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THE INFLUENCE OF DIETARY FAT ON THE
RESPONSE OF THE WEANLING ALBINO RAT
TO EXCESSIVE INTAKE OF
THIAMINE AND NIACIN

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ABSTRACT

THE INFLUENCE OF DIETARY FAT ON THE RESPONSE OF THE WEANLING ALBINO RAT TO EXCESSIVE INTAKE OF THIAMINE AND NIACIN

by Beverly Jane Klooster

Since high potency vitamin preparations are directly available to the consumer, it is possible to consume excess quantities of the water soluble vitamins. This experiment was undertaken to determine if consumption of excess quantities of thiamine or niacin were toxic when added to low or high fat diets.

Male albino weanling rats of the Sprague-Dawley strain were distributed among five experimental groups. Each group was fed (ad libitum) one of the following diets during the six-week experimental period.

Group I	20% casein, 5% fat.
Group II	20% casein, 5% fat, 0.1% thiamine.
Group III	20% casein, 40% fat.
Group IV	20% casein, 40% fat, 0.1% thiamine.
Group V	20% casein, 40% fat, 0.1% niacin.

At weekly intervals five rats from each group were sacrificed. Livers were removed, weighed, and homogenized with water in a Potter-Elvehjem homogenizer. The homogenate was evaporated to dryness, and ground in a Wiley mill with a 40 mesh screen. Fat was determined by ether extraction in the Goldfish apparatus. Nitrogen was determined by the macro-Kjeldahl method.

Standard errors were calculated for each mean, Students "t" test was used as a measure of significance.

Rats fed the high fat diets (groups III, IV, and V) gained less weight than the rats fed the low fat diets (groups I and II). Rats receiving the high fat-high thiamine diet (group IV) were heavier than those rats on the high fat control diet (group III) throughout the experimental period, but especially from the second through the fourth week.

Livers taken from the rats on the high fat diets were significantly smaller than those from rats on the low fat diets regardless of the vitamin composition of the diets.

No significant differences in liver moisture or liver nitrogen were found between any of the groups studied.

Increasing the fat content of the diet from 5% to 40% resulted in the accumulation of an excess quantity of fat in the liver. Control rats fed a diet containing 40% fat had a maximum liver fat level of 19% (at four weeks) as compared with a maximum of 11% (at two weeks) in control rats fed a 5% fat diet. The presence of 0.1% supplementary thiamine in the diet had no sustained effect on liver fat in either the high or low fat diets.

However, a marked increase in liver fat was observed when 0.1% niacin was added to the high fat diet. Rats fed this diet accumulated a maximum of 24% liver fat in one week. The accumulation of fat in the livers of animals fed the high fat-high niacin diet was rapid and sustained. At the end of six weeks, liver fat levels in these animals were still about 20%.

Under the conditions of this experiment, thiamine was relatively non toxic at the 0.1% level regardless of the fat content of the diet. However, a diet containing 40% fat and 0.1% niacin appeared to be toxic to weanling rats, as manifested by the accumulation of fat in the livers of the animals in this group.

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By

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INTRODUCTION

INTRODUCTION

Early investigators discovered some human and animal diseases were caused by a dietary lack of essential factors we now call vitamins. Since that time the minimum daily recommended allowances have been established and re-evaluated (Dann and Cowgill, 1934; Birch, 1939; Brown and Sturtevant, 1949).

Hypervitaminosis is a recent development as the quantity of vitamins naturally occurring in food is small. To consume an excess of vitamin, great quantities of a specific food high in that vitamin would have to be eaten. However, recent isolation and purification of vitamins with the production of potent vitamin preparations, have made hypervitaminosis a possibility.

Vitamins are of two types, fat-soluble and water-soluble. Repeated excess oral doses of the fat-soluble vitamins cause definite deleterious effects in the body (Sebrell and Harris, 1954, Vol. II; Nieman and Klein Obbink, 1954). Excess water soluble vitamins have received little consideration as their solubility resulted in their excretion from the body in the urine. However, high concentrations of these water-soluble vitamins may affect the body in some manner as yet unknown.

We are now concerned about excess oral consumption of water-soluble vitamins because of:

(1) Initiation of a widespread enrichment program. Many cereals and cereal products i.e. flour, bread, and rice, are now enriched with water-soluble vitamins.

(2) High potency vitamin preparations. Vitamin preparations containing high concentrations of water-soluble vitamins, especially niacin and thiamine, are directly available to the consumer. Usually the most potent preparation is advertised as the best preparation. These vitamin pills contain up to 2000 per cent of (or 20 times) the minimum daily requirement.

(3) Indiscriminant use of vitamins by doctors. Stern (1938) stated that intraspinal subarachnoid injection of synthetic vitamin B₁ should be tried in the treatment of cancer, and whenever symptoms of obscure origin failed to respond to the usual methods of treatment.

Since the possibility of consuming excess quantities of water-soluble vitamins now exists, this experiment was devised to determine if excess quantities of thiamine or niacin were toxic when added to low or high fat diets. The male albino weanling rat was used as the experimental animal.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

THIAMINE

Requirement

Early investigators using semi-purified diets adequate with respect to protein, fat, carbohydrates, minerals, and water demonstrated the existence of unknown dietary factors essential for normal growth and development. These factors were subsequently identified and named "vitamins." Since the discovery of thiamine as a dietary essential, the daily requirement was determined experimentally. Animals receiving insufficient quantities of thiamine were polyneuritic and grew at a slower rate than those fed a diet with adequate thiamine. These symptoms of thiamine deficiency became the criteria used to evaluate the thiamine adequacy of experimental diets. Thiamine requirement was expressed in terms of body weight or food consumption. Dann and Cowgill (1934) found female albino rats weighing from 90-240 grams required about 2.1 I.U.¹ thiamine per 100 grams of body weight. When thiamine was related to food intake, 80-100 micrograms per 100 grams of ration were needed for normal growth (Arnold and Elvehjem, 1938).

Earlier, Brodie and MacLeod (1935) related thiamine storage to thiamine intake in an attempt to develop another criterion for analyzing the thiamine adequacy of diets. They found livers and

¹One International Unit of thiamine is equivalent to 3 micrograms.

hearts taken from animals reared on a normal diet contained ten times as much thiamine per gram as skeletal muscle. Kidney contained about one-half and brain one-third the quantity of thiamine present in liver. Only traces of thiamine were found in blood, spleen, and lungs.² Therefore, blood thiamine levels were not indicative of thiamine adequacy.

Muralt (1947) stated that thiamine was essential for the normal function of nervous tissue. As a result, this tissue was one of the last to lose its thiamine content when a state of avitaminosis prevailed.

