine per kilo of diet was added to the ration of groups 1, 3 and 5, appetite was promptly restored and body weight increased. When the alloxan and cortisone-treated rats (groups 3 and 5) are compared to the positive control rats (group 1), it is observed that the

efficiency of food utilization was greater in the alloxan than in the cortisone-treated rats. The rats maintained on a thiamine-free diet (group 2) and those on a thiamine-free diet with alloxan (group 4) or cortisone (group 6) did not show any significant variation in food intake; otherwise their appetite was very poor and they showed a pronounced loss in body weight.

2. Effects on blood sugar (Table XI)

After the end of the deprivation period blood glucose averaged 92.8 mg. per 100 ml. of blood. Two mg. of thiamine added to the diet of the positive control rats (group 1) induced a progressive increase in the average blood glucose to 125.0 mg. percent, while the rats maintained on a thiamine-free diet averaged 93.9 mg. percent of blood glucose throughout the experiment.

When alloxan was given blood glucose levels rose to 381.0 mg. percent for the rats maintained on a thiamine-adequate diet (group 3). On the thiamine-deficient alloxanized rats (group 4), only a slight and probably not significant increase in blood glucose was observed. The cortisone-treated rats fed 2 mg. of thiamine per kilo of diet (group 5) showed a consistent and progressive increase in blood glucose during the experiment, reaching 172.6 mg. percent by the end of the experimental period. When cortisone was given to thiamine deficient rats (group 6), blood glucose only rose to 121.2 mg. percent.

3. Effects on urinary nitrogen (Table XI)

The total urinary nitrogen excretion per 100 gm. of body weight per 24 hours, after the end of the depletion period averaged 86.0 mg. When 2 mg. of thiamine were added to the diet of the positive control rats (group 1) urinary nitrogen slightly increased during the experiment, while it remained at about the same level for the thiamine-deficient control rats (group 2).

Alloxan-treated rats on a thiamine-adequate diet (group 3) showed a progressive increase in urinary nitrogen which by the end of the experiment averaged 162.0 mg. per 100 gm. of body weight. On the other hand, alloxan-treatment of thiamine-deficient rats (group 4) did not induce any increase in the excretion of nitrogen in the urine. Cortisone administration somewhat increased nitrogen excretion in both thiamine-adequate (group 5) and thiamine-deficient rats (group 6). The greater increase (149.0 mg./100 gm. body weight) was shown by group 5 (thiamine-fed) while the thiamine-deficient rats (group 6) increased only to 133.0 mg. per 100 gm. body weight.

4. Effects of insulin on blood sugar (Table XII)

The effects of insulin on blood sugar are summarized in Table XII. In groups 1, 3 and 5, which were fed on a thiamine-adequate diet, insulin induced the greatest percent decrease in blood glucose in the alloxan-treated rats (group 3), with an average decrease of 62.8 percent. The cortisone-treated rats (group 5) were the most resistent to the hypoglycemic action of insulin, showing only a 34.5 percent

decrease in blood glucose compared to 53.1 percent for the positive-control rats (group 1). The rats maintained on a thiamine-free diet (group 2) showed a 51.6 percent average decrease in blood glucose, while the alloxan treated, thiamine-deficient rats (group 4) showed a decrease of 35.2 percent and the cortisone-treated rats (group 6) a decrease of only 33.0 percent.

Conclusions

1. A deficiency of thiamine reduced food intake and body weight, and markedly reduced the efficiency of food utilization, as observed in previous experiments. Alloxan administration to rats fed a thiamine-adequate diet slightly reduced the efficiency of food utilization while cortisone decreased it markedly. No aggravated effects were noted in thiamine-deficient rats given alloxan, but when cortisone was given to thiamine-deficient rats an enhancement of the deficiency symptoms was observed.

2. Blood glucose was increased slightly or not at all in thiamine-deficient rats given either alloxan or cortisone, as observed in previous experiments, while in thiamine-adequate rats a significant increase in blood glucose was observed. It was reported previously by Lowry et al. (1945) that a reduction in glucosuria was observed in diabetic rats submitted to a 16-day thiamine-depletion period, and that administration of thiamine restored the glucosuria. This and the previous experiment confirms these observations. In the cortisone-treated, thiamine-deficient rats, hyperglycemia did not follow the

increased nitrogen excretion by these rats. It is possible that the decreased food intake hid any manifestation of gluconeogenesis from protein.

3. The increase of urinary nitrogen in alloxan-treated rats fed a thiamine-adequate diet, and in cortisone-treated rats has already been noted in previous experiments. In the former this was due to the absence or reduction in the amount of insulin available, which has an anti-catabolic effect on protein. In the latter, this was due to the well known catabolic effect of cortisone on protein metabolism. In thiamine-deficient rats, administration of alloxan did not result in any increase in urinary nitrogen. This effect is presumably due to the reduced food consumption associated with thiamine deficiency.

4. Insulin was less effective in lowering blood glucose in thiamine-deficient rats than in thiamine-adequate rats, with the possible exception of the thiamine-adequate controls. This suggests that thiamine is necessary for the full action of insulin, and is in agreement with the view that there is an increased resistance to insulin in B vitamin deficiencies (Samuels 1948). Burke et al. (1938) had previously reported a reduced effectiveness of insulin in the absence of thiamine.

The cortisone-treated groups showed an increased resistance to insulin both on the thiamine-adequate and deficient diets, confirming the findings of Ingle (1945) and Feng (1954) who showed that cortisone increased insulin resistance. Feng (1954) reported that insulin was twice as effective in reducing blood sugar in cortisone-treated rats

fed a diet abundant in vitamin B_{12} than on a diet deficient in vitamin B_{12}. However, since the same dose of insulin was not given to both groups in this experiment, it can only be assumed that insulin was more effective in the thiamine-treated group.

TABLE X

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN ON BODY WEIGHT, FOOD INTAKE AND EFFICIENCY OF FOOD UTILIZATION

Group	Treatment	Initial* Body Weight	Final Body Weight	Avg. Food Intake	
				Total	Per gm. Gain Body Weight
		gm.	gm.	gm.	gm.
1	2 mg. thiamine	197.2±6.9**	244.8±7.3**	193.5	4.06
2	No thiamine	196.1±6.3	155.7±5.6	38.5	-
3	2 mg. thiamine, alloxan	196.9±5.9	215.3±8.9	164.0	8.45
4	No thiamine, alloxan	198.2±7.2	159.1±8.1	42.3	-
5	2 mg. thiamine, cortisone	199.1±6.7	189.6±7.6	169.5	-
6	No thiamine, cortisone	198.0±7.5	139.5±5.8	41.8	-

* Average body weight after thiamine-depletion period.
** Standard error of the mean.

