THE EFFECTS ON THE WEANLING RAT OF THE ADDITION OF WATER TO THREONINE-DEFICIENT DIETS

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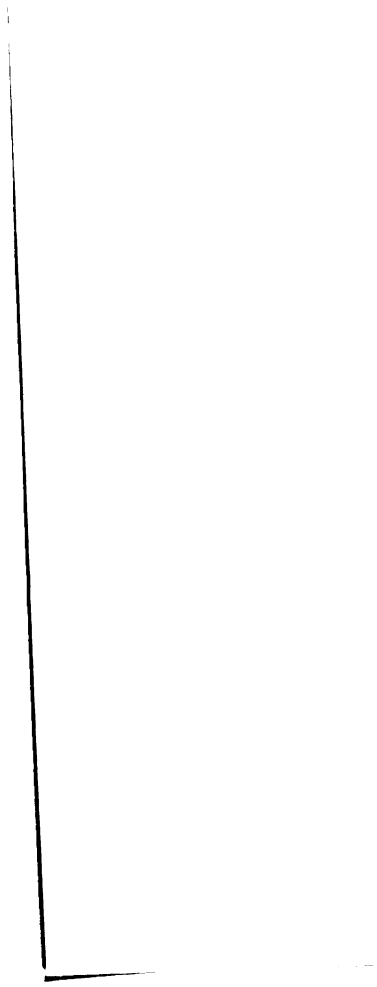

ABSTRACT

THE EFFECTS ON THE WEANLING RAT OF THE ADDITION OF WATER TO THREONINE-DEFICIENT DIETS

by Rosemary Louise Blyth

Weanling rats of the Sprague-Dawley strain were fed 9% casein diets supplemented with 0.3% DL-methionine and 0.1% DL-tryptophan, but not supplemented with threonine. Varying levels of water were added as a 1% agar solution to the diet. The experimental period ranged from four days to two weeks, food and water consumption and weight gain records were kept, and Protein Efficiency Ratios calculated. The animals were sacrified, and livers removed for analysis of moisture, fat and nitrogen content. The xanthine oxidase activity of liver tissues was also measured.

Additions of 20, 50 or 80% moisture to a 9% casein, threonine-deficient diet cause a decrease in weight gain, food and water consumption and Protein Efficiency Ratio in weanling rats. Moreover, liver lipids are elevated in animals fed the deficient diet plus 50% water.

These effects are apparently not caused by chemical interaction between water and the other diet components in the food cup prior to ingestion. When the diet containing

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50% moisture is allowed to remain at room temperature for 24 hours and then freeze-dried before feeding, liver lipids are not elevated as observed when the diets are fed wet.

The addition of water to the threonine-deficient diet increases the efficiency of utilization of the dietary amino acids as measured by the xanthine oxidase activity of the liver after four days of feeding the diet. This may appear to contradict the data obtained in Part I, in which the PER was decreased with increasing water in the diet. However, in the imbalanced animals receiving the wet diet, two different mechanisms are apparently in operation to maintain homeostasis. Initially the efficiency of utilization of the amino acids is increased in the animals fed the deficient diet wet as compared with those fed the same diet dry, using xanthine oxidase activity of the liver as the criterion. However, this initial increase in the efficiency of protein utilization is followed by a decrease in the food intake in response to the altered plasma amino acid pattern (Yoshida et al., 1966). This pattern is more rapidly distorted towards the "critical ratio" in the rats fed the wet diet than is the case with those fed the dry diet. Thus the increased availability of the amino acids in the imbalanced diet induced by the addition of water

serves only to aggravate the situation caused by the imbalance, and over the two week experimental period results in a significantly decreased food consumption, weight gain and PER.

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REVIEW OF LITERATURE

Harper (1959) has defined amino acid imbalance as "a change in the proportion of the amino acids of the diet that results in a depression of food intake or growth rate that can be completely prevented by a supplement of the amino acid present in the least amount in the diet in relation to the amount required for optimal performance." The evolution of this concept as defined by Harper has been long and tedious, and complicated by a great deal of confusing data.

The first instance of the phenomenon, noted in 1946 by Krehl and co-workers, was perplexing in that the addition of protein to diets deficient in that nutrient caused a growth <u>retardation</u> in rats. Krehl et al. added gelatin to diets deficient in tryptophan and niacin, and observed a significantly lower growth rate as compared with that of rats fed the unsupplemented diet. A similar retardation in growth was seen with certain amino acid supplements to low protein diets. When Hankes et al. (1949) added an amount of threonine equivalent to that in 6% casein to a 9% casein diet supplemented with 0.2% cystine, a growth depression

occurred. This could be reversed by the addition of 1.5 mg% niacin or 5.0% tryptophan. These workers suggested that the inhibition of growth occurring with the addition of threonine could be due to some reaction taking place in the intestinal tract or tissues between cystine, threonine and tryptophan, which utilized the tryptophan and left insufficient amounts for niacin synthesis. Thus these early attempts to correct deficient diets by supplementing at least one of the deficient amino acids resulted in a situation measurably worse than the original deficiency. The effort to explain this phenomenon occupied many years. First efforts centered around the documentation of various kinds of growth depressions induced by amino acid supplementation. These were followed by studies aimed at unearthing the underlying physiological and biochemical mechanisms.

Growth Depression Studies

Salmon in 1954 provided the first concrete data implicating the balance of amino acids in the diet as the critical factor. He found that 20 mg niacin/kg diet reversed the decreased growth seen in rats when 10% gelatin was added to a ration containing 20% casein, 40% corn grits

and 0.3% L-cystine. However, the same quantity of niacin added to a 7% casein, 40% corn grits ration had no effect. Only the addition of 0.2% DL-tryptophan increased growth. He suggested that niacin corrected the growth depression only when the addition was made to diets that contained a limited surplus of tryptophan above that needed for use as amino acid per se. This surplus would normally be converted to niacin, but when the tryptophan deficient protein was added, the conversion failed to occur. In this case, niacin was the limiting factor for growth. However, in the 7% casein diet, there was no surplus of tryptophan to begin with, and tryptophan was growth limiting. Then the addition of niacin had no effect.

Sauberlich and Salmon (1955) observed a slow rate of growth in animals fed a 7% casein diet deficient in niacin. Growth was further reduced with the addition of gelatin, and restored with a supplement of 0.05% DL-tryto-phan. The addition of niacin had no effect. When 20% oxidized casein (devoid of cystine, methionine and trypto-phan) was added to a 10% casein basal diet, the growth of animals fed this supplemented ration was depressed below the basal diet controls. Growth was restored in this case with 0.4% DL-tryptophan, and the authors concluded that the

tryptophan requirement of the rat was not constant, but related to the diet employed and the protein level.

In 1953, Henderson, Koeppe and Zimmerman suggested that niacin deficiency was really a result of amino acid imbalance, and substantiated this with diets composed of free amino acids. They produced a niacin deficiency in rats fed 10% acid-hydrolyzed casein rations supplemented with 0.2% L-cystine, and 0.1% DL-tryptophan. When the threonine content of the diet was increased from 0.33 to 0.38% of the L-isomer, growth was depressed. This was reversed with the addition of 2.5 mg% niacin. They suggested that the addition of other amino acids to diets containing a limited surplus of tryptophan for niacin synthesis caused this tryptophan to be used preferentially for protein synthesis, and conversion to niacin did not occur.

