INTERRELATIONSHIP OF EXCESS THIAMINE WITH NIACIN IN THE DIET OF THE ALBINO RAT

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ABSTRACT

INTERRELATIONSHIPS OF EXCESS THIAMINE
WITH NIACIN IN THE DIET OF THE
ALBINO RAT

by Marjorie B. Phillips

The tendency to prescribe massive doses of certain vitamins, either as a dietary adjunct or for therapeutic reasons, along with the popular consumption of vitamin supplements and the enrichment of certain foods have led to an increased consumption of vitamins by the population as a whole.

Newer knowledge of biological functions makes it desirable to review earlier concepts of the fate of the B vitamins in the body and their possible toxic effects when taken in large amounts. Because of the close interrelationship of the B vitamins, the possibility of an imbalance, precipitated by an excess of one of the group, seemed worthy of investigation.

The object of this study was to determine possible harmful effects of large amounts of thiamine alone and in the presence of elevated niacin. The niacin present in the high thiamine diet was increased to the point where the thiamine-niacin ratio of the basal ration was re-established.

Male albino weanling rats were allotted at random to five experimental groups. The control group was fed a basal 6% casein diet: supplements added to the four experimental groups were 0.4% thiamine, 1.0% thiamine, 0.4% thiamine plus 0.5% niacin, or 0.5% niacin. The animals were housed in individual wire-bottomed cages, with food and water provided ad libitum for the two-week experimental period. Records of weight changes and food consumption were made twice weekly.

At the end of the two-week period, the animals were sacrificed by decapitation. Livers were removed and analyzed for fat moisture and nitrogen.

Analysis of variance was carried out on mean values for weekly growth, total growth, liver fat, liver moisture, and liver nitrogen. Studentized range figures were used as measure of significance at the 5% level.

Addition of either 0.4% or 1.0% thiamine to a 6% casein diet depressed the growth rate by approximately 70% of the rate established by control animals. The addition of 0.5% niacin to the excess thiamine diet completely reversed this growth inhibition.

The groups on high thiamine exhibited markedly lower food efficiency ratios than the other three groups. This became apparent in the second week, when the food efficiency of the high-thiamine groups was approximately one-third that of the controls.

Analysis of liver fats revealed no statistical differences amongst any of the five groups. Significantly higher percentages of nitrogen were obtained in livers of rats in the high-thiamine groups. The figures were consistent with the lower growth rates of these animals.

A relationship amongst the B vitamins which may be analagous to that of the essential amino acids is suggested by these data. An interrelationship between thiamine and niacin is suggested by the fact that growth was restored to the control level when the balance between these two vitamins was resumed. To date, the nature of this relationship has appeared to be qualitative, but it is not inconceivable that with further research a quantitative pattern may emerge.

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By

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INTRODUCTION

INTRODUCTION

It has been generally accepted that dietary overdosage of the water-soluble B vitamins produces no harmful effects; that excess, beyond individual need, is excreted in the urine.

While the average North American diet seems unlikely to provide an excessive intake of the B factors, the tendency in recent times to prescribe massive doses of certain vitamins either as a dietary adjunct or in treatment of various diseases could conceivably impose strain on some of the body functions. The popular consumption of vitamin supplements in this country, and of enriched products, both on this continent and in developing areas in Africa, Asia, and Europe, have resulted in a higher level of vitamin intake in the population as a whole.

Newer knowledge of biological functions makes it desirable now to review earlier concepts of the fate of the B vitamins in the body and their possible toxic effects when taken in large amounts. The object of this study was to determine possible harmful effects of large amounts of thiamine when administered in the diet of the rat. Because of the close association of other members within the B group of vitamins, it was decided to study the toxic effects of thiamine in the presence of elevated niacin.

The niacin present in the high thiamine diet was increased to the point where the thiamine-niacin ratio of the basal ration was reestablished.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

DIETARY INTERRELATIONSHIPS

Amino Acid and Vitamin Imbalances

The concept of dietary interrelationships and imbalances is not new. Many studies have been published on amino acid; and amino acid-vitamin and hormone interrelationships. Some of these studies are pertinent to this discussion.

One of the most-widely studied interrelationships has been that of tryptophan and niacin.

As tryptophan is one of the "essential" amino acids, a dietary source is necessary. The amino acid has a dual role within the body: in synthesis of protein, and as a precursor of nicotinic acid.

The tryptophan requirement of the rat for maximum growth was reported by Osterling and Rose (1952) to be approximately 0.15%. When varied from 0.10 to 0.15% in a nicotinic acid-free diet the amino acid produced an average weight gain in direct relationship to the tryptophan increase. When the diet was inadequate in tryptophan, a definite need for nicotinic acid developed, indicating a relationship between these two nutrients.