I - 2 mg. thiamine
II - no thiamine
III - 2 mg. thiamine, alloxan
IV - no thiamine, alloxan
V - 2 mg. thiamine, cortisone
VI - no thiamine, cortisone

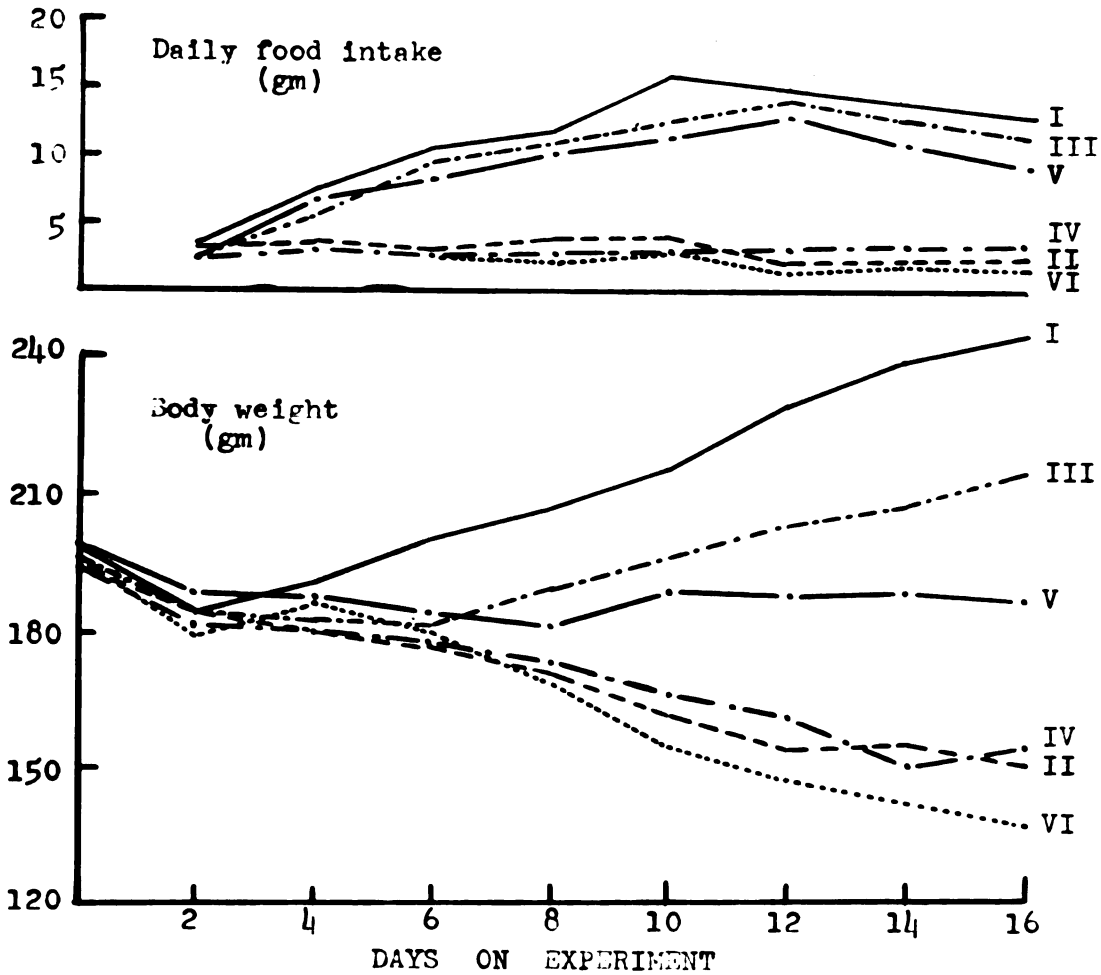


Fig. 5 - Effects of thiamine, cortisone and alloxan on food intake and body weight.

TABLE XI

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN ON BLOOD SUGAR AND URINARY NITROGEN[1]

Group	Treatment	Initial[2] mg.	5 days mg.	10 days mg.	15 days mg.
1	Positive controls				
	Blood glucose	92.6±4.2[3]	115.3±4.7[3]	120.6±4.1[3]	125.2±4.6[3]
	N/rat	171.4	242.6	269.3	276.6
	N/100 gm.	88.6	121.3	115.6	113.4
2	Negative controls				
	Blood glucose	94.8±5.5	93.7±3.4	92.9±3.1	94.1±4.5
	N/rat	151.7	149.6	146.0	139.9
	N/100 gm.	83.4	87.5	94.2	90.3
3	Adeq. thiamine Alloxan				
	Blood glucose	91.7±4.6	396.8±23.5	389.3±21.2	381.6±23.7
	N/rat	154.1	234.0	310.9	325.6
	N/100 gm.	85.6	129.3	153.2	162.8
4	No thiamine Alloxan				
	Blood glucose	93.9±4.7	103.9±3.6	112.0±5.7	116.2±5.8
	N/rat	149.5	153.2	154.1	141.6
	N/100 gm.	81.3	92.3	94.6	89.1
5	Adeq. thiamine Cortisone				
	Blood glucose	96.3±4.9	165.5±6.8	170.0±5.9	172.6±7.6
	N/rat	164.4	257.0	278.8	281.7
	N/100 gm.	89.4	135.3	146.2	149.1
6	No thiamine Cortisone				
	Blood glucose	87.8±4.1	118.6±3.8	121.3±4.6	121.2±3.9
	N/rat	159.6	211.0	194.4	196.0
	N/100 gm.	86.7	121.3	129.6	133.2

[1] Represents total nitrogen per 24 hours.
[2] At the end of the depletion period.
[3] Standard error of the mean.