Sauberlich (1956) suggested that it was the amino acid array in the diet that caused growth depression, and not a specific effect of one added amino acid or protein, or the vitamin interrelationship that might be involved, as was the case with niacin. By adding 20% oxidized casein to a 35% peanut meal ration supplemented with 0.2% L-lysine and 0.3% L-cystine and Vitamin B12, he made methionine the most limiting amino acid. Growth was depressed with the

addition of 0.2% DL-tryptophan and the depression reversed by adding 0.5% DL-methionine. He also created an imbalance with the addition of 15% hemoglobin to 75% corn diets. Corn is deficient in tryptophan, isoleucine, lysine, threonine and valine, and 75% corn diets when supplemented with these amino acids supported growth. The isoleucine content of the supplemented diet was barely adequate however, and when hemoglobin with its low isoleucine level was added, a severe inhibition of growth occurred. This was reversed by a supplement of 0.55% isoleucine to the diet. The fact that he caused an imbalance of an amino acid (isoleucine) without a known relationship to a vitamin suggested that the imbalance is a general phenomenon associated with amino acids.

That an amino acid imbalance is caused by the addition of the second most-limiting amino acid in the diet was established by Morrison and Harper (1960). They used 8 or 9% casein diets supplemented with 0.3% DL-methionine, and added separately a series of amino acids in attempts to produce a growth retardation. Only those animals fed diets supplemented with threonine, the second most-limiting amino acid, showed depressed growth.

Deshpande (1958a) created an imbalance in rats fed

6% fibrin diets. He found that four amino acids, leucine, isoleucine, valine and histidine were all about equal and first-limiting for growth, and methionine and phenylalanine about equally second most-limiting. The addition of 0.4% methionine and 0.6% phenylalanine to 6% fibrin diets caused a growth retardation that was reversed with the addition of all four of the most limiting amino acids.

The level of protein in the diet was found to be important in producing an imbalance. Harper (1959b) fed rats various levels of casein supplemented with 0.3% methionine. He added an amino acid mixture equivalent to the L isomers of 6% casein, but lacking in threonine to diets containing 4, 6, 8, 10 and 15% casein. The growth depression was greatest with the 6% casein diets. It was less at levels higher or lower than 6%, and very little depression of growth occurred when the amino acid mixture was added to the 15% casein diets. Rats fed 4, 7 and 10% casein diets supplemented with 0.3% methionine and the amino acid mixture lacking threonine suffered different magnitudes of a growth depression, the most severe being with the 7% casein ration, but all the depressions were reversed by the same amount of threonine, 0.1% of the DL isomer. Harper hypothesized that adding the same quantity of an amino

acid mixture which increased the requirement of the animal by 0.1% would always call for an equal supplement of the amino acid, even though the growth depression caused by the imbalance is less severe when it is produced with higher quality proteins.

The problem of amino acid imbalance has been complicated by the appearance of fatty livers in rats fed 9% casein diets supplemented with choline and niacin. Harper (1954a) found an increased deposition of fat in the livers of animals fed a 9% casein diet supplemented with methionine or cystine. When the sulphur-containing amino acids were not added, growth was retarded, but there were no fatty infiltration of the liver. Harper added 0.1% DL-methionine to 9% casein diets containing choline and 0.1% DL-tryptophan, and found an increase in liver fat on a dry weight basis from 13.0% to 24.6%. The inclusion of 0.36% DL-threonine reduced the liver lipid to 13.1%. Increments of methionine up to 0.6% of the DL isomer caused no further increase in liver fat. When rats receiving 9% casein diets containing choline and 0.3% DL-methionine were pair fed against the same diet with no methionine supplement, there was no difference found in the percent liver fat of either group, nor did the addition of methionine cause as great

an increase in growth as when the diet was fed ad libitum. Harper suggested that a 9% casein diet is primarily deficient in the sulphur-containing amino acids, and when methionine was added, the secondary deficiencies of threonine and tryptophan became evident. When tryptophan was added, the threonine deficiency appeared, and fat accumulated in the liver. When the intake of the diet containing methionine was limited to that of the unsupplemented group, threonine did not become sufficiently limiting to cause the deposition of fat.

Deshpande and Harper (1958b) suggested that the proportions of amino acids required for growth might differ from those required for physiological processes, such as fat metabolism. When the growth of amino acid imbalanced rats is stimulated by adding the most limiting amino acid, more of the other amino acids are used for protein synthesis and less for control of fat deposition. This agreed with Singal et al. (1953a) who fed rats an amino acid mixture containing all the amino acids provided by a 9% casein diet except for threonine. Consumption of this threonine-devoid diet caused a growth depression, but the animals had normal liver lipid levels. When 0.7% DL-threonine was added, bringing the level to that in 9% casein, growth improved

markedly, but there was an increased deposition of fat in the liver. Not until 1.1% DL-threonine was added and growth was optimum was a surplus of threonine available, and liver fat levels reduced to normal.

By this time, a significant number of amino acid imbalances had been identified, and attention in this field was turned toward an effort to identify the physiological and biochemical basis for the occurence of amino acid imbalances.

Physiological and Biochemical Studies

Deshpande (1958b) attempted to study the fate of the amino acids that could not be used for protein synthesis when one amino acid is limiting. Salmon in 1954 had suggested that the surplus of amino acids arising from an imbalance might stimulate amino acid catabolism, and result in some additional loss of the amino acid that is already in shortest supply. Deshpande (1958b) found some support for this theory. He fed 6% fibrin diets imbalanced with respect to leucine, isoleucine, valine and histidine by the addition of 0.4% methionine and 0.6% phenylalanine. He found that the imbalance led to a decrease in food intake, total nitrogen retained, and percent absorbed nitrogen

retained. Force feeding the diet in an attempt to elevate the food intake resulted in the death of the animals in 2 or 3 days. He hypothesized that the rapid fall in food intake and percentage of absorbed nitrogen retained might indicate a reduced ability of the rat to utilize the nitrogen present in the diet. However, Kumpta et al. in 1958 found the percent nitrogen retained by animals fed a 6% fibrin diet imbalanced in leucine, isoleucine, valine and histidine was not significantly different from that of animals fed the control 6% fibrin diets pair fed to the same low food intake as the imbalanced animals. Thus it was very hard to separate the effects of the imbalance from the effects of the decreased food consumption.

These workers found the food intake fell within 24 hours after feeding the imbalanced diet. However, in rats trained to eat a single daily feeding in a one or two hour period, the food intake did not fall until the second day, indicating that there was some sort of systemic effect in operation, and not merely a difference in palatability (Kumpta, 1958). They suggested that the plasma amino acid patterns might provide some answer. As early as 1955, Sauberlich and Salmon noted an altered plasma amino acid pattern in rats fed tryptophan-deficient diets.

They added 20% oxidized casein to 10% casein diets supplemented with L-cystine, and found a lowered plasma tryptophan and two to three times as much tryptophan in the urine of rats fed the imbalanced diets compared to those fed control diets to which no oxidized casein had been added, or corrected diets that were supplemented with 0.4% DL-tryptophan.

Kumpta and Harper (1962) studied the plasma amino acid pattern of rats fed an imbalanced diet of 6% fibrin and an amino acid mixture lacking histidine. Despite the fact that the animals had been pair fed so that both groups consumed exactly the same amount of histidine, the plasma histidine levels of the imbalanced rats fell far below those of the controls within an hour. The other amino acids in plasma from imbalanced rats reflected the dietary pattern.

Sanahuja and Harper (1962) investigated the problem of the decreased food intake of rats fed imbalanced diets in the light of these data. They proposed a homeostatic mechanism that responds to changes in the plasma amino acid pattern, and explored this possibility by means of experiments that allowed rats to choose between imbalanced and protein-free diets. When rats depleted in protein for a

seven day period were offered a choice between an imbalanced and a protein-free diet, they chose the latter within three days (Sanahuja and Harper, 1962). Correction of the imbalanced diet with the addition of histidine caused a reversal of the preference for the protein-free diet. Although rats that had not been protein depleted began by consuming more of the imbalanced diet, by the end of 24 hours, almost all of the animals were consuming the protein-free diet.