Maximum tissue storage of thiamine occurred in rats when the dietary intake of thiamine was about 30 I.U. per day. A further increase above this level resulted in no appreciable accumulation of reserves (Leong, 1937a). Leong reported the concentration of thiamine expressed as I.U. per gram tissue was five times greater in liver and heart than in muscle. Of the total quantity of thiamine stored in the body of the "saturated" rat, 50 per cent was found in the muscle while the liver contained 35 per cent.

Further study revealed the biologically active form of thiamine was the phosphorylated derivative. Permeability studies using the phosphorylated and free forms of the vitamin revealed only the free form was readily absorbed by the cell. Therefore,

²Rat bioassay was used to determine the quantity of thiamine present in the tissue.

phosphorylation must have occurred within the cell (Banga, Ochoa, and Peters, 1939). Liver and kidney tissue probably act as storage depots for thiamine because of their high phosphorylating power.

Fate of dietary thiamine

Urinary excretion of thiamine in rats receiving 120 I.U. thiamine per day was about 8 per cent of that ingested (Harris and Leong, 1936). The fate of the thiamine retained was possibly (1) storage in the tissues, (2) excretion in the feces, (3) destruction in the alimentary tract, or (4) conversion to other compounds during digestion (Leong, 1937b).

Storage of thiamine in the body tissues has been mentioned previously. The second possibility concerned the derivation of fecal thiamine. Fecal excretion of thiamine was measured in adult normal rats on a thiamine-free diet following a single test dose of thiamine given as the International Standard Acid Clay. Intake was varied from 0-50 I.U., however, the quantity of fecal thiamine remained relatively constant. This indicated the thiamine was readily absorbed, and fecal thiamine was not derived from food under these conditions. When the thiamine intake was greater than 80 I.U. appreciable amounts were excreted in the feces.

These data indicate fecal thiamine was not normally derived from dietary thiamine. The most probable source of fecal thiamine in the normal rat was bacterial synthesis in the lower part of the intestine (Leong, 1937b).

Function

Early investigators attempted to discover the function of thiamine by analyzing its effect upon the body composition of animals.

Two groups of rats were pair-fed a fat free ration deficient in thiamine. The average body composition of animals receiving thiamine was: water 64.9 per cent, fat 9.4 per cent, nitrogen 18.5 per cent; whereas those not receiving thiamine was: water 67.8 per cent, fat 3.3 per cent, and nitrogen 21.2 per cent (Whipple and Church, 1936). McHenry and Gavin (1938) also found rats fed a diet containing thiamine stored a greater amount of body fat than control animals pair-fed a fat free diet deficient in thiamine. They concluded thiamine was active in the synthesis of fat from carbohydrate.

However this apparent relationship between thiamine and fat synthesis was considered indirect by Stirn, Arnold, and Elvehjem (1939). They stated the thiamine deficient rat may preferentially metabolize fat as opposed to carbohydrate, thus reducing the fat reserves. But, when adequate quantities of thiamine were available, carbohydrate was metabolized normally both for energy and fat synthesis.

Only recently has the function of thiamine been elucidated. As stated previously the active form of thiamine is the phosphorylated form. Insulin was found active in the phosphorylation of thiamine by increasing the concentration of available adenosine triphosphate (ATP). Thiamine pyrophosphate (TPP) is a constituent of cocarboxylase, a coenzyme capable of removing carboxyl groups

from substrates. Many reactions are catalyzed by thiamine pyrophosphate containing enzyme systems; most are involved in the breakdown of pyruvic acid or other α keto acids (Jansen, 1949). Therefore, thiamine functioned directly in carbohydrate metabolism.

In recent studies Williams and Anderson (1959) found thiamine deficiency in the rat caused liver neutral lipid (except cholesterol) to fall rapidly to well below the level present in normal control animals. The sudden reintroduction of thiamine by injection caused total lipids to return to a normal level. Williams and Anderson regarded this as an indication that the actual deposition of lipid in the liver was controlled by thiamine, and that thiamine also functioned to allow repletion of liver lipid at a very rapid rate.

Therefore, it appeared that the function of thiamine was not simply its action in carbohydrate metabolism.

Effects of dietary constituents on thiamine requirement

Carbohydrate

A relationship between dietary carbohydrate and thiamine requirement was suggested by Vorhaus, Williams and Waterman in 1935. Vorhaus stated, the larger the carbohydrate intake the greater the demand for thiamine in the human and lower animals. Consequently a depletion of thiamine would take place more rapidly on a high carbohydrate than on a low carbohydrate diet.

The type of carbohydrate in the diet also influenced the dietary requirement for thiamine, but only when coprophagy was permitted (Guerrant and Dutcher, 1935). Thus the effect of

different types of dietary carbohydrate was indirect, being mediated through its acceleration or depression of bacterial thiamine synthesis. The metabolic thiamine requirement of the rat depended upon the quantity of carbohydrate and was independent of the type of dietary carbohydrate. Thus when coprophagy was permitted, animals consuming a carbohydrate that stimulated thiamine production in the gut actually had two sources of thiamine: thiamine in the diet and thiamine in the consumed feces.

Morgan and Yudkin (1959) believed the thiamine sparing action of sorbitol was due to the immediate absorption of the thiamine produced in greater quantities by the intestinal bacteria when sorbitol was included in the diet. They later discovered prevention of coprophagy caused the rats to lose weight and die with thiamine deficiency symptoms. These observations supported the theory that thiamine synthesized by intestinal flora was not readily absorbed from the gut, if at all.

Therefore, the quantity of carbohydrate in the diet directly influenced the dietary thiamine requirement, whereas the type of carbohydrate was only indirectly related.

Fat

Adult male rats consuming a low thiamine-high fat diet lost less weight than those consuming a low thiamine-low fat diet (Kemmerer and Steenbock, 1933). This seemed to indicate that fat was thiamine sparing. However, analysis of liver and muscle from both groups of animals indicated the concentration of thiamine was the same in both groups. In addition, the liver cocarboxylase level of rats

fed a low thiamine-high fat diet was similar to liver tissue from thiamine deficient polyneuritic rats (Stirn, Arnold, and Elvehjem, 1939).

An explanation for these data was given by Stirn, Arnold and Elvehjem (1939). The thiamine deficient rat may preferentially metabolize fat as opposed to carbohydrate. This allowed the animals on a low thiamine-high fat diet to lose less weight than those on a low thiamine-low fat diet. However, since the dietary intake of thiamine was low, the animal's thiamine stores were depleted.