TABLE XII

EFFECTS OF INSULIN ON BLOOD GLUCOSE AFTER PRETREATMENT WITH THIAMINE, CORTISONE OR ALLOXAN

Group	Treatment	Blood glucose, mg. percent	
		First Trial	Second Trial
1	2 mg. thiamine		
	Before insulin	117.5±4.2*	119.0±3.9*
	After insulin	55.2±5.6	54.7±4.8
	mg. decrease	62.3	64.3
	Percent decrease	52.1	54.1
2	No thiamine		
	Before insulin	94.3±5.5	91.8±3.4
	After insulin	46.2±6.3	44.0±4.8
	mg. decrease	48.1	47.8
	Percent decrease	51.1	52.1
3	2 mg. thiamine, alloxan		
	Before insulin	246.2±20.3	249.4±21.9
	After insulin	95.0±8.6	93.7± 9.3
	mg. decrease	151.2	155.7
	Percent decrease	61.5	62.4
4	No thiamine, alloxan		
	Before insulin	90.2±4.7	98.1±5.1
	After insulin	60.6±6.3	61.3±6.5
	mg. decrease	29.6	36.8
	Percent decrease	32.9	37.6
5	2 mg. thiamine, cortisone		
	Before insulin	147.5±6.7	164.2±7.2
	After insulin	101.3±7.3	102.5±7.6
	mg. decrease	46.2	61.8
	Percent decrease	31.4	37.7
6	No thiamine, cortisone		
	Before insulin	118.5±3.8	121.2±4.2
	After insulin	81.2±6.3	79.3±5.8
	mg. decrease	37.3	41.9
	Percent decrease	31.5	34.6

* Standard error of the mean.

Experiment VI and VII - Glucose utilization in normal, alloxan-diabetic and cortisone-treated rats as influenced by thiamine.

Purpose

Styron et al. (1942) demonstrated that glucose tolerance was lower in thiamine-deficient than in thiamine-adequate rats. These experiments were designed to observe the response of alloxan-diabetic and cortisone-treated rats to glucose administration, when maintained on diets either deficient or adequate in thiamine.

Methods

The rats from Experiment V were used in this study. After 20 days of the treatment outlined in the previous experiment, the rats were starved for 12 hours and blood samples were taken for initial glucose measurements. A total of 750.0 mg. of glucose dissolved in 5 ml. of physiological saline was injected into each rat intraperitoneally. Blood samples were obtained 1 and 2 hours later and glucose was determined as in the previous experiments.

Results of Experiment V (Table XIII and Figure 6)

The results are summarized in Table XIII and Figure 6. All thiamine-deficient rats (groups 2, 4 and 6) showed a lower ability to utilize the injected glucose than the corresponding groups fed on a thiamine-adequate diet (groups 1, 3 and 5). The alloxan-treated rats fed the thiamine-adequate diet (group 3) and thiamine-deficient diet (group 4) showed the highest blood glucose level after the first hour and these

values were only slightly reduced by the second hour. Such a glucose tolerance curve is characteristic of diabetic rats. The percent increase in blood sugar was greater in the thiamine-deficient rats (group 4), indicating that thiamine is essential even in diabetic rats for the limited carbohydrate utilization which takes place. The cortisone-treated, thiamine-deficient rats (group 6) showed as little ability to utilize glucose as the alloxanized rats, whereas the cortisone-treated, thiamine-adequate rats showed much greater ability to utilize glucose. The positive control rats (group 1) showed greater ability to utilize glucose than the negative controls (group 2), although even the latter rats metabolized more glucose than either of the alloxanized or cortisone-treated groups. This is to be expected since diabetes and cortisone both interfere with carbohydrate utilization.

Results of Experiment VII (Table XIV and Figure 7)

The results in this experiment were essentially the same as in Experiment VI and hence require no further comment.

Conclusions

1. These experiments confirm the view that a thiamine deficiency interferes with the utilization of glucose. Cortisone was shown to impair glucose utilization in both thiamine-deficient and adequate rats. However, cortisone impaired glucose utilization more in the thiamine-deficient rats than in the thiamine-adequate rats. This is to be expected

since either cortisone or a thiamine deficiency interferes with glucose metabolism.

2. A lack of insulin, induced by administration of alloxan, produced the greatest inhibition of glucose utilization observed in these experiments, particularly in the thiamine-deficient rats. It appears that thiamine is more essential for carbohydrate utilization than insulin, since some degree of glucose metabolism can proceed in the absence of insulin but not in the complete absence of thiamine. Of course, none of the rats in this study were completely lacking in thiamine.

3. From these experiments it can be concluded that in the absence of insulin or following the administration of large doses of cortisone, there is still a need for thiamine to carry on at least a minimal degree of carbohydrate metabolism. Essentially the same has been shown to be true for vitamin B_{12} (Feng, 1954) under similar circumstances and the same probably applies to other vitamins which assist in the metabolism and utilization of carbohydrate. More of these vitamins are needed for the maximum effectiveness of insulin and to overcome certain catabolic effects of large doses of cortisone. Apparently more of these vitamins are also needed during adrenal cortical insufficiency. Dumm and Ralli (1948, 1953) and Meites (1953) showed that administration of large doses of B vitamins to adrenalectomized rats greatly prolonged life and induced some degree of body growth. It is possible that an increased intake of B vitamins would also be beneficial in diabetes, since it would insure at least a minimal degree of carbohydrate metabolism and utilization.

TABLE XIII

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN
ON GLUCOSE TOLERANCE TEST

Group	Treatment	Blood Glucose, mg. Percent		
		Initial*	1 Hour	2 Hours
1	2 mg. thiamine	117.5±4.7**	225.5±6.8**	130.6±6.2**
	% increase	--	83.4	11.1
2	No thiamine	92.5±2.4	254.3±7.2	204.3±6.1
	% increase	--	174.1	120.8
3	2 mg. thiamine, alloxan	241.8±22.3	641.3±35.8	588.7±28.9
	% increase	--	165.2	143.4
4	No thiamine, alloxan	96.3±3.6	379.4±5.9	366.3±6.3
	% increase	--	293.9	280.3
5	2 mg. thiamine, cortisone	157.5±6.9	348.7±17.3	257.5±12.3
	% increase	--	121.4	63.5
6	No thiamine, cortisone	114.5±3.7	295.0±7.5	255.8±7.2
	% increase		157.6	123.4

* After 12 hours fasting.
** Standard error of the mean.