The difference in behavior of the depleted rats was only in the time at which the response occurred, and was ascribed to the plasma amino acid pattern. As soon as the non-depleted rats consumed the imbalanced diet, plasma levels of the limiting amino acid would fall rapidly, and the ratio between the level of this amino acid and all the others would become very great. On the other hand, this ratio would <u>not</u> be so great in the depleted rats because although the histidine level would be very low, the levels of the other amino acids would not reach as high a level as quickly as they would in the non-depleted animals. According to this theory, the homeostatic mechanism would be activated by the "critical ratio" of the limiting amino acid to the others, and would prompt the animals to begin consuming a protein-free diet that would promote a more

normal plasma amino acid pattern.

These experiments on the amino acid pattern of the blood prompted a more thorough examination of the immediate fate of the most limiting amino acid in the diet. Yoshida et al. (1966) created amino acid imbalances of threonine and histidine using casein diets. Six percent of casein was imbalanced with respect to threonine by the addition of an amino acid mixture lacking in threonine, and a histidine imbalance created with the addition of an amino acid mixture minus histidine to a 5% casein diet. Additions of threonine $-C^{14}$ and histidine-C^{14} to each diet as tracers were of quantities too small to affect growth.

There was no difference in the excretion of the labelled amino acids in CO_2, urine or feces between the balanced and the imbalanced rats. However, rats fed the imbalanced diets retained more radioactivity in the liver and carcass. There were no observed differences between the radioactivities of the gastrointestinal contents of the groups, so the imbalance did not affect absorption. The blood plasma showed significantly less threonine-C^{14} and histidine-C^{14} in rats fed the imbalanced diets than those fed the control. They hypothesized the following succession of events. The imbalanced meal was digested and absorbed

normally, causing a flow of all but one of the essential amino acids to the liver, where protein synthesis was stimulated. In the imbalanced animals, more of the amino acid in shortest supply was incorporated into protein so that the amount in the peripheral system was reduced, and food intake depressed. Sidransky and Farber (1958) have shown that pathological lesions developed when severely deficient diets were force-fed to the rat. This depression in food intake is apparently a protective response on the part of the animal.

These data effectively eliminated Salmon's theory of increased catabolism of the limiting amino acid, but supported Henderson, who had earlier (1953) suggested an increased protein synthesis accounted for the lack of tryptophan conversion to niacin when tryptophan was not the limiting amino acid. Sidransky and Farber (1958) found that force-feeding an amino acid diet devoid of threonine caused an increase in the RNA content of the liver, and that the incorporation of amino acids into liver protein was increased.

Yoshida et al. did not find conclusive evidence of enhanced protein synthesis in organs other than the liver, but there was no depression of labelled amino acid incor-

poration into any other compartment, and the same alterations would occur in the plasma amino acid pattern if the synthesis were only accelerated in a few organs, and proceded at a normal rate in others. Of course, protein synthesis over a longer period of time would be decreased once the initial rapid rate had resulted in the decreased food intake and depressed growth.

Kumpta and Harper (1961) showed that when the food intake of rats fed a 6% fibrin diet, imbalanced with respect to leucine, isoleucine, valine and histidine by the addition of methionine and phenylalanine, was increased by the injection of insulin, the animals grew well, and showed no adverse effects. Thus, provided that the food intake can be maintained, the limiting amino acid would be used as efficiently, if not more so, than the same amino acid in a control diet (Yoshida et al., 1966). The response of the animal, the depressed food intake, is a response to a plasma amino acid pattern that looks like that of a much more severely deficient diet than the imbalanced diet actually is, and if the food intake can be stimulated by some method such as insulin injection, the animals should grow well and show no ill effects.

Since the problem of amino acid imbalance has there-

fore been suggested by Harper (1964) as being primarily one of overcoming the depressed food intake, any method that increases the availability of the amino acids should accomplish the same end. In 1962, Keane and Denton found that they could increase the food consumption, weight gain and efficiency of utilization of protein of rats fed low protein diets by the addition of water. It was therefore decided to attempt the correction of the threonine-imbalanced, low protein diets used in this laboratory with the addition of varying levels of water.

GENERAL METHODOLOGY

Male weanling rats of the Sprague-Dawley strain were used in all experiments. The initial weight ranged from 43 to 52 grams, and the animals were divided into groups so that the mean weights of the groups did not differ more than ± 0.5 grams. They were individually housed in screen bottom cages, in a temperature controlled room, and weighed twice weekly over the experimental period. Weight gain was reported as grams gain per week.

The basal diet was of the following percent composition: sucrose, 81.2; casein, 9.0; salts W,[1] 4.0; fat (corn oil),[2] 5.0; vitamin mix,[3] 0.25; choline chloride, 0.15; DL-methionine, 0.3; DL-tryptophan, 0.10. Alphacel was added to the diet to the level of 10% to retard diarrhea. In those experiments where threonine was supplemented to this basal diet, 0.36% of the DL isomer replaced an equal weight of sucrose.

The addition of water to the basal diet was accom-

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[1]Wesson modification of Osborne and Mendel salt mixture. L. G. Wesson, Sci., 75, 339 (1932).

[2]Containing 75 mg α-tocopherol per kilogram diet.

[3]For composition of vitamin mix see L. L. Rikans, D. Arata and D. C. Cederquist, J. Nutri., 82, 83 (1964).

plished with a 1% agar[4] solution. The agar was disolved in water with heat, and allowed to cool before being added by weight to the desired percentage of the diet solids. The mixture formed a thick gel when set, which prevented the separation of the solid and liquid components of the diet, thus ensuring the consumption of the proper proportion of solids to water. The wet diets were mixed fresh daily, while the basal dry diet was replenished only when necessary. Food consumption was recorded as grams of diet solids consumed, and the animals were allowed to eat ad libitum, except where the pair feeding technique was used. Individual water consumption was not measured. The animals were allowed to drink ad libitum, but the bottles were filled from a weighed reservoir for the group. The difference between this amount and the total remaining in the bottles at the end of one week was recorded as the total weekly consumption of the group. This figure divided by the number of animals gave the average weekly consumption per rat. These data were totalled for the experimental period, and the average consumption per week per rat computed.

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[4]Bacteriology grade, General Biochemicals, Chagrin Falls, Ohio. (Lot #54395)

At the end of the two week experimental period, the animals were killed by decapitation and exsanguinated. The livers were excised, weighed and homogenized in distilled water in a Potter-Elvehjem homogenizer.[5] They were placed in a forced air drying oven at 85°C for 12 hours, and then removed to a vacuum oven at 50°C where they were allowed to come to constant weight. The moisture content of the liver was calculated, and the dried tissue ground in a Wiley mill. Fat content of the liver was determined by continuous extraction with ether of the dried ground tissue for three hours on a Goldfisch apparatus. The data were calculated as the percent of the dry weight of the tissue. Nitrogen was determined on 0.10 gram samples of the fat extracted tissue, and calculated as the percent of fresh tissue. Standard errors of the means were calculated for all data except water consumption where there were no individual values, and the Student's t test used to measure significance. Unless otherwise stated, a probability of 0.01 or less was used as a criterion of significance.

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[5] V. R. Potter and C. A. Elvehjem, J. Biol. Chem., 114, 499 (1936).