In an effort to determine some of the factors affecting the need for exogenous niacin, Salmon (1947) observed that the rat had maximum need for nicotinic acid when the diet was high in sugar or low in fat. Diets containing 9% casein provided insufficient tryptophan for niacin requirement and for protein synthesis in the growing rat.

In addition to these exogenous sources, such as the diet and the conversion from tryptophan, niacin may be supplied endogenously. Synthesis of niacin by intestinal bacteria provides certain animals, including the rat, with a source of the vitamin. If the bacterial activity is impaired, this supply of niacin is reduced, and an increased dietary requirement for the vitamin results. This increased requirement can be satisfied directly by the vitamin itself, or indirectly by tryptophan, as a result of the metabolic conversion of the amino acid within the body.

Experiments were conducted by Goldsmith and others (1961) to determine the efficiency of the tryptophan-niacin conversion in man. Fourteen male subjects were maintained in a metabolism ward with controlled diets of low or moderate niacin and tryptophan content. Diets low in these nutrients provided approximately 5 milligrams of niacin and 200 milligrams of tryptophan daily; those containing moderate amounts furnished approximately 10 milligrams of niacin and 1000 milligrams of tryptophan per day.

Urinary excretions of niacin and tryptophan metabolites were determined during a control period and during experimental periods in which the diet was supplemented with 10 to 30 milligrams of nicotinamide or 2 to 6 grams of DL-tryptophan daily. Efficiency of conversion of tryptophan to niacin was calculated by comparison of the excretion of niacin metabolites following administrations of nicotinamide with those from tryptophan administration. The conversion ratio varied considerably among the subjects, but the average was found to be 55.8 milligrams tryptophan to 1 milligram niacin. An average of 3.3% of the tryptophan was converted to niacin.

B-vitamin interrelationships

Human studies

Thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, folinic acid, biotin, and vitamin B_{12} play important roles as cofactors in certain enzyme systems. This would explain why any one member of the B complex should be interrelated with other members of the B group. Reports in the literature have provided evidence of these relationships.

Interrelationships amongst several of the water-soluble vitamins were demonstrated by Sebrell and Butler (1938) in a study on human subjects fed synthetic diets providing all the necessary nutrients. After three months of daily supplements of 30 milligrams of ascorbic acid and 3.3 milligrams of crystalline vitamin B_1, 56% of the

subjects developed symptoms of ariboflavinosis. Administration of 100 milligrams of nicotinic acid aggravated the condition. Clearly an imbalance of the vitamins had been produced; the condition was corrected by riboflavin therapy.

Spies and co-workers (1939) reported that at the Hillman Hospital in Cincinnati the majority of patients who suffered symptoms of nutritional disorders resulting from unbalanced or inadequate diets exhibited signs of more than one deficiency syndrome. Symptoms of thiamine, riboflavin, and niacin deficiencies frequently were present simultaneously in the same patient. Dietary supplements of thiamine tended to delay, retard, or prevent symptoms of beriberi, but not those of pellagra, riboflavin deficiency, or other deficiency states. In pellagrins treated with nicotinic acid and continuing their usual diets, the associated deficiencies of thiamine and riboflavin often became worse.

Similar observations were made by Sydenstricker and others (1940) who noted that ocular lesions typical of ariboflavinosis appeared in hospitalized pellagrins. These symptoms appeared in from two weeks to one month after instigation of nicotinic acid therapy and were alleviated by addition of riboflavin. To avoid effects of more than one deficiency, it was necessary to administer a daily dietary supply of 20 milligrams of thiamine, 300 milligrams of nicotinic acid, 50 milligrams of ascorbic

acid, and 4 millilitres of U.S.P. cod liver oil or 5000 International Units of vitamin A.

Animal studies

Evidence supplied in the clinical literature on imbalances and antagonism in the B vitamins led Richards (1945) to conduct studies on some of these interrelationships in the rat. Definite adverse effects resulted from disturbing the B-vitamin balance. The basal synthetic diet was planned to be approximately equal in calories to a poor human diet containing a large proportion of white bread. Additions of aneurin at low, medium and high levels were made which corresponded to the thiamine contents of different breads. The basal diet contained the vitamin at a level corresponding to that in white unenriched bread; the medium level corresponded to the thiamine content of national wheat meal flour, and the high level was ten times this amount. No marked evidence of untoward effects on growth, arising from the variations in the diet, was observed. Richards submitted that growth, and even reproduction, may not be a suitable criterion of unfavorable effects of an adequate diet; inadequacies might be more manifest in lactating animals. She observed a marked interference in lactation as the level of vitamin B_1 increased. The mortality rate of the nursing rats in the high vitamin B_1 group was high; the few survivors were low in weight and poor in condition. Evidences of other

deficiencies in the mothers were noted, such as skin lesions, alopecia, and numerous body sores, which have been related to riboflavin deficiency. It was the author's contention that excess vitamin B_1 upset the B-vitamin balance, producing deficiency of one or more factors in the B group.