I - 2 mg. thiamine
II - no thiamine
III - 2 mg. thiamine, alloxan
IV - no thiamine, alloxan
V - 2 mg. thiamine, cortisone
VI - no thiamine, cortisone

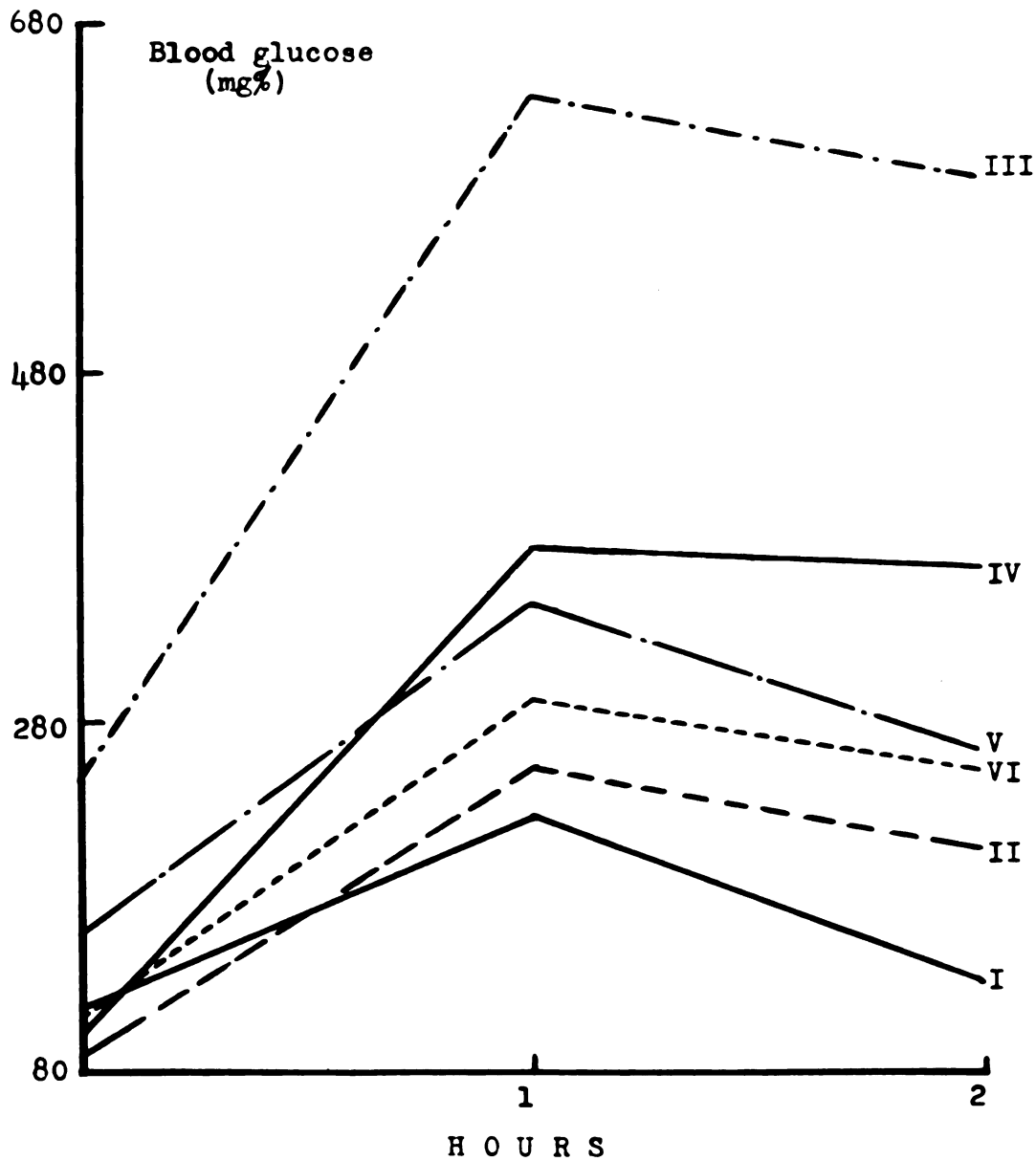


Fig. 6 - Glucose tolerance curves in normal, alloxanized and cortisone-treated rats as influenced by thiamine.

TABLE XIV

EFFECTS OF THIAMINE, CORTISONE AND ALLOXAN ON GLUCOSE TOLERANCE TEST

Group	Treatment	Blood Glucose, mg. Percent		
		Initial*	1 Hour	2 Hours
1	2 mg. thiamine	119.8±4.1**	214.5±7.6**	125.0±6.1**
	% increase	--	79.05	12.7
2	No thiamine	96.2±	258.5±8.8	201.4±6.9
	% increase	--	168.5	109.3
3	2 mg. thiamine, alloxan	248.1±23.1	695.0±31.4	579.1±30.6
	% increase	--	180.1	133.4
4	No thiamine, alloxan	99.4±2.9	393.1±7.8	361.8±7.6
	% increase	--	295.4	263.9
5	2 mg. thiamine, cortisone	154.4±7.1	359.1±19.3	242.5±18.1
	% increase	--	132.5	57.1
6	No thiamine, cortisone	120.0±4.2	308.8±8.6	273.1±9.3
	% increase	--	157.2	127.5

* After 12 hours fasting.
** Standard error of the mean.

I - 2 mg. thiamine
II - no thiamine
III - 2 mg. thiamine, alloxan
IV - no thiamine, alloxan
V - 2 mg. thiamine, cortisone
VI - no thiamine, cortisone