PART I

THE EFFECT OF VARYING QUANTITIES OF WATER ADDED TO LOW PROTEIN, THREONINE-DEFICIENT DIETS

INTRODUCTION

Keane, Smutko, Krieger and Denton in 1962 studied the effects of the addition of water to low protein diets. They fed male weanling rats 9% casein diets containing choline and added varying amounts of water. The water was added alone or in combination with such diet thickeners as agar and guar gum. In the absence of any thickener, the addition of water at the 20% level produced a highly significant increase in PER compared with the dry control diet. In the presence of agar, the addition of water at the 20 and 50% levels also produced a highly significant ($P < 0.01$) increase in PER over control values, but comparable to water alone. An increased food consumption was noted, but when animals were pair fed a diet containing 20% water to animals receiving a 9% casein diet dry the PERs of the "wet" group were still significantly increased, despite the same protein consumption (Keane et al., 1963). The effects of the addition of different levels of water to various levels of protein in the diet were also studied (Keane et al., 1962). With 6 and 9% casein diets,

PERs were significantly increased ($P < 0.01$) with additions of 20% water. There was no increase in PER with 18% casein diets. The authors suggested that the osmotic pressure in the gastrointestinal tract prompted the increased utilization of protein in the low protein diets.

Since Keane and Denton worked with a low protein diet similar in composition to the 9% casein diet used to produce a threonine imbalance in this laboratory, it was suggested that the addition of water to this low protein, threonine-deficient diet might increase the food consumption, weight gain and efficiency of utilization of protein in rats fed these diets. Harper (1964) suggested that the main problem in overcoming an amino acid imbalance is in counteracting the depressed food intake. Increasing the level of water in the diet might increase the efficiency of utilization of the amino acids in the deficient diet and achieve the same end as improving the food intake. Experiments studying these possibilities are described in the following sections.

METHODS

Five groups of ten animals each were fed the basal diet plus five different levels of added water. Data from replicate groups were combined at the end of the study. The groups were as follows:

Group I - basal diet, no added water.

Group II - basal diet, 10% water.

Group III - basal diet, 20% water.

Group IV - basal diet, 50% water.

Group V - basal diet, 80% water.

Weight gain, food consumption and water consumption were recorded and the Protein Efficiency Ratio calculated (weight gained in grams per week per gram of protein consumed) for each group. Livers were analysed for moisture, fat and nitrogen.

In the first experiment conducted in this series, animals were given these diets ad libitum. Rats fed the wet diets ate significantly less on a solids basis than did those fed the dry diet. In order to eliminate this complicating factor, the pair feeding technique was used

in succeeding experiments. Since the greatest effect on liver lipid from the addition of moisture was seen in the 50% wet group, it was decided to pair feed to this group on a solids basis. Three groups of ten animals each were used in the study, and data from replicate groups combined. Group I was fed the basal diet with no added moisture. Group IV received the basal diet containing 50% moisture, and Group VI received the basal diet with no added moisture, pair fed to the solids consumption of Group IV (50% wet). The analytical procedures were the same as for the ad libitum studies.

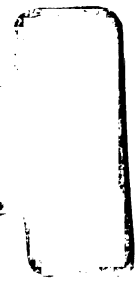
RESULTS

Weight gain, food consumption, PER and water consumption data for the ad libitum studies are presented in Table 1. A significant growth depression from the control ($P < 0.01$) is obtained with the addition of 20, 50 and 80% water. Food consumption is significantly reduced from the control at each of these levels. The depression of the food intake is of equal magnitude in Groups III and IV, with a more severe decrease as the water content of the diet is increased from 50 to 80%. As would be expected, the water consumption of rats in each group varies inversely with the water content of the diet. When the total water consumed, including that provided by the diet, is calculated, there still remains a difference between the amount consumed by the animals fed the basal dry diet, and those fed the wet. Group V (80% moisture) is an exception, the total consumption being very nearly the same as that of Group I. The PERs are significantly reduced with the addition of 20 and 80% moisture, and since there are no significant differences between these two

groups and Group IV (50% wet), the reduced PER with 50% moisture must also be significant. The addition of 10% moisture causes no significant decrease in PER.

Liver composition data are presented in Table 2. Livers taken from rats in Group IV (50% wet) have significantly less moisture than do those from control animals, but there are no significant differences among the other groups. The addition of 50% moisture (Group IV) to the basal diet (Group I) causes a significant elevation in liver lipid from 25.7 to 30.1%. However, a further increase in moisture content of the basal diet to 80% (Group V) does not elevate the lipid content of liver tissues above the control value. There are no significant differences in the percent liver nitrogen between groups.

The results from the pair feeding trials are reported in Tables 3 and 4. Increasing the moisture content of the basal diet to 50% results in a significant decrease in food consumption and PER (Table 3). These results agree with those from previous experiments (see Table 1). When the basal diet is pair fed with Group IV, an identical decrease in growth, food consumption and PER is observed, and the water consumption rises, as would be expected, since the diet is fed dry.



The animals fed the 50% wet diet ad libitum (Group IV) have livers significantly lower in moisture and higher in fat compared to the basal dry controls. These results corroborate data from the previous studies (see Table 2). However, animals consuming the same amount of dry diet as those in Group IV (50% wet) show liver moisture levels comparable to controls, and liver fat reduced even below control levels. No significant differences are found between any of the three groups in percent liver nitrogen.

Table 1. The effects of varying levels of water added to low protein, threonine-deficient diets on weight gain, food consumption, PER and water consumption of weanling rats.

Group	# N	Diet	Weight Gain g/wk	Food Consumption g/wk	PER[1]	Water Consumption g/wk	Total Water Consumption[4] g/wk
I	30	basal + no added water	19.6 ± 0.7[2]	60.4 ± 1.4[2]	3.44 ± 0.08[2]	66.7	66.7
II	10	basal + 10% water[3]	20.1 ± 1.7	61.9 ± 2.8	3.40 ± 0.15	-	-
III	20	basal + 20% water[3]	14.8 ± 1.0*	50.4 ± 2.0*	3.05 ± 0.01*	47.8	51.5
IV	30	basal + 50% water[3]	16.3 ± 1.0*	53.7 ± 1.6*	3.14 ± 0.01*	33.6	49.9
V	20	basal + 80% water[3]	12.0 ± 1.1*	44.3 ± 1.3*	2.79 ± 0.02*	17.1	65.1

*Significant difference from Group I at 1% level.

[1]Grams gain/gram protein consumed.

[2]SE

[3]Water added as 1% agar solution.

[4]Total includes water provided by the diet.

Table 2. The effects of varying levels of water added to low protein, threonine-deficient diets on the liver composition of weanling rats.

Group #	N	Diet	Liver Composition		
			Moisture %	Fat[1] % Dry Weight	Nitrogen % Fresh Weight
I	30	basal + no added water	66.5 ± 0.4[2]	25.7 ± 1.0[2]	2.27 ± 0.05[2]
II	10	basal + 10% water[3]	67.9 ± 0.6	22.2 ± 1.3	2.27 ± 0.08
III	20	basal + 20% water[3]	66.7 ± 1.0	24.6 ± 1.0	2.29 ± 0.05
IV	30	basal + 50% water[3]	64.2 ± 0.5*	30.1 ± 1.3*	2.18 ± 0.04
V	20	basal + 80% water[3]	66.3 ± 0.4	24.4 ± 1.6	2.33 ± 0.08

*Significant difference from Group I at 1% level.

[1]Ether soluble lipid.

[2]SE

[3]Water added as 1% agar solution.

Table 3. The effect of pair feeding on weight gain, food consumption, PER and water consumption of weanling rats fed wet or dry low protein diets deficient in threonine.

Group #	Diet	N	Weight Gain g/wk	Food Consumption g/wk	PER[1]	Water Consumption g/wk	Total Water Consumption[4] g/wk
I	basal + no added water	20	19.6 ± 0.7[2]	60.4 ± 1.4[2]	3.44 ± 0.08[2]	66.7	66.7
IV	basal + 50% water[3]	20	14.1 ± 1.1*	50.1 ± 1.6*	2.94 ± 0.15 *	37.4	38.8
VI	basal + no added water pair fed to IV	20	13.3 ± 0.6*	47.9 ± 1.3*	2.93 ± 0.07*	58.4	58.4

*Significantly different from Group I at 1% level.