Salmon and Goodman (1937) analyzed the vitamin B sparing action of various natural fats and synthetic esters. The effectiveness of esters of single fatty acids in alleviating the thiamine deficiency symptoms in rats depended upon the length of the fatty acid carbon chain. Maximum effectiveness was found at the 8 carbon fatty acid with longer or shorter chains showing less effectiveness.

The beneficial effect of fat in a low thiamine diet was an alleviation of metabolic stress on the animal by decreasing the carbohydrate intake.

Protein

The effect of protein on the dietary thiamine content was similar to the effect of fat. Increasing the protein content at the expense of carbohydrate reduced the thiamine requirement.

On a high protein diet (64 per cent casein) the rat required 20 micrograms of thiamine per day, whereas on a high carbohydrate diet (64 per cent sucrose), 33 micrograms per day were required for normal growth (Wainio, 1942).

Likewise, the concentration of thiamine in the carcass and liver was not affected by the quantity of protein in the diet.

Temperature

Diets containing adequate vitamins for the maintenance of rats adapted to temperate coolness contained insufficient thiamine for rats maintained at tropical warmth. This was true because the rats did not consume enough ration at the high temperature to meet the dietary thiamine requirement (Mills, Cottingham, and Taylor, 1948). Using growth after partial depletion as the criterion of adequacy, Hegsted and McPhee (1950) found rats maintained at a low temperature (55° F) required 50 per cent more thiamine per day than rats maintained at a high temperature (78° F). Animals maintained at the lower temperature were consuming 25 per cent more calories which accounted for only part of the increased requirement for thiamine. At 78° F rats required 0.164 - 0.168 milligrams thiamine per 1000 non-fat calories compared with 0.191 - 0.203 milligrams per 1000 non-fat calories at 55° F.

Metabolism

The use of thiamine labeled with S³⁵ allowed Khmelevskii (1959) to trace thiamine metabolism in liver and kidneys of rats. Tests were made at various times after thiamine S³⁵ administration to identify thiamine decomposition products. During the entire period of study the concentration of labeled thiamine decomposition products remained at a low level. Khmelevskii regarded this as an indication that organ tissues did not store the decomposition products. The ratio between the concentration of free thiamine

S^{35} and thiamine S^{35} phosphoesters in liver and kidney showed no fluctuation, indicating an active balance between phosphorylated and free thiamine. Urine contained large quantities of S^{35} labeled decomposition products, while the feces contained small quantities of free thiamine, phosphoesters, and thiamine decomposition products.

Analysis of rabbit urinary and fecal excretions for 4 to 6 24-hour periods following administration of radiothiamine, gave an average total recovery of 77 per cent of the S^{35} after administration by stomach tube, 86 per cent after intramuscular injection, and 54 per cent after intravenous injection. The neutral sulfur fraction of urine contained more than 50 per cent of the recovered S^{35} . The greatest portion of this was recovered in the first 24-hour period following oral administration (Verrett and Cerecedo, 1958). Isolation of the main metabolites during the first 24 hours following oral administration indicated that unchanged thiamine S^{35} and the thiazole S^{35} moiety accounted for approximately 95 per cent of the S^{35} fraction of the urine. Thiamine S^{35} and thiazole S^{35} were excreted in a ratio of 2:1.

Grebennik and Zakharova (1959) found after subcutaneous injection of thiamine S^{35} , 81.73 per cent was in the urine and 7.12 per cent in the feces. Only 0.69 per cent of the S^{35} was excreted in the oxidized form.

These experiments indicated that thiamine in the blood and tissue fluids was easily filtered out in the kidney and appeared in the urine. In addition, a very small portion of the thiamine re-entered the digestive tract to be excreted with the feces.

Toxicity

Many substances required by the body in small quantities become toxic when administered in large quantities. Administration of excess quantities of the fat soluble vitamins results in toxicity.

Vitamin D

Excessive intake of vitamin D was characterized by the development of specific symptoms of hypervitaminosis. The histological changes were similar in children and adults, although fatalities seemed to occur more frequently in the young (Sebrell and Harris, 1954, Vol. II). There was a diffuse calcinosis in the joints, synovial membranes, kidneys, myocardium, pulmonary alveoli, parathyroid glands, large and medium sized arteries, conjunctivae, cornea, and the acid secreting portion of the stomach. The abnormal calcification could be seen grossly as a whitish, chalky material. In the early stages of hypervitaminosis the bones may show accelerated calcification of the provisional zone of calcification with thickening of the periosteum in more advanced cases. In later stages diffuse demineralization of the bones and interference with cartilage growth can be observed.

Vitamin A

Nieman and Klein Obbink (1954) reported excess vitamin A suppressed normal keratinization. Continued excessive intakes caused spontaneous fracture of the tibia and femur. The fractures resulted from acceleration of longitudinal bone growth. Reduced formation of dentine was found as well as hemorrhage and inflammation of mucous membranes. Hypothrombopenia and degenerative effects

in heart, kidney, and liver were noted as well as reduced erythrocyte counts due to a hyperplastic bone marrow. In general, lowering the vitamin intake reversed the toxic effects.

Continued administration of toxic doses of vitamin A to rats during a period of several days caused symptoms of chronic intoxication: weight loss, muscular weakness, loss of hair, soreness and bleeding in the skin, swelling of the palpebrae, exophthalmos, stiffness of the limbs, limping, spontaneous fractures, internal hemorrhages, and eventually death (Rodahl, 1950).

Thiamine

To obtain any toxic effects from thiamine in experimental animals maintained on adequate diets, parenteral doses of several thousand times the daily requirement must be given (Sebrell and Harris, 1954, Vol. III). Since thiamine preparations used for injection contained a preservative, it was necessary to determine which component (thiamine or preservative) was responsible for the toxic effects. Haley and Flesher (1946) found the toxic effects observed in rabbits were due to the thiamine in the preparation. They added that this was not an anaphylactic response caused by hypersensitivity to thiamine.

Excess thiamine in the blood following injection repressed the respiratory center in the medulla, decreasing the oxygen content of the blood causing asphyxial convulsions and cardiac arrhythmia. Death followed due to respiratory arrest. Experimentation with dogs revealed if artificial respiration was maintained after injection of thiamine until the concentration of thiamine in the

blood fell to a tolerable level, spontaneous respiration was resumed (Smith, et al., 1948).

Parenteral doses of thiamine in man also resulted in toxic reactions. Laws (1941) and Schiff (1941) both reported almost fatal responses in man following repeated injection with thiamine hydrochloride. Both patients had received repeated thiamine injections previously without any untoward effects. A hypersensitivity to thiamine may have developed causing an anaphylactic shock when the thiamine injection was given.