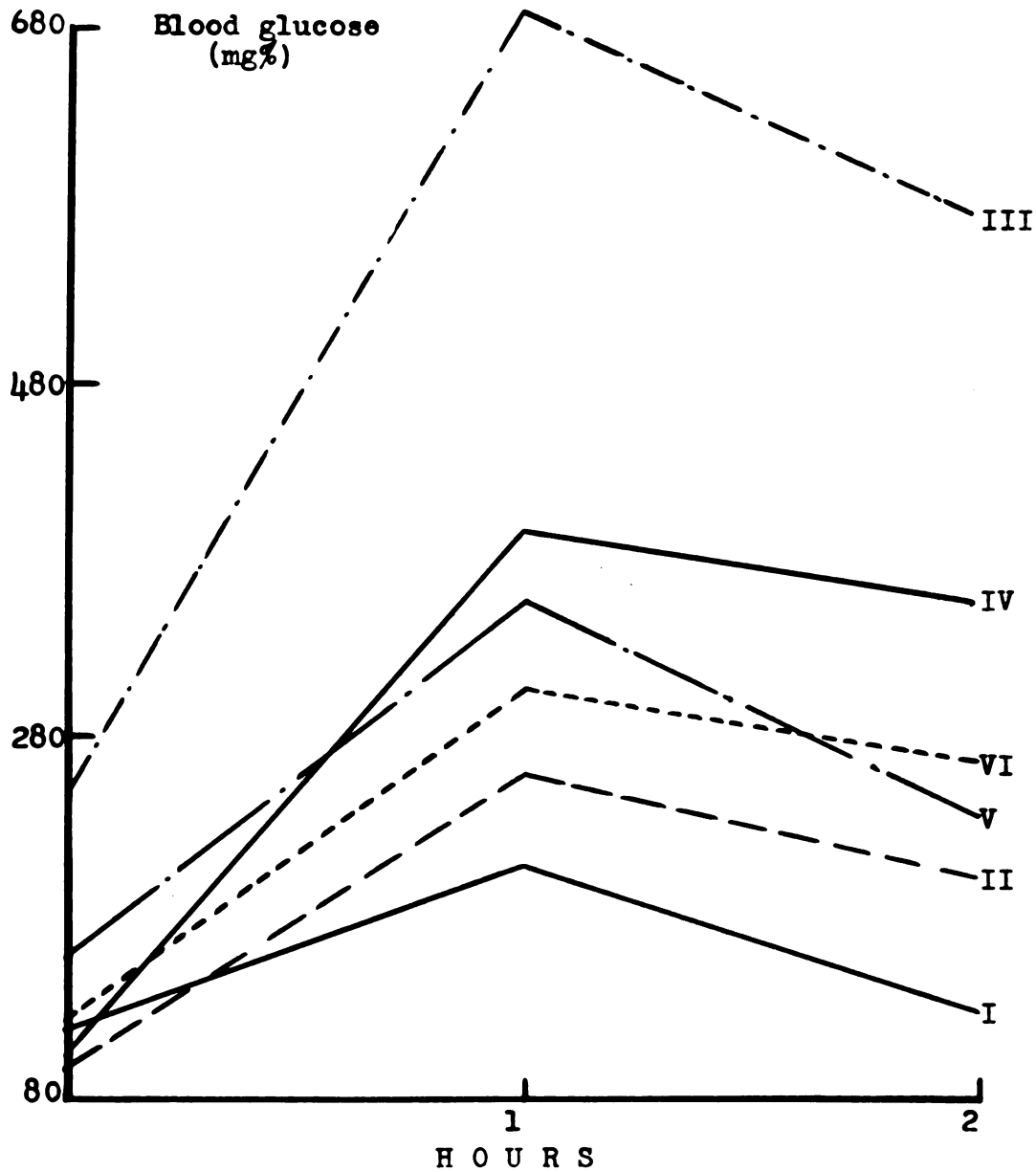


Fig. 7 - Glucose tolerance curves in normal, alloxanized and cortisone-treated rats as influenced by thiamine.

DISCUSSION

A large dose of cortisone injected into young rats fed a diet just adequate in thiamine completely inhibited further body growth, depressed appetite slightly and considerably reduced the efficiency of food utilization. However, when five times or more than the normal requirement for thiamine was fed, it partially counteracted the inhibitory effects of cortisone on body growth and efficiency of food utilization. It was observed that on a thiamine-deficient diet, cortisone further aggravated the symptoms of thiamine-deficiency but did not do so in rats fed 40 mg. of thiamine per kilo of diet and permitted only a very limited food intake.

Alloxan did not appear to markedly alter growth in either thiamine-deficient or adequate rats. After administration of alloxan to thiamine-adequate rats, body weight was decreased during the first 4 to 5 days but was partially recovered during the remainder of the experiment. The efficiency of food utilization was lower in these rats but appetite was not depressed. Thus the requirement for thiamine does not appear to be increased in alloxanized rats. Lowry et al. (1945) also concluded that there was no increase in the requirements for this vitamin in alloxan-diabetic rats. Charalampous et al. (1948) similarly reported that the requirement for riboflavin was not increased in alloxan-diabetic rats. Feng (1954) found that alloxan-diabetes reduced body

growth and the food/gain ratio of rats on a vitamin B_{12} deficient but not on a vitamin B_{12}-supplemented diet.

A deficiency of thiamine has been reported to produce enlargement of certain organs such as the adrenals, heart and kidney (Dunn et al. 1947; Pecora, 1953) and atrophy of the thymus, testes and seminal vesicles (Skelton, 1950). Cortisone also, when given in high doses has been shown to induce atrophy of the thymus and adrenals (Antopol, 1950; Meites, 1951), testes and seminal vesicles (Antopol, 1950).

In this study it was observed that the kidney and the heart were enlarged in thiamine-deficient rats and in cortisone-treated rats fed either adequate or thiamine-deficient diets. The increase was more pronounced however in the thiamine-deficient rats. This action of cortisone may have been due in part to its limited salt and water retaining action. It has also been attributed to a negative nitrogen balance (Skelton, 1950). Walter et al. (1939) have presented evidence that changes in the weight of the kidney and heart are determined mainly by changes in the amount of work done by these organs. With continued starvation and consequent loss of weight the burden of the heart and kidneys may be reduced, permitting loss of weight of these organs. The functional demands on the kidneys and heart however, are closely related to the rate of metabolism. Since the metabolic rate and surface area of an animal diminish proportionally less than its body weight, the functional burden of the kidneys and heart and consequently the weights of these organs may diminish proportionally less than body weight.

The adrenals were greatly enlarged in the thiamine-deficient rats, whether or not they received cortisone. A thiamine-deficiency and the concomittant inanition constitute a stress, and it has been demonstrated by Selye (1937) that in any stressing situation the adrenals are stimulated by the adrenocorticotrophic hormone of the anterior pituitary gland. A dose of 1 mg. of cortisone daily did not completely overcome the enlargement of the adrenals in the thiamine-deficient rats. Cortisone however, reduced the weight of the adrenals of thiamine-adequate rats.

The thymus gland was reduced in both cortisone-treated and in thiamine-deficient rats, with the greater reduction occurring in the latter. A decrease in size of the thymus can be induced directly by cortisone (Meites, 1951) or indirectly by prolonged inanition (Selye, 1937). Deane et al. (1947) reported that the involution of the thymus was due to stimulation of the adrenal cortex by ACTH, resulting in increased secretion of corticosterone-like hormones. In pair-feeding experiments, it was found that the stimulation of the zona fasciculata occurred earlier in thiamine-deficiency than with a comparable degree of inanition (Deane et al. 1947). Meites (1951, 1952, 1953) found that a large intake of vitamin B_{12} counteracted the effects of large doses of cortisone on thymus involution. In this study it was found that large doses of thiamine produced no significant counteraction of the inhibitory effects of cortisone on thymus weight.