[1]Grams gain/gram protein consumed.

[2]SE

[3]Water added as 1% agar solution.

[4]Total includes water provided by the diet.

Table 4. The effect of pair feeding on liver consumption of weanling rats fed wet or dry low protein diets deficient in threonine.

Group #	Diet	N	Liver Composition		
			Moisture	Fat[1] % Dry Weight	Nitrogen % Fresh Weight
I	basal + no added water	30	66.5 ± 0.4[2]	25.7 ± 1.0[2]	2.27 ± 0.05[2]
IV	basal + 50% water[3]	20	64.4 ± 0.6*	31.0 ± 1.7*	2.22 ± 0.05
VI	basal + no added water, pair fed to IV	20	67.1 ± 1.4	20.7 ± 1.1*	2.35 ± 0.10

*Significantly different from Group I at 1% level.

[1]Ether soluble lipid.

[2]SE

[3]Water added as 1% agar solution.

DISCUSSION

The addition of 10% moisture to a low protein, threonine-deficient diet has no significant effect on the parameters measured in this study. However, at all levels above 10%, there is a significant decrease in food consumption, weight gain and PER. There is no significant change in the liver composition until the moisture content of the diet reaches 50%, at which point moisture is decreased and fat elevated. The low lipid levels in livers from rats in Group V (80% water) may result from the greatly depressed food intake. The threonine deficiency may have been too severe to allow the appearance of fatty livers. Singal et al. (1953a) fed weanling rats an amino acid diet to simulate 9.0% casein. It contained 0.7% DL-threonine, and produced liver fat levels of 13.9% of the fresh tissue weight. When he reduced the amount of threonine to 0.55%, the liver lipid was 11.7%, and at 0.4% threonine, it fell to 8.1%. The addition of 1.1% DL-threonine brought the liver fat to 5.9%, and the complete omission of threonine produced levels of 5.7%. There was a range between the 0.7% level of thre-

onine and the complete lack of the amino acid when growth was retarded to a greater degree, and less fat deposited in the liver than was observed when higher levels of the amino acid were fed. In this experiment, the animals fed the diet with 80% moisture added consumed only 73.5% as much threonine as the control animals, and it is possible that this reduction brings the threonine intake within the range where fatty livers do not appear.

The results from the pair feeding studies indicate that the addition of moisture, rather than the reduction in food intake is responsible for the increase in liver fat. Livers from animals consuming the same quantity of the dry ration as those fed the 50% wet diet (on a solids basis) contain significantly less fat than those fed the wet diet.

The PER data from the ad libitum studies indicate that the addition of water to 20% or more of the diet has an adverse effect on the absorption and/or utilization of the diet. However, in the pair feeding trials the PERs of the animals fed the wet diet are very nearly identical to those of the animals fed the same quantity of dry solids, and are significantly lower than controls. It is difficult to define which are the effects of the diet and which the

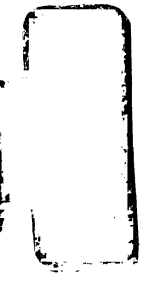
decreased food intake. These data are contrary to those reported by Keane and Denton (1962). They observed increased growth and higher food efficiency resulting from increasing the water content of the diet. They hypothesized that the difference in osmotic pressure of the gastric contents of the animals fed the wet diets caused the increase in weight gain, food consumption and PER. There is in fact evidence that altered tonicity of the gastric contents affects gastric motility. Hunt (1959) states that there are receptors in the duodenum that are sensitive to the osmotic pressure of the chyme. Thomas (1934) suggests that the mechanism is a vagal reflex that lowers the tone of the stomach muscle and the strength of the peristaltic contractions, rather than shutting down the pylorus to prevent the entrance of a hyper- or hypotonic chyme. If this mechanism is operative, the rate of flow through the gastrointestinal tract would be slowed, and there would be time for more thorough absorption. This would produce a higher efficiency of utilization of the diet solids, and account for the increased weight gain and PER found in the experiments of Keane and Denton. The decreased weight gain, food consumption and PER found in this experiment are not in agreement with their work, but these

diets are low in protein and imbalanced with respect to threonine, while Keane and Denton's experiments were not complicated by the presence of an amino acid imbalance. Furthermore, PER values are relatively gross measurements, compounding the error from two parameters, and this combined with an experimental period of only two weeks may make the PER a somewhat less than ideal method for measuring protein utilization under these conditions.

These grounds do not seem sufficient to eliminate entirely osmotic pressure as a consideration in the studies under discussion, and it may operate in conjunction with some other factor. The effect of added moisture to the diet may be reduced to two possibilities; either the effect is a chemical one, resulting from the interaction of the water with the diet solids prior to ingestion by the animal, or a physical one *via* the water's presence in the gastro-intestinal tract. Experiments exploring both of these possibilities are discussed in the following sections.

SUMMARY

When 20, 50 or 80% water are added to low protein, threonine-deficient diets (9% casein supplemented with 0.3% DL- methionine and 0.1% DL- tryptophan) a significant decrease ($P < 0.01$) is observed in the weight gain, food consumption and PER of animals fed these diets. Alteration in the liver composition does not occur until 50% water is added, when moisture is decreased and fat elevated. In the animals receiving 80% moisture in the diet, fatty livers do not appear, but this may be due to the low food and hence threonine intake. The effect of the water may be mediated through either chemical alterations in the diet solids caused by their contact with the water over a 24 hour period prior to ingestion, or through the physical presence of the water in the animal's gastrointestinal tract. Experiments exploring these possibilities will be described.



PART II

EFFECTS OF FREEZE-DRYING ON THE RESPONSE OF THE WEANLING RAT TO WET VERSUS DRY THREONINE-DEFICIENT DIETS

INTRODUCTION

Adding water to dry, low protein, threonine-deficient diets may cause some chemical change in the diet solids that would produce the effects described in Part I when fed to the animal. The wet diets remain in the cages at room temperature throughout a 24 hour period, and under these conditions microbial action, for instance, might alter one or more of the dietary constituents.

This experiment was designed to preserve in the diet solids any changes that might have occurred during this time period. If the diets are allowed to stand at room temperature for 24 hours, and then freeze-dried, any changes should remain in the solid components, as the process only removes the water with no effect on the rest of the diet mixture. In order to eliminate the possibility that the addition of agar as a vehicle for the water has an effect on its own, wet diets were prepared with the usual agar solution, and also with water alone.

If the added water has a chemical effect on the diet solids prior to ingestion by the animal, the data

from the freeze-dried groups should compare favourably with those of the animals fed the wet diets.

METHODS

Four groups of ten animals each were used in the experiment. Group I received the basal diet dry,[1] and Group IV the basal diet with 50% moisture added. The ration for Group VII (wet diet, freeze-dried with agar) was prepared exactly as for Group IV (50% moisture) except that the quantity calculated to last for the entire experimental period was prepared at once. The mixture was allowed to stand for 24 hours at room temperature, and then frozen at -20°C, and freeze-dried in a Stokes freeze-drier (Model Number 2003F-2, lot p6569, serial number p65753) for 72 hours. The internal pressure fell to a minimum of 100 microns.

Rats in Group VIII were given a diet prepared by the same procedure, with the exception that the 50% moisture was added as water alone, not as a 1% agar solution. The mixture was shaken in a mechanical shaker over the 24 hour period, in order to keep the water in constant contact

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[1] For composition of basal diet, see p. 18.

with the diet solids. The mixture was frozen and freeze-dried as above for 48 hours.