Further evidence of B-vitamin interrelationship was provided by Morrison and Sarett (1959a) who gave male weanling rats otherwise adequate diets but with one B vitamin at a low level in combination with a second B vitamin at low, adequate, or high level. The doses ranged from one twentieth of an adequate dose to 50 times the requirement. As would be expected, deficiencies of thiamine, riboflavin, pyridoxine, and pantothenic acid retarded growth. No influence of high thiamine on weight gain was observed in pyridoxine-, pantothenic acid-, or riboflavin-deficient animals. Administration of a high level of these four B vitamins to animals deficient in the other three vitamins produced no adverse effects as measured by weight gain and food efficiency in any cases except those fed riboflavin. Administration of high quantities of riboflavin, in the presence of low levels of thiamine, pyridoxine, or pantothenic acid resulted in higher mortality rates than did the same level of riboflavin in control animals.

THIAMINE

Historical

In the early years of the twentieth century, shortly after McCollum and Davis (1915) designated vitamins as "fat soluble" and "water soluble," research on the B group was restricted because of difficulty in obtaining sufficient quantities of the purified vitamins. This obstacle was removed in the case of thiamine when Williams and Cline (1936) synthesized the vitamin, thereby providing access to a plentiful, inexpensive supply of vitamin B_1 for therapeutic and investigational use. This opened the door to the field of thiamine research.

Early studies by McHenry (1936) led him to suggest that thiamine exerted a specific effect upon fat metabolism. On a basal 10% casein diet, choline-free, containing vitamins A and D, and daily supplements to the diet of 5 milligrams of vitamin B_1, albino rats showed liver fat deposition of 16.5% as compared to 7.5% in thiamine-deficient control animals. Daily addition of 5 milligrams of choline to the experimental ration reduced the liver fat to a level comparable to that of the control animals. McHenry suggested that the increased liver fat in the vitamin-supplemented animals might be due to the effect of the vitamin upon transport of fat.

Casein was known to have a lipotropic action (Best, et al. 1935). An effort was made to measure the relationship between the protein, thiamine, and choline levels in the diet and the appearance of fatty livers in experimental animals. Engel (1942) fed two groups of rats 18% casein diets to which increasing amounts of from 2 to 10 milligrams of choline were added. When 20 micrograms of thiamine were added, liver fats increased from 5% in control animals to 33%. It required at least 10 milligrams of choline to reduce fat content of livers from thiamine-deficient rats to that of the control animals whose diets contained no added thiamine. The author suggested increased liver fat observed in thiamine-fed animals should be considered a deficiency of choline rather than a harmful effect of thiamine.

In the opinion of Popper and Schaffner, (1957) the administration of thiamine in a choline-deficient diet increased liver fat because the vitamin increased food intake, with a resulting increase in the requirement for choline. This explanation implied an interrelationship between these two food factors.

Metabolism and functions

Plants and a number of microorganisms can synthesize the vitamin. Animal tissues have only an infinitesimal capacity to do so, and the major portion of thiamine requirement for the animal must be supplied either by the oral route, or by microbial synthesis in the gut.

Thiamine is absorbed primarily from the small intestine, although a measurable quantity is absorbed from the large intestine. When thiamine-S^{35} was administered to rats, approximately one-fifth of the vitamin was absorbed from the large, and four-fifths from the small intestine (Gassmann and Ketz, 1961).

Although there is some free thiamine in tissues and blood, the active form in the body is the phosphorylated thiamine pyrophosphate (TPP), composed of a pyrimidine ring and a phosphorylated thiazole ring. According to Ochoa and Peters (1938) the major site of phosphorylation of thiamine is in the liver, and to a lesser extent, brain and muscle. Pigeon intestinal mucosa showed no phosphorylating activity, which led to the conclusion that thiamine was absorbed into and transported by the blood stream for activation in the aforementioned locations.

Phosphorylation is thought to occur at the terminal alcohol group of the thiazole ring, with ATP and magnesium ions necessary factors in the conversion to the coenzyme (Fruton and Simmons, 1959). Neither cytidine nor guanosine could replace adenosine in the nucleoside triphosphate, and of several cations tested, only manganese and magnesium were effective as activators in this reaction (Lewin and Brown, 1961).

The major contribution of thiamine to body function is through its coenzyme activity (Jansen, 1954). In this capacity it is associated with the breakdown of

carbohydrate, protein, and fat. Thiamine pyrophosphate, as cocarboxylase, catalyzes the decarboxylation of pyruvic acid to acetyl CoA, and of a-ketoglutaric acid to succinyl CoA (Banga, et al., 1939). In both these reactions, the conversion proceeds through a series of intermediary steps, utilizing lipoic acid as hydrogen carrier and DPN as hydrogen acceptor.