The weight of the testes was not significantly affected by cortisone at any level of thiamine intake but was reduced in size when

thiamine was absent from the diet. Thiamine may have exerted some beneficial effect on the size of the testes which was unrelated to food intake, since the testes of the limited-food rats given thiamine were larger than in the thiamine-deficient rats.

The size of the seminal vesicles was about the same in the cortisone-treated rats fed 2 mg. of thiamine per kilo of diet and in the control rats which received the same amount of thiamine. This is in disagreement with the findings of Antopol (1950) who reported atrophy of the testes and seminal vesicles in cortisone-treated mice. In the cortisone-treated rats fed higher levels of thiamine, the seminal vesicles were increased in weight but this appeared to be due primarily to increased food intake and not to cortisone. A thiamine-deficiency alone induced a decrease in the weight of the seminal vesicles, confirming the observations of Skelton (1950). He considered the failure of development of sex organs in thiamine-deficiency to be due to decreased production of pituitary gonadotrophic hormone.

Administration of large doses of cortisone to thiamine-deficient rats and guinea pigs was unable to or only slightly increased blood glucose, but it was able to produce a pronounced hyperglycemia in thiamine-adequate animals. Protein catabolism was slightly increased in the former rats as shown by the small increase in urinary nitrogen excretion. Alloxan also was unable to induce any significant hyperglycemia or increase of urinary nitrogen in thiamine-deficient rats. Similarly, Lowry et al. (1945) observed that after a thiamine depletion

period of 16 days, glucosuria in alloxan-diabetic rats was significantly decreased. After injecting 50 to 200 mcg. of thiamine the excretion of glucose in the urine increased consistently. Charalampous et al. (1948) made similar observation in alloxan-diabetic rats fed a riboflavin-deficient diet. These authors observed a marked decrease in food intake on the deficient diet, and this probably accounted to a large degree for the decrease in the excretion of urinary glucose. It appears probable therefore, that food intake played a paramount role in the diabetogenic response to cortisone and alloxan in the conditions under which these experiments were carried out.

Apparently thiamine itself is partially responsible for hyperglycemia in alloxan-diabetic rats, since on a limited-food intake in which large amounts of thiamine are fed, there is a consistent hyperglycemia and increased urinary nitrogen excretion. This contrasts with the results noted in the cortisone-treated, limited-food rats given thiamine, since in these rats there was no pronounced hyperglycemia or increase in urinary nitrogen. Thus, reduced food consumption rather than thiamine deficiency seems to be responsible for the comparative absence of the diabetogenic response to cortisone in these rats.

Feng (1954) found that 1 to 4 mg. of cortisone acetate given daily to vitamin B_{12}-deficient rats induced a progressive increase in urinary nitrogen and increased blood glucose and glucosuria to greater levels than in rats fed on a diet containing 200 mcg. of vitamin B_{12} per kilo of diet. The cortisone-treated rats on vitamin B_{12}-deficient diet ate

half as much food as the rats on 200 mcg. of vitamin B_{12} per kilo of diet, but despite this the former rats showed values for blood glucose almost double that of the latter animals. Thus the diabetogenic action of cortisone is not influenced in the same way by thiamine and vitamin B_{12}. Feng (1954) also found that alloxan-diabetic rats showed higher levels of blood glucose when fed a vitamin B_{12}-adequate diet as compared to a vitamin B_{12}-deficient diet. This is similar to the results observed in the present experiment with thiamine and cortisone.

The hyperglycemia induced by cortisone in this study was considerably lower than that reported by Feng (1954). The diets used may have accounted at least in part for these differences. In the present study a semi-synthetic diet containing 62 percent glucose and 24 percent casein was employed. Long et al. (1940) and Engle (1949) noted that the administration of large amounts of carbohydrate to cortisone-treated rats prevented the protein catabolic action of cortisone, and it is therefore possible that the readily available glucose in the diet reduced gluconeogenesis in these experiments.

In the thiamine-deficient rats, glucose utilization was reduced and insulin resistance was increased. This is in agreement with the previous reports of Burke et al. (1938) and Styron et al. (1942) who showed that rats maintained on a thiamine-free diet had less ability to utilize injected glucose and showed increased resistance to insulin. The administration of thiamine decreased the hypoglycemic action of insulin and increased glucose utilization, as indicated by increase in

body weight. Cortisone-treated rats also showed increased resistance to insulin as had been noted previously by others (Grattan et al. 1940).

It was the purpose of this thesis to determine the nature of and the possible relationships between the actions of thiamine, cortisone and insulin on body growth, carbohydrate and protein metabolism. Apparently such relationships exist. Cortisone has been shown to stimulate pancreatic islet function in rats (Baker et al. 1952) and guinea pigs (Hausberger et al. 1953), and thiamine has been demonstrated to be necessary for maximum insulin action. Cortisone increases gluconeogenesis from protein but at the same time interferes with the utilization of glucose by insulin (Ingle et al, 1945). Insulin is essential for the conversion of glucose into energy, glycogen and fat (Long, 1954). Thiamine is essential for the intermediate metabolism of glucose. Of all the factors involved in carbohydrate metabolism only the site of action of thiamine is known. Thiamine apparently can function to a limited extent even in the absence of the pancreas (Soskin et al. 1952), indicating that insulin and also probably cortisone influence the rate of the reaction rather than the reaction itself. However, the ability of cortisone and insulin to influence carbohydrate metabolism depends in part on the level of thiamine and other B vitamins concerned with carbohydrate metabolism.

Does cortisone increase the requirements for thiamine in the young rat or guinea pig? Draper and Johnson (1953) reported that neither pyridoxine nor riboflavin metabolism was influenced by cortisone in

the rat. They found that cortisone did not affect the survival time of young rats or the excretion of pyridoxine in the urine on a pyridoxine-deficient diet. Likewise, no effect was observed on the excretion of riboflavin or the riboflavin content of the liver on a riboflavin-adequate diet. Dhyse et al. (1953) found no change in the excretion of pantothenic acid, biotin, riboflavin, niacin, pyridoxine or folic acid in the rat liver following either adrenalectomy or cortisone injection on a diet adequate in these vitamins.