An instance of sudden death following intravenous injection of thiamine hydrochloride in man was reported due to anaphylactic shock by Rinegold and Webb (1946).

Stiles (1941) suggested a solution of 5 milligrams thiamine hydrochloride per milliliter be used for a cutaneous sensitivity test prior to injection. However, because of the critical nature of the concentration employed in the intradermal test, a positive test was not conclusive proof of sensitivity to thiamine (Kalz, 1942). In man, no toxic effects have been reported following oral administration of thiamine.

The addition of high levels of thiamine (50 times the adequate level) to a diet deficient in riboflavin, pyridoxine, and pantothenate had no significant influence on weight or food efficiency of rats when compared to a diet with adequate thiamine and deficient in riboflavin, pyridoxine, and pantothenate (Morrison and Sarett, 1959).

In another study, four groups of rats were fed for six months on diets containing 0, 40, 200, or 1000 parts thiamine

β-hydroxyethyl disulfite respectively per one million parts of basal diet. Even at the highest level of intake, weight gain, food intake, food efficiency, and organ weights were not affected. Anatomical and histological examination revealed no differences between groups (Ishikawa et al., 1959). The toxicity of thiamine β-hydroxyethyl disulfite was negligible in rats at a dose over 2500 times the usual intake of human beings in relation to body weight.

Niacin

Requirement

Niacin is also one of the water soluble B vitamins. Niacin requirements first were studied in the same manner as thiamine using growth as a measurement of adequacy. Birch (1939) found that complete absence of niacin from a 20 per cent casein diet for over 150 days was not accompanied by any specific physiological dysfunction in the rat such as was seen in dogs and swine. This did not, however, exclude the possibility that niacin was necessary for normal growth, but indicated dietary niacin was not required. Evidence for synthesis of niacin by the rat was given by Dann and Kohn (1940) and by Dann (1941). Brown and Sturtevant (1949) questioned whether niacin should be considered an essential dietary nutrient for the rat since it can be synthesized by the rat.

Relation to amino acids

Since it was proven that the rat was capable of synthesizing niacin, experimentation was conducted to determine the dietary precursors of niacin. Early experiments relating protein to

niacin requirement led Huff and Perlzweig (1942) to suggest, "the tissues of the rat are capable of synthesizing nicotinic acid from the simplest of ammonium salts, amines, and amino acids, and that any contribution of the intestinal bacteria to this synthesis is of small order of magnitude." Niacin synthesis was actually performed by the tissues of the rat, whereas, thiamine synthesis was a function of intestinal bacteria.

When large quantities of corn were included in a low niacin diet, pellagra resulted, whereas casein in the diet failed to produce pellagra. This action of casein could not be explained by the presence of niacin in casein. Conversion of tryptophan to niacin was responsible for the action of casein. Growth retardation caused by inclusion of 40 per cent corn grits in a 9 per cent casein diet could be counteracted by addition of either 50 milligrams of tryptophan or 1.0 milligram of niacin per 100 grams of diet (Krehl et al., 1945).³ Growing rats maintained on a ration in which tryptophan was the limiting amino acid showed a marked decrease in niacin synthesis. When tryptophan was added to the diet, niacin synthesis increased (Hundley, 1947).

Niacin synthesis from dietary tryptophan followed this scheme as cited by Lushbough and Schweigert (1958) in their review article:

Tryptophan — kynurenine — 3-hydroxy kynurenine — 3-hydroxy anthranilic acid — 1-amino-4-formyl-1, 3-butadiene —
1, 2-dicarboxylic acid — quinolinic acid — nicotinic acid.

³In man, 55.8 mg tryptophan is equivalent to 1 mg niacin. (Goldsmith, Miller, and Unglaub, 1961).

It is well established that tryptophan and niacin are interconvertible.

Function

Niacin occurs in animals mainly as the amide which is generally found in the form of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). DPN was formerly called Coenzyme I and TPN Coenzyme II. These compounds act as hydrogen carriers, undergoing reversible oxidation reduction reactions. In the presence of a dehydrogenase DPN accepts hydrogen forming reduced DPN. Reduced DPN is oxidized by an appropriate flavin nucleotide in the initial reaction of the electron transport system.

Rat liver pyridine nucleotide was below normal when the diet of the adult contained 1.5 milligrams per cent niacin but no tryptophan. However, if tryptophan was fed with 20 milligrams per cent niacin, liver pyridine nucleotide increased to the same level as that found in rats fed tryptophan and 1.5 milligrams per cent niacin. This indicated high levels of niacin had little effect upon liver pyridine nucleotide content (Williams, Feigelson, and Elvehjem, 1950). However, in young rats, dietary niacin had no effect upon liver pyridine nucleotide either in the presence or absence of dietary tryptophan. In young rats dietary tryptophan appeared more important than niacin in maintaining liver pyridine nucleotide levels.

Dietary tryptophan added to the non-protein ration increased liver pyridine nucleotides almost to normal in both young and adult rats. In adult rats, however, niacin had no effect on the liver

pyridine nucleotides, even when fed at very high levels (650 mg per cent). In young rats fed the non-protein rations, high dietary niacin appeared to spare liver pyridine nucleotides. The effect was not as marked as with equivalent levels of dietary tryptophan. (Williams, Feigelson, Shahinian, and Elvehjem, 1951).

Even during severe niacin deficiency in the dog, the DPN level of the blood, kidney cortex, and brain remained constant, whereas the DPN content of liver and muscle was lower (Axelrod, Madden, and Elvehjem, 1939).

The blood DPN level in man may have been increased by ingestion of large amounts of niacin. However, the results were variable depending upon the quantity of niacin ingested. Therefore, in borderline cases of deficiency disease diagnosis could not be made from blood DPN levels.

Metabolism

Six urinary metabolites were demonstrated chromatographically after injection of radioactive nicotinic acid and nicotinamide: N-methylnicotinamide, nicotinuric acid, nicotinic acid, N-methyl-6-pyridone-3-carboxylamide and nicotinamide. Another unknown compound was separated. This compound has not been identified; it was not trigonelline, nicotinic acid N-methyl betaine (Leifer et al., 1951).

Toxicity

Niacin toxicity was studied in rats and dogs by Chen, Rose, and Robbins (1938). Two dogs were fed daily per os two grams of nicotinic acid in a capsule. One dog died after 19 days though the medication was stopped on the twelfth day. Fatty metamorphosis

of the liver was apparent in both animals. Symptoms of toxicity noted prior to death were bloody feces, anorexia, and convulsions. However, Unna (1939) found none of these toxic effects in dogs receiving excess oral doses of niacin which had been neutralized prior to administration. Unna suggested the acidity of the niacin may have been responsible for the toxic reaction observed by Chen et al.