High thiamine dosage

Excess by injection

Effects of various methods of thiamine administration have been studied in a number of species (Unna, 1954). In the rat, intravenous injection of 250 milligrams per kilogram of body weight caused death due to depression of the respiratory centre. Intravenous injection of thiamine of less than lethal doses produced untoward effects in these animals, such as inhibition of skeletal muscular nerve impulses, and, in large concentrations, inhibition of cholinesterase.

Vitamin shock was produced by Mouriquand and co-workers (1951) in both normal and thiamine-deficient adult pigeons by administering 100 milligrams of thiamine intravenously into the thigh. Symptoms, which appeared almost instantly, were those of profound coma, complete immobility of the feet and wings, and disappearance of the corneal reflex. An oral dose of three times the strength of the intravenous dose produced no harmful effects.

There have been several reports in the clinical literature concerning harmful effects of large doses of thiamine administered parenterally to humans. Steinberg (1938) treated cases of chronic arthritis with large doses of thiamine. The vitamin was given as 800 I.U.[1] orally and 2000 I.U. intravenously per week. Distressing lesions of herpes zoster, irritation of peripheral nerve plates, and spasm of smooth muscle appeared in several of the treated patients. When thiamine therapy was discontinued, the symptoms disappeared.

Untoward effects of 25-milligram intravenous injections of thiamine were recorded by Leitner (1943). The vitamin therapy induced insomnia, headache, dizziness, and palpitation, similar to thyroid overdosage. A second patient, receiving a similar amount of the vitamin to counteract malnutrition, exhibited a typical allergic reaction with edema and itchiness. Relief from these symptoms was experienced with removal of the thiamine treatment.

Excess by ingestion

Reports in the literature of thiamine toxicity as a result of oral ingestion of the vitamin are rare. An extensive investigation was made by Unna and Clark (1942) into effects of feeding excesses of B vitamins to rats deficient in other vitamins of the B group. Three groups

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[1] I. U. = the thiamine activity of 3.0 micrograms of crystalline thiamine hydrochloride.

of rats were given basal rations containing 18% casein and 1% choline chloride, but no B vitamins. Daily supplements of thiamine at 10-microgram and 1-gram levels were supplied to two groups. Symptoms of avitaminosis developed, characteristic of the missing factors, with accompanying retarded growth in all groups. Prolonged administration of thiamine up to 100 days failed to aggravate manifestations of the deficiency states or to produce significant influence on the growth rate of the deficient animals.

Assessment of thiamine and pyridoxine excess on growth and reproduction in rats was made by Morrison and Sarett (1959b). Diets composed of 18% casein, containing 150 micrograms per cent each of thiamine and pyridoxine were supplemented with excess thiamine and pyridoxine at 50 times the levels in the basic diet. These excesses produced no effect on weight gain, reproductive performance, levels of solids, total lipid, or concentrations of riboflavin, pantothenate and vitamin B_{12} in livers after parturition and lactation.

Thiamine toxicity has for the most part been the outcome of administration of the vitamin by injection. Reports of harmful effects of overdosage by the oral route, on the other hand, have generally been associated with syndromes of other B vitamin deficiencies. Thus the concept of an interrelationship between thiamine and other B vitamins has emerged as knowledge of vitamin functions

has been elucidated. To date, the nature of this relationship has appeared to be qualitative, but it is not inconceivable that with further research a quantitative pattern may become evident.

NIACIN

Metabolism and functions

Niacin is available to the body from three sources: the diet, conversion of tryptophan, and synthesis by intestinal microorganisms. As the liver contains more niacin than do other organs, it is the probable storage place of the vitamin (Popper and Schaffner, 1957). While nicotinic acid and nicotinamide are interchangeable in the nutrition of animals, the amide is the form more naturally-occurring (Fruton and Simmonds, 1959). Little is known of the mechanism of conversion of the acid to the amide form.

The main role of nicotinic acid is in oxidation-reduction systems as diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN)[1]. These dehydrogenases alternate between oxidized and reduced states by accepting hydrogen from certain substrates and transferring

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[1]The report of the Enzymes Commission which was presented to the International Union of Biochemistry at the Fifth International Congress of Biochemistry at Moscow in August 1961 recommended the chemical name, nicotinamide-adenine dinucleotide (NAD) be used rather than diphosphopyridine nucleotide (DPN), and NADP rather than TPN.

it to other hydrogen acceptors such as the flavin enzymes (Hundley, 1954). In this way, the vitamin is an integral part of intermediary metabolism.