The above reports appear to cast some doubt as to whether large doses of cortisone can actually alter requirements for thiamine in the young rat, although neither group of investigators worked with thiamine. To the writer, it appears logical that large doses of cortisone should alter the metabolism of the vitamins concerned with carbohydrate metatolism since cortisone interferes with carbohydrate utilization (Ingle et al. 1945). The vitamins essential for carbohydrate metabolism should therefore be eliminated more rapidly through the kidneys. It will be noted that in the work of Draper et al. (1953) and Dhyse et al. (1953), the only vitamins directly concerned with carbohydrate metabolism are riboflavin, niacin and pantothenic acid and these were fed in more than adequate quantities in their experiments. On the other hand, Wahlstrom and Johnson (1951), Chow (1954) and Feng (1954) have demonstrated that on a vitamin B_{12}-deficient diet, large dose or cortisone increased the urinary excretion of vitamin B_{12}. It would have been worth-while to study the metabolism of thiamine as influenced by large doses of cortisone.

The results of the present study did show however, that large doses of cortisone aggravated the condition of young rats on a thiamine-deficient diet and that this could be at least partially counteracted by feeding large amounts of thiamine.

If large doses of cortisone interfere with the utilization of glucose in the body, and increase urinary losses of vitamin B_{12} and possibly thiamine, how can it be claimed that requirements for these vitamins are increased? Evidence has already been presented elsewhere in this thesis that large doses of cortisone increases pancreatic islet function (Baker et al. 1952), and that insulin increases requirements for thiamine (Samuels, 1948 and this thesis) and vitamin B_{12} (Feng, 1954). It is suggested that a condition is produced by large doses of cortisone whereby at one and the same time there is both a lesser and greater requirement for the above vitamins. Supplementation of the diet with large amounts of vitamin B_{12} or thiamine are beneficial, apparently because they increase the action of insulin. Whatever the barrier which cortisone places in the way of insulin action, large amounts of these vitamins help overcome this barrier. This is the best hypothesis which the writer can present at this time to explain the interactions between large doses of cortisone, insulin and thiamine observed in this study.

SUMMARY

1. When young rats were maintained on a thiamine-free diet, symptoms of thiamine deficiency developed within 15 to 20 days. Supplementation of the diet with 2 mg. of thiamine per kilo of diet increased appetite and body weight gains, slightly increased blood sugar and greatly increased glucose tolerance.

2. When 4 mg. of cortisone acetate daily were injected into thiamine-deficient rats, there was a slight increase in the excretion of urinary nitrogen, a slight or no increase in blood glucose, decreased glucose tolerance, reduction in body weight gains and reduced appetite. When 2 mg. of thiamine per kilo of diet (or higher levels of thiamine) were fed to cortisone-treated rats and they were allowed to eat ad libitum, urinary nitrogen increased greatly, blood glucose increased moderately, glucose tolerance was partially improved and body weight was maintained at the same initial level or was slightly increased.

3. Thiamine at high levels, fed to rats on a limited-food intake, largely prevented the development of thiamine-deficiency symptoms but was unable to increase the blood glucose of cortisone-treated rats. It slightly increased urinary nitrogen excretion. It is concluded that large doses of thiamine, greater than normal requirements for growing rats, can partially counteract the protein catabolic action of cortisone by increasing food consumption and increasing the availability and utilization of carbohydrate by the organism.

4. Cortisone partially interferred with the favorable action of large doses of thiamine on the efficiency of food utilization for body growth. Hyperglycemia, glucosuria, increased nitrogen excretion and increased insulin resistance were noted, and therefore less carbohydrate was available to exert a "sparing action" on protein for transformation into body weight gains.

5. (a) When young rats were fed a thiamine-free diet, the weights of the kidneys, heart and adrenals were increased and the weights of the thymus and seminal vesicles were greatly decreased. When 1 mg. daily of cortisone acetate was injected into thiamine-deficient rats, a still greater increase in the weight of the kidneys and heart was noted, and a slight increase was found in the weight of the testes and adrenals. The low thymus weight was not decreased further by cortisone treatment, while the seminal vesicles weighed twice as much as those of thiamine-deficient rats.

(b) When thiamine was fed to cortisone-injected rats, the kidneys, heart and testes showed a slight increase in weight. The thymus showed less involution, the adrenals were reduced in weight and the seminal vesicles were slightly but not significantly increased in size. The increases in thymus and seminal vesicles weights apparently were not due to thiamine per se but to the concomittant increase in food intake.

6. Alloxan-diabetes did not further reduce the efficiency of food utilization of thiamine-deficient rats or rats on a limited-food intake, but slightly reduced the efficiency of food utilization of thiamine-adequate rats. In the latter there was a consistent increase in blood

glucose and urinary nitrogen, while in the thiamine-deficient rats there was neither an increase in blood glucose nor of urinary nitrogen. Rats on a limited food intake but fed thiamine showed a consistent increase in both blood glucose and urinary nitrogen which decreased progressively as chronic inanition developed. When the treatment of the thiamine-deficient rats was reversed, by administering large amounts of thiamine, there was a pronounced hyperglycemia and an increase in urinary nitrogen excretion. It is concluded that thiamine, by increasing food intake, permits hyperglycemia to develop in alloxan-diabetes.

7. When guinea pigs were maintained on a thiamine-free diet, symptoms of thiamine-deficiency developed within 25 days. Supplementation of their diet with 16 mg. of thiamine per kilo of diet increased appetite and body weight gains. Injections of 5 or 10 mg. of cortisone acetate daily did not appear to reduce body weight significantly in thiamine-deficient guinea pigs in contrast to rats. When cortisone was injected into thiamine-deficient guinea pigs there was no increase in urinary nitrogen or blood glucose, as in rats. When 16 mg. of thiamine or more per kilo of diet were fed to cortisone-treated guinea pigs, only a slight increase in blood glucose was observed with 5 mg. of cortisone injected daily and a consistent increase with 10 mg. of cortisone daily. Cortisone did not increase blood glucose of thiamine-deficient guinea pigs at any level.

8. Insulin was much more effective in reducing blood glucose in normal and alloxanized rats than in cortisone-treated rats maintained

on either a thiamine-deficient or adequate diet. A thiamine-deficient diet reduced the hypoglycemic action of insulin, indicating that thiamine is essential for the maximum action of insulin. The greatest resistance to insulin was found in cortisone-treated rats, confirming the observation that cortisone increases insulin resistance.