A group of ten six-week old rats was fed one gram of sodium nicotinate per kilogram daily over a period of 40 days. The weight of the experimental animals increased as regularly as the controls. When the rats were sacrificed, gross and microscopic examination showed no pathological changes in heart, lungs, spleen, kidneys, intestinal tract, bone marrow, and genital organs. No symptoms of toxicity were observed.

Acute toxicity was of minor concern to practical vitamin therapy, however, chronic toxicity deserved more attention since vitamins were likely to be taken over a prolonged period and without supervision by a physician. Daily feeding of several hundred times the maintenance doses of niacin over the entire life span of rats, failed to produce gross toxic effects (Molitor, 1942).

Nicotinamide was demonstrated to be several hundred times more toxic than nicotinic acid. Inclusion of 1 per cent nicotinamide in a 10 per cent casein diet almost completely inhibited the growth of rats of both sexes. One per cent nicotinic acid had no effect upon growth but did induce fatty livers. Even 2 per cent nicotinic acid had only a slight effect upon growth (Handler and Dann, 1942). The explanation given for fatty liver induction was a deprivation of methyl groups because of trigonelline synthesis.

However, Leifer et al. (1951) found trigonelline was not a metabolite of niacin metabolism. Sarett (1943) stated N-methyl nicotinamide is closely related to trigonelline and may comprise a large part of what has been measured as trigonelline in earlier work, thus Handler and Dann may have had the correct idea even though they may have incorrectly identified the end product.

The growth inhibition due to nicotinamide was prevented by the administration of methionine and choline plus homocystine, but not by choline, betaine, homocystine or cystine alone. Fatty liver formation induced by nicotinic acid and nicotinamide was prevented by feeding methionine, choline and betaine, but increased when cystine or homocystine were fed (Handler and Dann, 1942).

Brazida and Coulson (1946) found methylation decreased the toxicity of nicotinamide but had little or no apparent influence on the toxicity of nicotinic acid. Therefore, methylation was not the only factor concerned in the toxicity of these compounds. They stated the toxicity of non-methylated compounds appeared to be directly related to structure, rather than a depletion of the body stores of methyl groups in the process of detoxication.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

The percentage composition of the basal diet (1) fed the control group (I) was as follows: sucrose, 71; vitamin-free casein, 20; corn oil, 5; salts W,⁴ 4; vitamin mix, 0.25; and choline chloride, 0.15. The vitamin mixture contained in milligrams per kilogram of ration: Vitamin A, 25.0; calciferol, 1.0; thiamine hydrochloride, 4.0; riboflavin, 8.0; niacin, 5.0; pyridoxine, 2.5; calcium pantothane, 20.0; inositol, 10.0; folic acid, 0.2; menadione, 4.0; vitamin B₁₂, 0.02; biotin, 0.1; p-aminobenzoic acid, 2.0; -tocopherol, 75.0. Diet 2 was identical with diet 1 except that diet 2 contained an additional 0.1 gram thiamine per 100 grams of ration.

Diets 3, 4, and 5 were prepared by increasing the corn oil of the basal diet from 5 per cent to 40 per cent at the expense of sucrose. Diet 3 served as the control diet in the high fat series, with a vitamin mixture identical with that in the basal diet. Diets 4 and 5 contained an additional 0.1 per cent thiamine and 0.1 per cent niacin, respectively.

Male albino weanling rats of the Sprague-Dawley strain were distributed by weight among five experimental groups. Each group was composed of 30 animals with the average weight of any one group not exceeding that of any other by more than one gram.

The animals were housed individually in cages with one-half inch raised wire-mesh bottoms. Food and water were provided ad libitum during the six week experimental period. The room was air conditioned and maintained between 74 and 76° F. The animals were

⁴Wesson modification of Osborne and Mendel salt mixture. Science 75:339, 1932.

weighed weekly.

At weekly intervals five rats from each experimental group were sacrificed by decapitation. Livers were removed, rinsed in water, blotted free of excess moisture and weighed. The livers were then homogenized with water in a Potter-Elvehjem homogenizer, and stored in the frozen state.

Prior to analysis, the frozen homogenates were allowed to thaw for one hour at room temperature, transferred quantitatively to an evaporating dish and evaporated to dryness (12 hours) in a drying oven at 95° C. The dried residues were weighed and ground in a Wiley mill with a 40 mesh screen. One gram samples were weighed for fat extraction in the Goldfish apparatus. About 0.3 gram of the fat extracted liver was weighed for nitrogen determination by the macro-Kjeldahl method.

Standard errors were calculated for each mean, Student's "t" test was used as a measure of significance.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

No effect of high quantities of thiamine upon growth was observed in the low fat series (Table 1). Body weights of rats in group II (high thiamine) were not significantly different from those of rats in group I (control). In the high fat series, no significant difference in growth was observed between group III (control) and group V (high niacin). Throughout the experiment these groups did not vary by more than 3 grams. However, the rats in group IV (high thiamine) were consistently heavier than group III throughout the entire experiment (Figure 1). The two groups were significantly different from the second through the fourth week ($P < 0.01$). After the fourth week, although the animals in group IV were consistently heavier than those in group III, the difference between these two groups was not statistically significant. However, this same trend in weight between groups III and IV was observed in a pilot study. The failure to demonstrate significant differences between these groups after the fourth week of this experiment was probably due to two factors. Each week the size of the population decreased,¹ with a resulting decrease in the number of degrees of freedom with which the two groups could be compared. At the same time, as the animals increased in size, individual variations between animals within a group increased.

¹Five animals were sacrificed from each group at weekly intervals.

Thus, the decreased size of the sample and the increased individual variation resulted in higher standard errors and lower degrees of freedom. Both factors served to reduce the level of significance between means after the fourth week.

Increasing the fat content of the diet from 5 per cent to 40 per cent did decrease the growth rate. Rats fed the high fat diets grew at a significantly slower rate than did rats fed the corresponding low fat diets (compare group I vs III and group II vs IV in Figure 1). This reduced rate of growth in rats fed a high fat diet is in agreement with the findings of Barboriak et al. (1958) and Harrill et al. (1959).