High niacin dosage

Excess by injection

In an attempt to assess the toxicity of niacin, Unna (1939) administered nicotinic acid or nicotinamide orally or subcutaneously to mice and rats. Death occurred within 12 to 36 hours after subcutaneous injection of 1.8 grams of nicotinamide per kilogram. The lethal dose of nicotinic acid was shown to be twice this amount. Oral administration followed the same pattern, except that the tolerance threshold was higher. The author concluded that nicotinamide was twice as toxic to these animals as nicotinic acid, regardless of the mode of administration.

Janes and Brady (1949) observed a ketogenic effect from niacin injection in normal rats. After five days of fasting, the animals were given one intraperitoneal injection daily of 5, 20, 35, or 50 milligrams of niacin, dissolved in 5 millilitres of physiological saline. Control animals received 5 millilitres of saline minus the vitamin. The treated animals varied in response to the drug, some failing to show ketonuria, but the 35- and 50-milligram levels seemed to be the most ketogenic.

Under similar conditions ketonuria was produced in rats receiving 35 milligrams of niacin in physiological saline (Janes, 1953). Urinalyses on controls and treated animals revealed that niacin raised the excretion of ketone bodies, and increased liver glycogen and liver nitrogen levels.

Excess by ingestion

Rat studies conducted by Handler and Dann (1942) indicated certain toxic effects of excess dietary nicotinic acid and nicotinamide. Test animals received basal 10% casein rations, to which were added 1% supplements of nicotinic acid or nicotinamide. Control animals receiving no supplements of niacin gained weight at the rate of 1.10 grams per day. Nicotinic acid supplements had no effect on growth but did induce elevated liver fat deposition averaging 24.1% as compared to 17.2% in the niacin-deficient controls. Inclusion of 1% nicotinamide almost completely inhibited growth, but reduced liver fat to 8.5%. The authors submitted that nicotinamide, rather than nicotinic acid, was an immediate precursor of the breakdown product trigonelline[1] in rats, and suggested inhibition of rat growth by nicotinamide was due to deprivation of methyl groups by trigonelline[1] synthesis.

Toxicity of niacin in humans seems restricted to a few individual abnormalities, as no generalized untoward

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[1] More recently identified as N-methylnicotinamide

effects have been reported. Acher (1957) reported that a small percentage of human hypercholesterolemia cases, treated with nicotinic acid in doses varying from 1.5 to 6.0 grams per day for periods up to two years, developed severe gastrointestinal disturbances, accompanied by flushing and pruritis, anorexia and vomiting. In the majority of hypercholesterolemia cases, however, the identical treatment resulted in a significant and sustained decrease in concentration of cholesterol, with none of the above-mentioned undesirable effects. These data confirmed results of niacin therapy by earlier workers, and led the author to conclude that administration of nicotinic acid in large amounts to reduce increased concentration of blood lipids "appeared effective, practical, and probably free from serious harmful effects."

Recent studies in this department have indicated that thiamine, when fed at a level of 0.4% in a diet containing 6% casein, resulted in lower growth rates in weanling albino rats, when compared to controls without added thiamine. Because of the close interrelationship of the B vitamins, the possibility of an imbalance, precipitated by excess of one of the B group, seemed worthy of investigation. It was therefore decided to explore the possibilities of an interrelationship between high thiamine intake and that of an elevated level of one other B vitamin, niacin.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Male albino weanling rats of the Sprague-Dawley strain, weighing between 45 and 55 grams, were allotted at random to five experimental groups. Each group was composed of ten animals, with the average weight of any one group not exceeding that of any other by more than one gram. The animals were housed in individual wire-bottomed cages, with food and water provided ad libitum for the two-week experimental period. Records of weight changes and food consumption were made twice weekly.

The basal control diet (I) was made up in the following percentages: vitamin-free casein, 6, sucrose, 84, corn oil[1], 5, Wesson salts, 4, vitamin mixture, 0.25, choline[2], 0.15. The vitamin mixture contained in milligrams per kilogram of ration: vitamin A powder, 25.0 (20,000 I.U. per gram), calciferol, 1.0, thiamine hydrochloride, 4.0, riboflavin, 8.0, niacin, 5.0, pyridoxine, 2.5, calcium pantothenate, 20.0, inositol, 10.0, folic acid, 0.2, vitamin B_{12}, 0.02 (20 milligrams of a 0.1 per cent trituration with mannitol), para-amino benzoic acid, 2.0, menadione, 4.0, sucrose to make 2.5 grams.

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[1] containing 75 milligrams of a-tocopherol.

[2] 1.0 millilitres of 15% choline solution.

The other four experimental diets were prepared with supplements to the basal diet as follows:

Diet II 0.4% thiamine hydrochloride

Diet III 1.0% thiamine hydrochloride

Diet IV 0.4% thiamine hydrochloride and 0.5% niacin

Diet V 0.5% niacin

The weight differences were adjusted with sucrose.