9. It is suggested that the over-all effect of large doses of cortisone in young rats, by virtue of its ability to interfere with carbohydrate utilization but at the same time increase the secretion of insulin, is to increase the need for thiamine. The beneficial action of large intake of thiamine in cortisone-treated rats is believed to be brought about by its ability to increase carbohydrate intake and utilization in the presence of hyperinsulinism.

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APPENDIX

1. Blood Glucose Determinations

a. Somogyi-Shaffer-Hartman Method (Hawk et al. 1951)

A volume of 0.2 ml. of blood was drawn from the tail of each rat by a Folin micropipette and was mixed into 5.8 ml. of water in a 25-ml. Erlenmeyer flask. The pipette was then rinsed several times with the lacking water. A volume of one ml. of 1.8 percent zinc sulfate and one ml. of 0.1 N of sodium hydroxide were added and mixed. After shaking, the contents were filtered through No. 1 dry filter paper.

Five ml. of the Shaffer-Hartman copper reagent were measured into a 25 x 250 mm. test tube and 5 ml. of the blood filtrate was mixed into it, shaken and covered with a glass bulb, and placed in a boiling water bath for 15 minutes. It was then cooled, 1 ml. of 5 N sulfuric acid was added, and it was titrated with 0.005 N sodium thiosulfate. Starch was added as an indicator. A blank was run on 5 ml. of the copper reagent after boiling with an equal volume of water. In the calculation the blank titration was subtracted from the titration of the unknown, and this gave the ml. of thiosulfate required for the unknown. For the glucose equivalent, the Table (page 525) in Hawk et al. (1951) was consulted. Since this table applies to the usual 1:10 dilution of blood, and in the present case, a 1:40 dilution was used, the mg. of glucose in 100 ml. of blood given in the table were multiplied by four.

b. Folin and Malmros Method (Hawk et al. 1951)

A volume of 0.1 ml. of blood was drawn from the tail of each rat with a Folin micropipette and transferred to a centrifuge tube

containing 10 ml. of dilute tungstic acid. This was mixed and centrifuged. Four ml. of the water-clear supernatant liquid were transferred to a test tube graduated at 25 ml. To this, 2 ml. of a 0.4 percent potassium ferricyanide solution and 1 ml. of a cyanide-carbonate solution were added. The contents were heated in a boiling water bath for 8 minutes and cooled in running tap water for 2 minutes. Then, 5 ml. of ferric iron solution were added and mixed. Two minutes later, the contents were diluted with water almost to the 25-ml. mark, two drops of alcohol were added to prevent foaming, and water was added exactly to the 25-ml. mark, and mixed. The solution was placed in a Fisher electrophotometer 10 minutes later and read. A green plate filter of 525 mu. wavelength was used. The photometer was initially set to zero density with water.

The preparation of the standard solution of sugar, a stock solution of 1 percent glucose, was made up in saturated benzoic acid. The stock solution was diluted to 0.01 mg. and then to 0.1 mg. per ml. The optical density for each amount was read on the photometer and a standard curve was drawn from these values.

The calculation of blood glucose was as follows:

$$\text{mg. percent glucose} = \frac{\text{density of unknown}}{\text{density of standard}} \times 0.04 \times \frac{10}{4.0} \times \frac{100}{0.1}$$

2. Determination of Total Urinary Nitrogen

Koch and McMeekin Method (Hawk et al. 1951)

One ml. from a 24-hour urine specimen was diluted to 50 ml. and mixed in a volumetric flask. One ml. of the dilute urine was pipetted

into a micro-Kjeldahl flask, and 1 ml. of 50 percent sulfuric acid was added and mixed. The flask was heated over a gas flame for 10 minutes, after which 3 drops of 30 percent hydrogen peroxide were added. The flask was heated for 6 more minutes until all the sulfuric acid fumes disappeared. It was cooled for 30 minutes and diluted to 75 ml. with water. Fifteen ml. of Nessler's reagent were added and the whole was diluted to exactly 100 ml. This was left to stand for 10 minutes and was then read on a Fisher electrophotometer in which a green plate filter of 525 mu. wavelength was used.

For a standard nitrogen preparation, 0.4714 gm. of ammonium sulfate was dissolved in one liter of water together with a few drops of concentrated sulfuric acid as a preservative. This solution contained 1 mg. of nitrogen per 10 ml. It was used in amounts of 0.1 ml. to 1 ml. of the stock solution, and was diluted with 15 ml. of Nessler's reagent and water to 100 ml. The values were read on the photometer and a standard curve was drawn.

The calculations were as follows:

$$\frac{\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{mg. N in the standard} \times \text{urine volume}}{\text{body weight}} =$$

total nitrogen expressed as mg./100 gm. body weight/24-hour urine specimen

3. Semi-synthetic Diet for Rats

Ingredients	Amount (gm/kilo)	Ingredients	Amount (mg./kilo)
Cerelose	620.0	Thiamine HCl	2.0
Casein (vitamin free)	240.0	Riboflavin	5.0
Corn oil	100.0	Pyridoxine	2.0
Mineral mixture No. 4	40.0	Niacin	10.0
Choline Chloride	1.0	Calcium pantothenate	28.0
		2-Me-1,4-naphthoquinone	0.4
		Vitamin B_{12}	0.02
		Vitamin A acetate	2000.0 I.U.
		Vitamin D	250.0 I.U.

4. Semi-synthetic Diet for Guinea Pigs (Reid et al. 1953)

Ingredients	Amount (gm./kilo)	Ingredients	Amount (mg./kilo)
Casein (vitamin free)	300.0	Thiamine HCl	16.00
Corn oil	73.0	Riboflavin	16.00
Sucrose	103.0	Pyridoxine HCl	16.00
Cellophane spangles	150.0	Calcium pantothenate	40.00
Starch (corn)	200.0	Niacin	200.00
Cerelose	78.0	Biotin	0.60
Potassium acetate	25.0	Folic acid	10.00
Magnesium oxide	5.0	Vitamin B_{12}	0.04
Salt Mixture No. 4	60.0	Vitamin A acetate	6.00
Choline chloride	2.0	Vitamin D	0.04
Ascobic acid	2.0	Alpha tocopherol acetate	20.00
Inositol	2.0	2-Me-1,4-naphthoquinone	2.00

Date Due

~~JUN 9 1962~~			