Liver weight data are recorded in Table 2. The livers from rats fed the high fat diets (groups III, IV, and V) were significantly smaller than those from rats fed the low fat diets (groups I and II). The addition of excess thiamine to either the low or the high fat diet and the addition of excess niacin to the high fat diet did not alter this observation. Since the animals on the high fat diets were smaller (Table 1), liver weight was calculated per 100 grams of body weight (Table 3) to determine whether or not the smaller livers observed in the high fat series were just a function of total body weight. Liver weight per 100 grams body weight of animals in the high fat groups (III, IV, and V) was significantly lower than the low fat groups (I and II) after the second week of the experiment. This suggested that the presence of a large quantity of fat in the diet inhibited growth of liver tissue to a greater extent than it retarded body growth. Addition of excess thiamine to either the high fat or low fat series, or the

addition of excess niacin to the high fat series caused no significant change in liver weight expressed in terms of body weight.

The animals on the high fat diets ate a smaller quantity of ration than those on the low fat diets. Food consumption records kept during a two week pilot study indicated that rats on the high fat diets ate an average of 100 grams during the two weeks, whereas, rats on the low fat diets consumed an average of 151 grams.

Liver moisture and liver nitrogen data are presented in Tables 4 and 5 respectively. In the low fat series, addition of 0.1 per cent thiamine to the basal diet did not alter either the moisture or nitrogen content of the livers. Likewise, in the high fat series, excess thiamine or niacin had no effect upon liver moisture or nitrogen.

Liver fat in both control group (I and III) increased during the first two weeks post-weaning (Table 6). The rate at which liver fat was deposited in the low fat control group (I) was virtually identical with the rate of liver fat deposition in the high fat control group (III) for the first three weeks (Figure 2). However, at the fourth week a significant rise ($P < 0.01$) in liver fat was observed in group III. The reason or reasons for this observation are not clear.

The addition of 0.1 per cent thiamine to the basal diet containing either low fat or high fat had no significant effect on liver fat levels. The liver fat curves from animals fed an excess quantity of thiamine (Groups II and IV) roughly followed the liver fat curves of the respective control animals (Figure 2).

However, when rats were fed the high fat basal diet supplemented with 0.1 per cent niacin, the increase in liver fat above the

control animals was significant at the 1 per cent level. The deposition of liver fat in the high niacin-high fat group (V) was particularly rapid during the first week post-weaning. The liver fat level declined slightly during the second week and remained relatively constant thereafter.

Since the composition of livers taken from rats in group V did not differ from control rats with respect to (a) weight of liver, (b) liver moisture, or (c) liver nitrogen, the constituent which was replaced by fat in group V is unknown.

When 0.1 per cent niacin was added to the high fat diet, the balance between fat synthesis, transport, storage, and degradation was in some way disturbed. Which one of these factors, or combination of factors, was responsible for the increased deposition of liver fat in this group has not been determined.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Five groups of 30 male albino weanling rats per group were fed five experimental diets:

Group I	20% casein, 5% fat.
Group II	20% casein, 5% fat, 0.1% thiamine.
Group III	20% casein, 40% fat.
Group IV	20% casein, 40% fat, 0.1% thiamine.
Group V	20% casein, 40% fat, 0.1% niacin.

Five animals from each group were sacrificed weekly during the six-week experimental period.

Rats fed the high fat diets (group III, IV, and V) gained less weight than rats fed the low fat diets (groups I and II). Growth of rats on the high fat-high thiamine diet (group IV) was greater than that of rats on the high fat control diet (group III) throughout the experimental period, but especially from the second through the fourth week.

Livers from rats on the high fat diets were significantly smaller than those on the low fat diets regardless of the vitamin composition of the diets.

No significant differences in liver moisture and liver nitrogen were found between any of the groups studied.

Increasing the fat content of the diet from 5 per cent to 40 per cent resulted in the development of moderately fatty livers. Control rats fed a 40 per cent fat diet had a maximum liver fat level of 19 per cent at 4 weeks, as compared with a maximum of 11 per cent at two weeks in control rats fed a 5 per cent fat diet.

The presence of 0.1 per cent supplementary thiamine in either the high fat or low fat diet had no sustained effect on liver fat.

However, a marked response was observed when 0.1 per cent niacin was added to the high fat diet. Rats fed this diet deposited a maximum liver fat of 24 per cent in one week. The response of the animal to excess quantities of niacin was rapid and sustained. At the end of six weeks, liver fat levels in these animals were still about 20 per cent.

Under the conditions of this experiment, thiamine was relatively non toxic at the 0.1 per cent level regardless of the fat content of the diet. However, a diet containing 40 per cent fat and 0.1 per cent niacin appeared to be toxic to weanling rats, manifested by the appearance of fatty livers in this group.

TABLES

TABLE 1
Weight records of animals¹ on experimental diets

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² gm	Group II ³ gm	Group III ⁴ gm	Group IV ⁵ gm	Group V ⁶ gm
0	48 + 1 ⁷	47 + 1 ⁷	49 + 1 ⁷	48 + 1 ⁷	49 + 1 ⁷
1	75 + 2	71 + 2	67 + 1	68 + 1	66 + 1
2	112 + 2	108 + 2	94 + 1	100 + 1	93 + 2
3	149 + 3	143 + 3	117 + 2	125 + 2	115 + 2
4	186 + 4	174 + 5	145 + 3	158 + 4	147 + 4
5	227 + 8	208 + 8	179 + 4	190 + 4	176 + 5
6	256 + 15	249 + 8	209 + 6	219 + 6	211 + 8

¹Each value is a mean of the entire number of animals remaining in the group. Each group originally consisting of 30 animals 5 of which were sacrificed each week.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 40% fat.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

TABLE 2
Liver weight¹

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² gm	Group II ³ gm	Group III ⁴ gm	Group IV ⁵ gm	Group V ⁶ gm
1	4.49 ± 0.25 ⁷	4.33 ± 0.23 ⁷	3.67 ± 0.37 ⁷	3.79 ± 0.22 ⁷	3.57 ± 0.18 ⁷
2	6.82 ± 0.29	6.43 ± 0.25	5.49 ± 0.14	5.38 ± 0.16	5.24 ± 0.32
3	7.67 ± 0.55	8.30 ± 0.82	5.36 ± 0.32	5.55 ± 0.21	4.98 ± 0.16
4	10.05 ± 0.33	9.76 ± 0.70	6.43 ± 0.11	7.53 ± 0.52	7.01 ± 0.45
5	12.08 ± 0.62	10.54 ± 0.93	7.92 ± 0.54	8.85 ± 0.45	7.62 ± 0.33
6	12.65 ± 0.84	12.10 ± 0.42	8.63 ± 0.31	9.06 ± 0.34	8.50 ± 0.44

¹Each value is a mean of 5 observations, expressed as fresh weight of liver.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 50% fat, 0.1%.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