At the end of the two-week experimental period, the animals were sacrificed by decapitation. Livers were removed, rinsed in water, blotted free of excess moisture, weighed, and stored in the frozen state. Prior to analysis, the frozen livers were allowed to thaw for one hour, then were homogenized with water in a Potter-Elvejhem homogenizer. The homogenates were transferred quantitatively to an evaporating dish and evaporated to dryness for twelve hours in a drying oven at 95° centigrade. The residues were weighed and ground in a Wiley mill with a 40-mesh screen. Approximately 0.500-gram samples of the dried, ground livers were weighed to the nearest milligram for fat analysis. The samples were subjected to continuous ether extraction for three hours in a Goldfisch apparatus. Fat was reported as per cent of dry weight of liver. Per cent moisture in liver samples was determined by difference.

The liver residues, following ether extraction, were re-dried to constant weight. Samples weighing 0.250 grams were taken for nitrogen analysis, using the

macro-Kjeldahl procedure. Liver nitrogen data were reported as per cent nitrogen, calculated on the basis of fresh weight of tissue.

Analysis of variance was carried out on mean values for weekly growth, total growth, liver fat, liver moisture, and liver nitrogen. Studentized range figures were used as a measure of significance at the 5% level.

RESULTS

RESULTS

Average growth figures for the animals are recorded in Table 1. During the first week of the experiment no statistically significant difference in rate of growth was observed between any one group and its appropriate control. The apparent decrease in weight gain in group III (1.0% thiamine) as compared to that in the control group (I) was borderline, with respect to statistical significance. As this was marginal statistically, the author considered the difference questionable.

The second week revealed a marked differentiation in weight gain amongst the groups. Animals fed diets containing excess levels of thiamine (groups II and III) grew at a significantly reduced rate compared to the control animals. Increasing the thiamine content of the diet from 0.4% to 1.0% (groups II and III) did not influence the growth rate significantly. Thus the addition of either 0.4% or 1.0% thiamine to a 6% casein diet depressed the growth rate during the second week by approximately 70% of the rate established by control rats. The growth-inhibiting effect of excess thiamine was sufficiently great the second week to maintain the same pattern for the entire two-week period. Addition of 5% niacin to the excess thiamine diet completely reversed this growth inhibition (compare groups IV and II).

There were no statistical differences in the weight gains of the control animals compared with those of the two groups whose diets included excess niacin (groups IV and V). Growth curves of the five groups are illustrated on page 33.

The quantities of food consumed are recorded in Table 2. The rate of weight gain in all groups was fairly consistent with amount of food eaten throughout the experimental period. An exception to this was in group II (0.4% thiamine) in the second week, when the food consumption was equal to that of the controls, but the weight gain was significantly depressed in the vitamin-supplemented animals. The reason for this is not clear.

The ratio of weight gain to food eaten (indicated as "food efficiency") gives a clearer picture of the utilization of the food. From these data it was apparent that the groups on high thiamine (II and III) exhibited lower food efficiency than did the other three groups. This effect was most marked in the second week, when food efficiency of the high thiamine groups was approximately 33% that of the control animals.

Analysis of liver fats revealed no statistical differences amongst any of the groups (Table 3).

Moisture content of livers from rats fed 1.0% thiamine was significantly lower than that of livers from control rats (Table 3). The percentage of liver moisture in rats fed a diet containing 0.4% thiamine was significantly elevated over that of the control. No explanation

is offered of this reversal.

The addition of 0.5% niacin (group IV) to the diet containing 0.4% thiamine (group II) resulted in a reduction of liver moisture to a level comparable with those of groups III and V.

Rats fed diets containing high levels of thiamine (groups II and III) had a higher liver nitrogen content than those fed the remaining three diets (Table 4). The values of 2.4 and 2.5 grams for these groups were significantly higher in comparison with the figure for control animals. The addition of niacin to a diet containing excess thiamine (group IV) lowered liver nitrogen to a level comparable to the controls.

DISCUSSION

DISCUSSION

Retarded growth was a direct outcome of the addition of excess thiamine to a 6% casein diet. Growth depression as a result of this treatment was not conclusive in the first week, but was firmly established in the concluding week of the experiment.

The action of niacin in reversing the growth inhibition is interesting. That this action of niacin was not reflective of a growth-stimulatory effect of niacin alone in this restricted diet is supported by the absence of any effect on growth in rats fed diet V as compared to control rats.

The significantly higher percentages of nitrogen observed in livers of rats fed diets containing high levels of thiamine were consistent with the lower growth rates of these animals. When nitrogen concentration in livers was calculated on the basis of milligrams of liver nitrogen per 10 grams of body weight, virtually identical figures were obtained for all groups (Table 4).