The animals in Groups I and IV were permitted to eat ad libitum, but the animals in Groups VII and VIII (the freeze-dried diets) were pair fed to the solids consumption of the animals in Group IV (50% moisture).

At the end of the experimental period, the animals were sacrificed, and the livers removed for analysis. The entire experiment was repeated with a second batch of rats, and the results are reported separately.

RESULTS

Experiment I

The results from this first study are presented in Tables 1 and 2. There are differences in some biological compartments at the 1% level. The weight gains of the rats in Group VII (freeze-dried with agar) are significantly greater than those of Group VIII (freeze-dried without agar). The rats fed the 50% wet diet consume significantly more diet solids than controls, and the PER values for the rats in Group VII are significantly increased over both the controls and those of Group IV (50% moisture).

The liver analysis data are presented in Table 2. The percent moistures of the livers from rats in Group I (basal diet dry) and Group IV (basal diet wet) are significantly lower than those from Groups VII and VIII (freeze-dried with and without agar). No significant differences are seen among groups in percent fat or nitrogen.

Since liver weights vary more than usual between groups in this experiment (those of Group IV significantly

heavier ($P < 0.01$) than those of Groups I and VIII) total values for moisture, fat and nitrogen have also been calculated and tabulated. No significant differences are observed either in total moisture or total nitrogen between groups, but Group IV (50% wet) has a significantly higher level of total fat than either Group VII or VIII (freeze-dried with and without agar). Since there are no differences among Groups I, VII and VIII with respect to liver fat, liver lipids in Group IV must be significantly greater than those in Group I as well.

Experiment 2

The data from the replicate experiment varies sufficiently from the initial that it is reported separately in Tables 3 and 4. Although the same trends may be observed in food consumption, weight gain and PER (Table 3), the significant differences found in the previous study are lost.

The liver composition data (Table 4) also show trends comparable to the initial experiment. Significant differences are seen between groups in percent nitrogen that do not appear previously. The animals in Group VII have percent nitrogen values significantly higher than

Groups I and IV, and those in Group VIII have values significantly increased over Group IV.

The liver weights in this experiment show the same variation seen previously. This time a significant increase in liver weight is found in the animals of Group IV (50% wet) over all other groups, and those of rats in Group VIII are significantly reduced from control weights. The same trends in total liver values are seen - significant differences in total moisture are retained, and the total fat levels of the animals in Group IV are increased ($P < 0.01$) over the levels observed with Groups VII and VIII. Since there is no significant difference between Groups I, VII and VIII in this perameter, Group IV must be increased significantly over Group I as well.

Table 1. A comparison of the effect of adding 50% water to low protein, threonine-deficient diets, and the same diets freeze-dried with and without agar on the weight gain, food consumption, PER and water consumption of the weanling rat.

Group #	Weight Gain g/wk	Food Consumption g/wk	PER[1]	Water Consumption g/wk
I basal, dry	23.2 ± 1.0[2]	64.3 ± 1.6[2]	3.82 ± 0.08[2]	92.0
IV basal + 50% water	28.2 ± 1.6	72.5 ± 1.9	4.16 ± 0.15	51.2
VII basal + 50% water freeze-dried with agar[3, 4]	27.8 ± 1.2	69.2 ± 2.0	4.27 ± 0.08	105.3
VIII basal + 50% water freeze-dried without agar[3, 4]	22.8 ± 1.1	65.7 ± 1.7	3.67 ± 0.11	94.2

[1]Grams gain/grams protein consumed.

[2]SE

[3]Diets were incubated at room temperature 24 hours before drying.

[4]Pair fed to Group IV.

Table 2. A comparison of the effect of adding 50% water to low protein, threonine-deficient diets, and the same diets freeze-dried with and without agar on the liver composition of the weanling rat.

	Group I Basal, dry	Group IV Basal + 50% water	Group VII Freeze-dried with agar[2,3]	Group VIII Freeze-dried without agar[2,3]
Liver Moisture				
Percent	66.5 ± 0.5[1]	66.4 ± 0.6	68.1 ± 0.3	68.6 ± 0.5
Total grams	4.02 ± 0.08[1]	4.86 ± 0.25	4.54 ± 0.27	4.04 ± 0.21
Liver Fat				
% dry weight	24.2 ± 1.5[1]	27.8 ± 1.5	23.4 ± 0.9	24.1 ± 1.1
Total grams	0.50 ± 0.05[1]	0.70 ± 0.06	0.49 ± 0.03	0.45 ± 0.05
Liver Nitrogen				
% fresh weight	2.05 ± 0.03[1]	1.90 ± 0.07	2.11 ± 0.06	2.11 ± 0.08
Total grams	0.124 ± 0.002[1]	0.137 ± 0.005	0.139 ± 0.006	0.122 ± 0.004
Wet Liver Weight				
Grams	6.05 ± 0.15[1]	7.32 ± 0.36	6.66 ± 0.40	5.89 ± 0.33

[1] SE

[2] Diets were incubated at room temperature 24 hours before drying.

[3] Pair fed to Group IV.

Table 3. A comparison of the effect of adding 50% water to low protein, threonine-deficient diets, and the same diets freeze-dried with and without agar on the weight gain, food consumption, PER and water consumption of the weanling rat.

Group #	Weight Gain g/wk	Food Consumption g/wk	PER[1]	Water Consumption g/wk
I basal, dry	22.0 ± 0.81[2]	63.8 ± 1.8[2]	3.66 ± 0.05[2]	85.5
IV basal + 50% water	22.5 ± 1.0	63.2 ± 1.7	3.77 ± 0.08	58.4
VII basal + 50% water freeze-dried with agar[3,4]	22.0 ± 0.8	61.5 ± 1.7	3.80 ± 0.08	90.6
VIII basal + 50% water freeze-dried without agar[3,4]	19.9 ± 0.7	59.3 ± 1.5	3.58 ± 0.08	85.7

[1]Grams gain/grams protein consumed.

[2]SE

[3]Diets were incubated at room temperature 24 hours before drying.

[4]Pair fed to Group IV.

Table 4. A comparison of the effect of adding 50% water to low protein, threonine-deficient diets, and the same diets freeze-dried with and without agar on the liver composition of the weanling rat.

	Group I Basal, dry	Group IV Basal + 50% water	Group VII Freeze-dried with agar[2,3]	Group VIII Freeze-dried without agar[2,3]
Liver Moisture				
Percent	66.5 ± 0.4[1]	65.9 ± 0.4[1]	68.5 ± 0.5[1]	68.0 ± 0.3
Total grams	3.73 ± 0.11	4.26 ± 0.13	3.37 ± 0.10	3.33 ± 0.09
Liver Fat				
Percent	20.4 ± 0.9	22.7 ± 1.3	21.2 ± 1.8	20.6 ± 1.4
Total grams	0.39 ± 0.03	0.50 ± 0.04	0.34 ± 0.04	0.29 ± 0.02
Liver Nitrogen				
Percent	2.04 ± 0.01	1.92 ± 0.02	2.38 ± 0.05	2.38 ± 0.06
Total grams	0.118 ± 0.002	0.122 ± 0.004	0.119 ± 0.002	0.113 ± 0.002
Wet Liver Weight				
Grams	5.59 ± 0.17	6.46 ± 0.19	4.93 ± 0.16	4.90 ± 0.15

[1] SE

[2] Diets were incubated at room temperature 24 hours before drying.

[3] Pair fed to Group IV.