TABLE 3
Liver weight per 100 grams body weight¹

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² gm/100gm	Group II ³ gm/100gm	Group III ⁴ gm/100gm	Group IV ⁵ gm/100gm	Group V ⁶ gm/100gm
1	5.69 ± 0.20 ⁷	5.55 ± 0.17 ⁷	5.29 ± 0.03 ⁷	5.22 ± 0.17 ⁷	5.30 ± 0.14 ⁷
2	5.52 ± 0.16	5.53 ± 0.16	5.50 ± 0.17	5.23 ± 0.06	5.22 ± 0.23
3	5.32 ± 0.23	5.69 ± 0.26	4.64 ± 0.07	4.44 ± 0.08	4.29 ± 0.12
4	5.66 ± 0.16	5.67 ± 0.19	4.55 ± 0.08	4.76 ± 0.09	4.60 ± 0.10
5	5.32 ± 0.13	5.26 ± 0.15	4.52 ± 0.17	4.60 ± 0.13	4.45 ± 0.14
6	4.94 ± 0.11	4.86 ± 0.06	4.13 ± 0.04	4.14 ± 0.04	4.04 ± 0.21

¹Each value is a mean of 5 observation.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 40% fat.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

TABLE 4
Per cent moisture in liver¹

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² %	Group II ³ %	Group III ⁴ %	Group IV ⁵ %	Group V ⁶ %
1	70.4 ± 0.2 ⁷	72.4 ± 1.1 ⁷	70.0 ± 1.5 ⁷	71.0 ± 0.6 ⁷	69.8 ± 0.6 ⁷
2	70.7 ± 0.3	70.1 ± 0.3	69.5 ± 0.4	69.4 ± 0.3	68.2 ± 0.9
3	69.4 ± 0.2	69.6 ± 0.1	70.7 ± 0.2	68.8 ± 0.4	68.0 ± 0.7
4	70.8 ± 0.3	71.3 ± 0.2	70.0 ± 0.2	69.6 ± 0.4	68.7 ± 0.4
5	69.4 ± 0.7	69.8 ± 0.4	69.3 ± 0.5	68.7 ± 0.2	68.2 ± 0.3
6	70.9 ± 0.1	68.9 ± 0.4	69.2 ± 0.3	68.4 ± 0.6	68.2 ± 0.7

¹Expressed as per cent of fresh liver, each value is a mean of 5 observations.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 40% fat.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

TABLE 5
Per cent nitrogen in liver¹

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² %	Group II ³ %	Group III ⁴ %	Group IV ⁵ %	Group V ⁶ %
1	9.30 ± 0.14 ⁷	9.73 ± 0.07 ⁷	10.25 ± 0.46 ⁷	10.24 ± 0.18 ⁷	9.42 ± 0.28 ⁷
2	9.27 ± 0.19	9.16 ± 0.22	9.15 ± 0.20	9.67 ± 0.18	9.18 ± 0.18
3	8.68 ± 0.19	8.24 ± 0.31	9.45 ± 0.14	9.29 ± 0.24	9.22 ± 0.06
4	8.87 ± 0.28	9.51 ± 0.21	9.59 ± 0.19	9.47 ± 0.08	9.73 ± 0.09
5	9.40 ± 0.14	9.59 ± 0.18	9.46 ± 0.36	9.72 ± 0.08	9.98 ± 0.15
6	9.27 ± 0.13	9.06 ± 0.17	9.46 ± 0.22	9.28 ± 0.13	9.61 ± 0.23

¹Expressed as per cent dry weight of liver. Each value is a mean of 5 observations.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 40% fat.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

TABLE 6
Per cent fat in liver¹

Weeks on diet	Low Fat Series		High Fat Series		
	Group I ² %	Group II ³ %	Group III ⁴ %	Group IV ⁵ %	Group V ⁶ %
0 [*]	6.2 ± 1.1 ⁷	6.2 ± 1.1 ⁷	6.2 ± 1.1 ⁷	6.2 ± 1.1 ⁷	6.2 ± 1.1 ⁷
1	9.4 ± 0.6	12.6 ± 2.4	12.3 ± 1.1	17.5 ± 1.2	24.1 ± 1.9
2	11.3 ± 0.8	10.3 ± 0.2	14.3 ± 1.1	15.3 ± 1.3	20.9 ± 3.0
3	8.9 ± 0.4	8.8 ± 0.6	11.7 ± 1.2	15.9 ± 1.8	20.0 ± 1.6
4	8.4 ± 0.5	9.7 ± 0.4	18.9 ± 1.0	18.4 ± 1.3	21.0 ± 0.6
5	7.6 ± 0.2	8.3 ± 0.4	16.7 ± 2.2	16.9 ± 1.2	19.7 ± 1.2
6	7.4 ± 0.4	7.0 ± 0.3	14.7 ± 0.6	16.3 ± 1.2	21.7 ± 2.5

¹Expressed as per cent dry weight of liver. Each value is a mean of 5 observations.

²Group I 20% casein, 5% fat.

³Group II 20% casein, 5% fat, 0.1% thiamine.

⁴Group III 20% casein, 40% fat.

⁵Group IV 20% casein, 40% fat, 0.1% thiamine.

⁶Group V 20% casein, 40% fat, 0.1% niacin.

⁷Standard error of the mean.

^{*}Zero time observations were made in this laboratory under the same dietary conditions.
(See Carroll, 1960).