Because thiamine plays an integral part in carbohydrate metabolism, it is conceivable that addition of vitamin B_1 to the extent of 100 times the requirement for normal growth might upset the glycolytic mechanism. Carbohydrate may be metabolized at a faster rate when excess thiamine is present. Malaguzzi-Valerie and co-workers (1950) found that basal metabolic rate in human subjects

increased 20% as a result of large doses of 100 milligrams of aneurin or 1 gram of nicotinic acid per day. Clearly, further investigations into the enzymic alterations induced by excess thiamine and nicotinic acid on the growth pattern of the animal would bear investigation.

A relationship amongst the B vitamins which may be analagous to that of the essential amino acids is suggested by the data presented in this paper. An inter-relationship between thiamine and niacin is indicated by the fact that growth was restored to the control level when the balance between these two vitamins was restored. The addition of an excess of one vitamin may result in an imbalance of one or more of the remaining components in the group of B vitamins. Certainly such a relationship appears to exist, under the conditions of this experiment, between thiamine and niacin. Whether riboflavin or pyridoxine would be just as effective as was niacin in reversing the grwoth inhibition caused by the high doses of thiamine is a question only further work on this problem can answer.

SUMMARY

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Male albino weanling rats were fed low protein experimental diets to investigate possible toxic effects of high thiamine and the effect of the thiamine-niacin ratio on this toxicity. Five diets were supplied, with the following percentage composition:

	Diet I	Diet II	Diet III	Diet IV	Diet V
Casein	6.0	6.0	6.0	6.0	6.0
Sucrose	84.6	84.2	83.6	83.7	84.1
Corn oil	5.0	5.0	5.0	5.0	5.0
Salt mix	4.0	4.0	4.0	4.0	4.0
Vitamin mix	0.25	0.25	0.25	0.25	0.25
Choline	0.15	0.15	0.15	0.15	0.15
Thiamine	-	0.4	1.0	0.4	-
Niacin	-	-	-	0.5	0.5

Compared with control rats, animals maintained on diets with added thiamine at levels of 0.4 and 1.0% showed lowered food intake, increased liver nitrogen, and depressed growth rate. Growth depression was not conclusive the first week, but was firmly established by the second and final week of the experimental period. The inhibition of growth during the second week, due to addition of excess thiamine to a 6% casein diet, was marked, amounting to an approximate 70% decrease below the rate established by control rats.

No differences were observed in levels of liver fat in any of the groups as compared with the controls.

Addition of 0.5% niacin to the diet containing excess (0.4%) thiamine reversed the toxic symptoms.

The possibility of an ideal balance within the group of B vitamins, analagous to that existing amongst the essential amino acids is considered.

TABLES AND FIGURE

TABLE 1

Average growth figures (in grams) for rats fed 6% casein diets with supplements of thiamine and/or niacin

No. of animals	Diet		Weeks		
	No.	Description	1st	2nd	Total
39	I	Basal, 6% casein	6.3	7.5	13.7
30	II	Basal + 0.4% thiamine	5.9	2.3	8.2
10	III	Basal + 1.0% thiamine	3.8	1.9	5.7
18	IV	Basal + 0.4% thiamine + 0.5% niacin	5.7	5.8	11.5
10	V	Basal + 0.5% niacin	8.0	5.1	13.1

TABLE 2

Average food intake of rats fed 6% casein diets with supplements of thiamine and/or niacin

No. of animals	Diet		1st Week		2nd Week		Total	
	No.	Description	Food eaten (grams)	F.E.[1]	Food eaten (grams)	F.E.[1]	Food eaten (grams)	F.E.[1]
39	I	Basal, 6% casein	42.5	14.8	50.7	14.8	93.2	14.7
30	II	Basal + 0.4% thiamine	40.8	14.5	49.7	4.6	90.4	9.1
10	III	Basal + 1.0% thiamine	35.7	10.6	38.0	5.0	73.7	7.7
18	IV	Basal + 0.4% thiamine + 0.5% niacin	43.8	13.0	44.7	13.0	88.6	13.0
10	V	Basal + 0.5% niacin	43.7	18.3	40.3	12.6	84.1	15.6

[1] F.E. (Food Efficiency) = $\frac{\text{Weight Gained}}{\text{Food Eaten}} \times 100$

TABLE 3

Average liver fat and moisture figures for rats fed 6% casein diets with supplements of thiamine and/or niacin