DISCUSSION

The data from the initial and the replicate experiment have been tabulated separately because of the differences observed in some of the biological compartments measured. Differences that are significant at the 1% level in the initial experiment are no longer so in the replicate, but the same trends are observed. Absolute values in these two experiments are slightly different as well from the data in the previous studies, but again, the same trends may be seen. Although the food consumption, weight gain and PERs of the group fed the 50% wet diet are much higher than previously observed, the liver composition is not very different when the total values are considered. The increase in deposition of fat in the livers of the rats of Group IV is significant over all other groups.

If the addition of water has a chemical effect on the diet solids, the liver fat levels of the animals fed the freeze-dried diets should be close to those of the rats in Group IV (50% wet). They are more comparable, however, to those of the Group I controls, and are significantly

reduced from the liver fat values of Group IV. This suggests that the effect of increased diet moisture on liver fat concentration is not the result of chemical interaction between nutrients in the food cup.

There is a significant difference found between Groups VII (freeze-dried with agar) and VIII (freeze-dried without agar) in the PER values in the initial experiment, and this cannot be ascribed to the diet treatment. In this experiment, the food intake of rats in Group VIII is lower than those in the group to which they were pair fed. As a consequence, Group VIII was in practice, not pair fed to Group IV, but rather fed ad libitum. There were no differences found between Group VII and VIII in the replicate experiment, and no differences in the liver composition in either experiment, so the lowered PER initially must be viewed as an isolated and unrepresentative situation. The presence of agar in the diet cannot be presumed to have any effect on the animal in the parameters measured in this study.

SUMMARY

Freeze-drying a 9% casein, threonine-deficient diet to which has been added 50% water with and without agar does not produce the same response in the weanling rat as feeding the same diet wet. The elevated liver fat levels observed previously upon feeding the wet diet do not appear when the diet is freeze-dried and pair fed to the solids consumption of the animals consuming the wet diet. Therefore, the effect of adding moisture to the diet cannot be caused by a chemical interaction between nutrients in the food cup prior to ingestion.

PART III

THE EFFECT OF ADDING 50% WATER TO LOW PROTEIN, THREONINE-DEFICIENT DIETS ON THE LIVER XANTHINE OXIDASE ACTIVITY OF WEANLING RATS

INTRODUCTION

Xanthine oxidase activity of the liver has been used by many investigators as an indication of the adequacy of dietary protein. Litwack et al. (1950) fed rats 18% casein diets supplemented with 0.25% DL-methionine for 7 days. At the end of this period, half the rats were changed to a protein-free diet with sucrose substituted for casein. Within two days, the xanthine oxidase activity fell to only 25% of controls, and within 5 days was completely lost. Muramatsu and Ashida (1962) experimented with varying levels of casein in the diet. They fed weanling rats 25% casein for 4 to 5 days followed by diets containing various amounts of casein from 0% to 50%, in 10% increments. The xanthine oxidase activity increased with each increment of casein, from zero activity at 0% casein to a maximum at 20% casein of 550 microliters of oxygen consumed per hour per gram of tissue. The curve obtained when the xanthine oxidase activity was plotted against the percent casein in the diet paralleled that when the liver nitrogen was plotted against percent casein in the diet. The authors concluded that the

xanthine oxidase activity was indicative of general protein metabolism in the liver.

Since the xanthine oxidase activity is altered with the level of dietary protein, and if there are differences in enzyme activity when the protein provided by two diets is the same, then this would indicate differences in the utilization of protein. If the addition of water to the basal diet does in fact increase the efficiency of protein utilization, then this should be reflected in an increased xanthine oxidase activity in the livers of the rats fed the wet diets. The colourimetric method of Litwack, Bothwell, Williams and Elvehjem (1953) was used to measure the activity of this enzyme system.

METHODS

There were two diets used in the first of these experiments, the basal diet fed dry (Group I), and the basal diet plus 50% moisture (Group II). A total of 84 animals was used. Immediately upon arrival, 12 animals were sacrificed. The livers were excised and weighed, and those from 6 rats homogenized with 5 volumes of a sodium-potassium phosphate buffer (pH 7.4) in a Potter-Elvehjem homogenizer[1] and assayed for xanthine oxidase activity by the colourimentric method of Litwack et al. (1953). These data were reported as "zero time" values. The livers of the remaining six were homogenized with distilled water and analysed for moisture, fat and nitrogen. The rest of the animals were divided into two groups and fed either the basal diet dry (Group I), or the basal diet wet (Group II). Twelve animals from each group were sacrificed at two days, seven days, and 14 days, six used for the enzyme assay, and six for moisture, fat and nitrogen analysis as described above.

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[1] V. R. Potter and C. A. Elvehjem, J. Biol Chem 114, 495 (1936).

Because of the extremely low enzyme activity (approaching zero) found in the livers from animals fed both diets I and II, any difference between these groups was completely obscured. The low protein, threonine-deficient diets, wet or dry, were apparently sufficiently severe nutritionally to cause a rapid and precipitous fall in the xanthine oxidase activity. In order to measure any differences which might in fact exist, a second experiment was designed in which all the rats were fed the basal diet supplemented with 0.36% DL-threonine at the expense of sucrose for a period of seven days. At the end of this time, 12 animals were sacrificed, six were used for the assay to provide "zero time" values, and six for moisture, fat and nitrogen analysis. Of the remaining animals, 12 were fed the basal threonine-deficient diet fed dry (Group I), and 12 the basal threonine-deficient diet fed wet (Group II). After four days of feeding these experimental diets, the animals were sacrificed and treated as described for the enzyme assay and the liver analysis.

Standard errors of the means were calculated for all data, and the Student's t test used as a measure of significance. Unless otherwise stated, $P < 0.01$ was accepted as a significant difference between means.

RESULTS

Experiment 1

The weight gain and food and water consumption data for the rats used in the enzyme analysis are presented in Table 1. The same data for the animals used for the liver analysis are seen in Table 2. No significant differences are observed between groups fed the basal diet dry or the basal diet plus 50% water for the same experimental period. Rats fed the dry diets consumed more water, as expected since the water consumption figures do not include the water provided by the diet.

Table 3 shows the liver composition data. No significant differences are observed between the wet and the dry groups in either percent moisture or nitrogen. The only significant difference in percent fat is found in those animals fed the wet diet for the 2 day period: liver fat is increased significantly ($P < 0.01$) over that of the group fed the dry diet.

The enzyme analyses are reported in Table 4 as micromoles of xanthine disappearing per hour per gram of liver

tissue. The zero time value is 5.67 micromoles per hour per gram tissue, and there is a rapid fall in enzyme activity in animals fed both the wet and the dry diets. At 2 days, the decrease in enzyme activity in both groups is significant at 5%, and at 7 days, significant at 1%. By 14 days, there is virtually no activity left, and it is not possible to calculate standard errors for these data. Though the enzyme activities are higher numerically in livers from rats fed the wet diet as compared with the dry, the differences were not significant. The failure to establish significance might be due to the extremely low activity of this system in both groups. Litwack et al. (1953) using the same 1 ml homogenate system, observed a range in enzyme activity for normal rats from 7.8 to 22.0 micromoles per hour per gram of tissue.[1] The threonine-deficient diet consumed by the animals in this study apparently causes such a severe reduction in the xanthine oxidase activity that any differences due to the feeding of the diet wet or dry are obscured. Consequently the experiment was repeated with modifications to obtain more readable results. In the second experiment,

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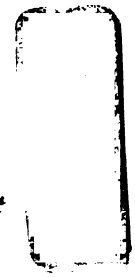
[1]These values are probably from adult rats, although the authors do not specify. In this laboratory a three week old stock rat had a xanthine oxidase activity of 8.8 micromoles per hour per gram tissue.



an attempt was made to place the animals in better nutritional condition prior to imposing the amino acid imbalance. To do this, the amino acid imbalance was corrected though the diets were still low in protein (9% casein). The animals were fed the basal diet supplemented with threonine for 7 days, then changed to the basal diet deficient in threonine, fed either wet or dry as before, for a period of four days. The following results were obtained.