FIGURES

FIGURE I
GROWTH CURVES

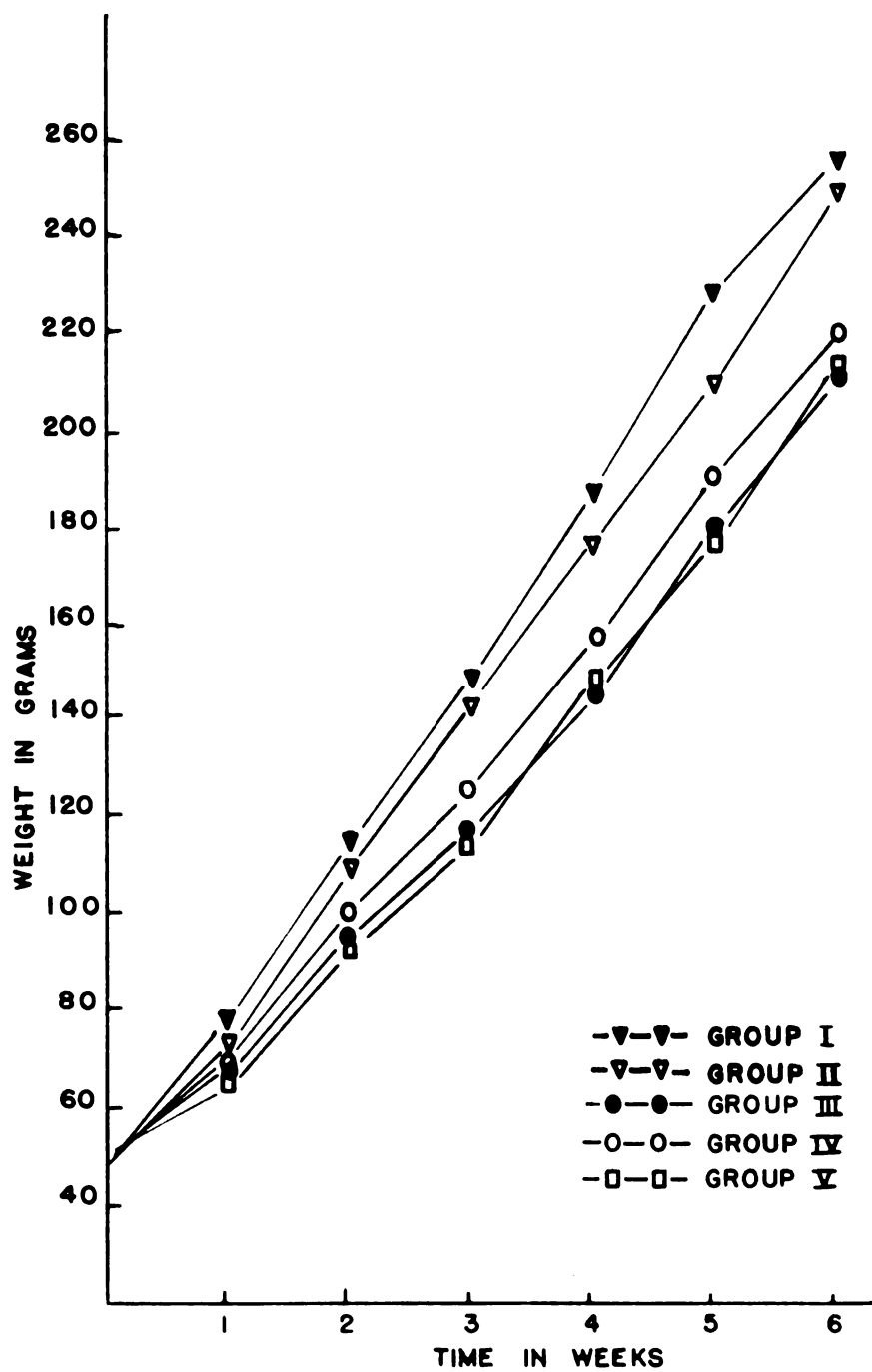
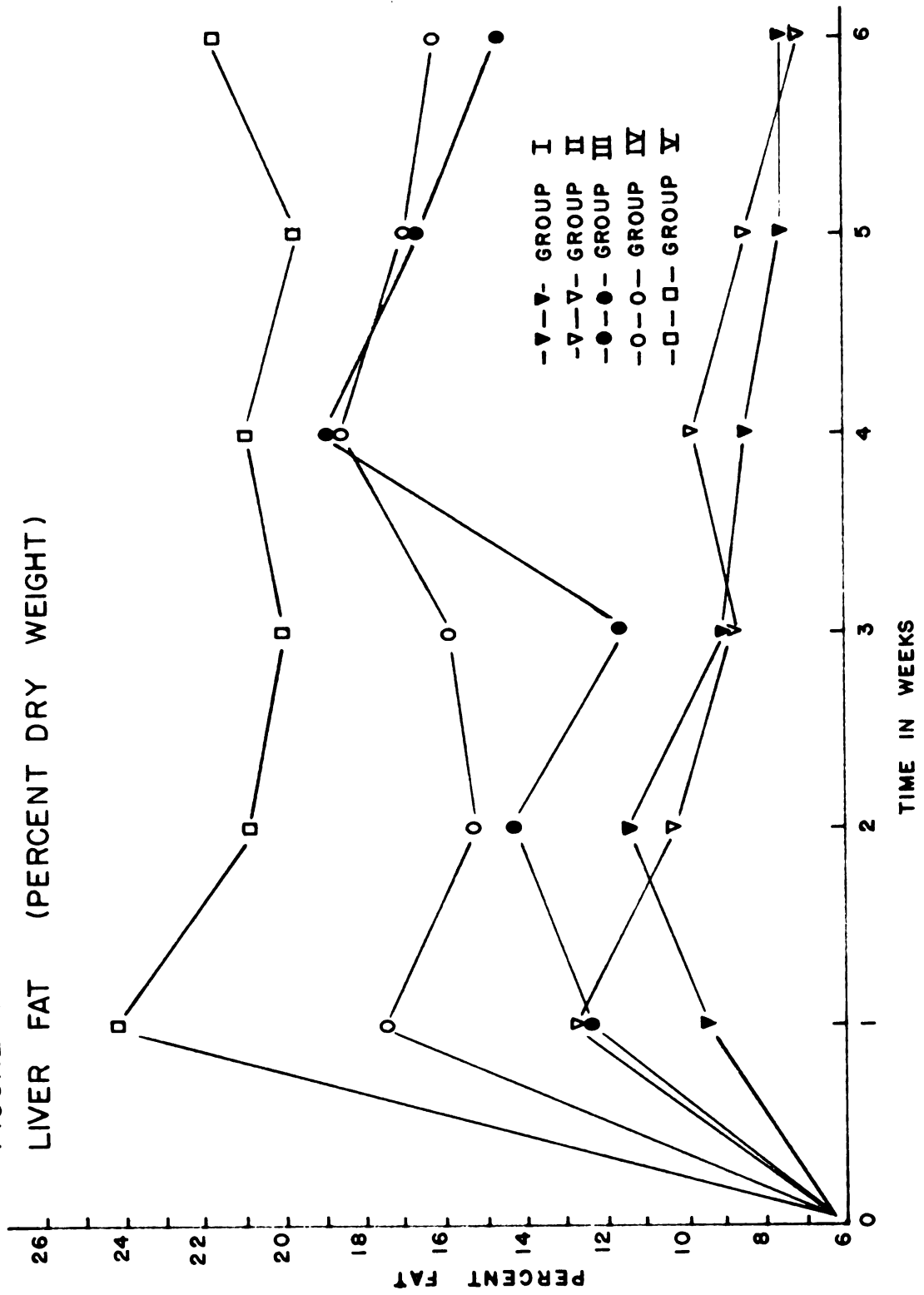


FIGURE 2
LIVER FAT (PERCENT DRY WEIGHT)



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LITERATURE CITED

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