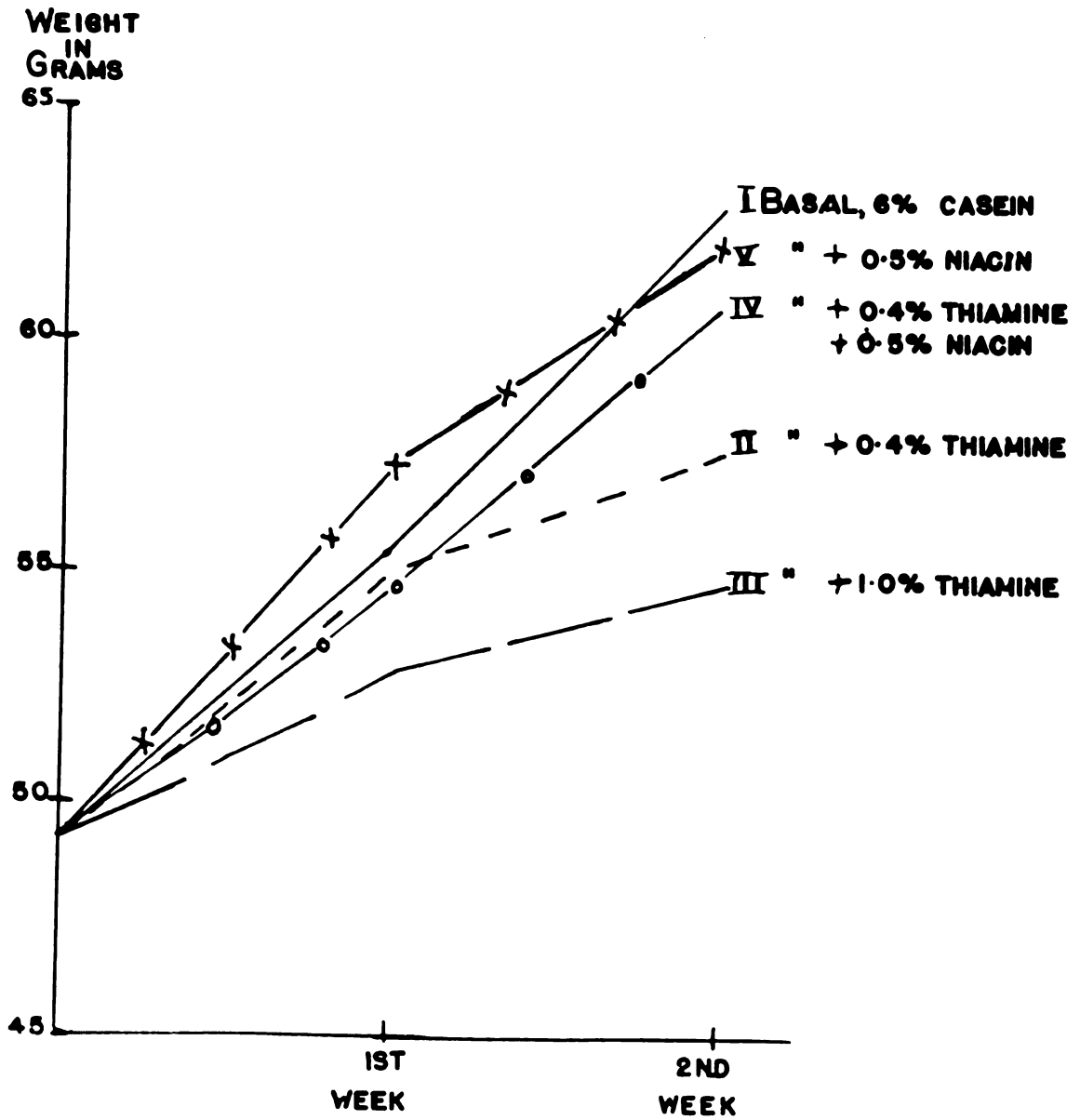
No. of animals	Diet		Per Cent	
	No.	Description	Fat (dry weight)	Moisture
39	I	Basal, 6% casein	12.6	72.3
29	II	Basal + 0.4% thiamine	10.3	74.1
10	III	Basal + 1.0% thiamine	11.3	71.2
18	IV	Basal + 0.4% thiamine + 0.5% niacin	13.6	71.1
9	V	Basal + 0.5% niacin	15.2	70.5

TABLE 4

Liver nitrogen data for rats fed 6% casein diets with supplements of thiamine and/or niacin

No. of animals	Diet		Liver		Liver Nitrogen (mg. per 10g. body wt.)
	No.	Description	Weight (grams)	Nitrogen % fresh wt.	
19	I	Basal, 6% casein	2.99	2.1	10.0
9	II	Basal + 0.4% thiamine	2.39	2.5	10.2
10	III	Basal + 1.0% thiamine	2.34	2.4	10.2
18	IV	Basal + 0.4% thiamine + 0.5% niacin	2.99	2.0	10.1
9	V	Basal + 0.5% niacin	2.89	2.1	10.2

FIGURE 1. GROWTH CURVES OF RATS FED 6% CASEIN DIETS WITH SUPPLEMENTS OF THIAMINE AND/OR NIACIN.



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