Experiment 2

Tables 5 and 6 show the weight gain, food and water consumption data for the animals used for the enzyme and liver analyses respectively. No significant differences are observed between the rats fed the deficient diet, wet or dry. These data corroborate those from Experiment 1.

Liver composition data are presented in Table 7. There are no significant differences between any of the groups in percent moisture or nitrogen. However, there is a significant increase ($P < 0.01$) in liver fat concentration in the group fed the wet deficient diet as compared with the dry deficient diet.

Table 8 shows the enzyme activity data. Feeding the basal diet supplemented with threonine causes no significant decrease in the enzyme activity from the "zero time" values.

When animals are then fed the deficient diet dry for 4 days, there is a decrease in enzyme activity significant at the 5% level. However, when rats are fed the wet threonine-deficient diet, this depression in xanthine oxidase activity is not seen. In these animals xanthine oxidase activity is maintained at the "zero time" level, even though the diet protein is less adequate.

Table 1. Food intake, water consumption and weight gain of rats[1] fed low protein, threonine-deficient diets with and without the addition of 50% water.

Length of Experimental Period	Diet Treatment	Total Weight Gain g	Total Food Consumption g	Total Water Consumption[2] g
2 days	dry	2.2 ± 1.0[3]	12.8 ± 0.3[3]	17.3[3]
	wet	0.5 ± 1.2	9.2 ± 0.8	19.0
7 days	dry	17.5 ± 1.0	58.8 ± 2.6	50.0
	wet	19.3 ± 1.1	57.1 ± 2.1	33.0
14 days	dry	39.5 ± 1.6	147.8 ± 4.0	148.3
	wet	45.3 ± 2.3	130.5 ± 4.1	72.3

[1]Livers taken from these rats were analysed for xanthine oxidase activity. Enzyme data are presented in Table 4.

[2]Total ad libitum water intake not including that provided by the diet.

[3]SE

Table 2. Food intake, water consumption and weight gain of rats[1] fed low protein, threonine-deficient diets with and without the addition of 50% water.

Length of Experimental Period	Diet Treatment	Total Weight Gain g	Total Food Consumption g	Total Water Consumption[2] g
2 days	dry	3.0 ± 0.6[3]	12.7 ± 0.8[3]	15.7[3]
	wet	1.0 ± 1.4	10.1 ± 1.0	8.8
7 days	dry	20.2 ± 1.3	65.5 ± 2.3	62.3
	wet	21.0 ± 1.7	59.5 ± 1.0	28.2
14 days	dry	48.5 ± 2.7	152.8 ± 2.3	156.8
	wet	57.2 ± 2.0	150.2 ± 2.5	87.4

[1]Livers taken from these rats were analysed for moisture, fat and nitrogen. These data are presented in Table 3.

[2]Total ad libitum water intake not including that provided by the diet.

[3]SE

Table 3. Liver composition of rats fed low protein, threonine-deficient diets with and without the addition of 50% water.

Length of Experimental Period	Diet Treatment	Liver Composition		
		Moisture %	Fat % dry weight	Nitrogen % fresh weight
2 days	dry	68.7 ± 0.6[1]	11.4 ± 0.9[1]	2.09 ± 0.03[1]
	wet	69.4 ± 1.0	15.3 ± 0.9*	2.18 ± 0.09
7 days	dry	68.5 ± 0.5	17.3 ± 2.0	2.31 ± 0.03
	wet	68.2 ± 1.0	21.4 ± 2.0	2.40 ± 0.11
14 days	dry	66.5 ± 0.4	25.1 ± 1.0	2.18 ± 0.06
	wet	65.3 ± 0.5	23.3 ± 2.3	2.09 ± 0.04

*Significant from dry group at the 1% level.

[1]SE

Table 4. Xanthine oxidase activity in rats fed low protein, threonine-deficient dietswith and without the addition of 50% water.

Days on Diet	Xanthine Oxidase Activity[1]	
	Dry Diet	Wet Diet
0	5.67 ± 0.69	
2	2.45 ± 1.0[2]*	3.09 ± 0.78[2]*
7	2.24 ± 0.9 **	2.27 ± 0.37 **
14	0.72	0.0

[1]Xanthine oxidase activity calculated as micromoles of xanthine disappearing per hour per gram of tissue.

[2]SE

*Difference significant from zero time value at $P<0.05$.

**Difference significant from zero time value at $P<0.01$.

Table 5. Food intake, water consumption and weight gain of rats[1] fed low protein, threonine-supplemented diets, and low protein, threonine-deficient diets with and without the addition of 50% water.

Group	Total Weight Gain g	Food Consumption		Total Water Consumption[2] g
		Total Supplemented g (week 1)	Total Deficient Diet g	
threonine-supplemented	17.0 ± 0.9[3]	51.3 ± 1.8[3]	-	58.5
threonine-deficient, dry	37.0 ± 1.5	58.4 ± 1.6	47.1 ± 1.7[3]	113.5
threonine-deficient, wet	36.0 ± 2.2	55.8 ± 2.3	44.7 ± 1.1	89.8

[1]Livers taken from these rats were analysed for xanthine oxidase activity. Enzyme data presented in Table 8.

[2]Total ad libitum water intake not including that provided by the diet.

[3]SE

Table 6. Food intake, water consumption and weight gain of rats[1] fed low protein, threonine-supplemented diets, and low protein, threonine-deficient diets with and without the addition of 50% water.

Group	Total Weight Gain g	Food Consumption		Total Water Consumption[2] g
		Total Supplemented g (week 1)	Total Deficient Diet g	
threonine-supplemented	17.0 ± 1.5[3]	50.2 ± 2.3[3]	-	59.7
threonine-deficient, dry	30.0 ± 2.0	53.3 ± 1.8	38.1 ± 2.1[3]	104.8
threonine-deficient, wet	33.0 ± 2.0	53.1 ± 1.8	41.4 ± 1.1	89.8

[1]Livers from these rats were analysed for moisture, fat and nitrogen. These data are presented in Table 7.

[2]Total ad libitum water intake not including that provided by the diet.

[3]SE

Table 7. Liver composition of rats fed low protein, threonine-supplemented diets, and low protein, threonine-deficient diets with and without the addition of 50% water.

Group	Liver Composition		
	Moisture %	Fat % dry weight	Nitrogen % fresh weight
threonine-supplemented	67.7 ± 3.0[1]	11.6 ± 2.1[1]	2.84 ± 0.3[1]
threonine-deficient, dry	71.8 ± 0.9	9.65± 1.0	2.30 ± 0.1
threonine-deficient, wet	69.6 ± 2.2	14.0 ± 0.7**	2.37 ± 0.1

[1]SE

**Significant difference from threonine-deficient dry group at $P < 0.01$.

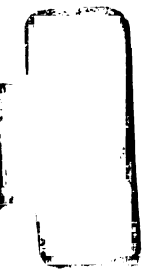
Table 8. Xanthine oxidase activity in rats fed low protein, threonine-supplemented diets, and low protein, threonine-deficient diets with and without the addition of 50% water.

Group	Xanthine Oxidase Activity[1]
initial	5.67 ± 0.69[2]
threonine-supplemented	5.46 ± 0.49
threonine-deficient, dry	4.07 ± 0.28*
threonine-deficient, wet	5.28 ± 0.60

[1]Xanthine oxidase activity calculated as micromoles of xanthine disappearing per hour per gram of tissue.

[2]SE

*Difference significant from zero time value at $P < 0.05$.



DISCUSSION

The xanthine oxidase activity of the liver is very sensitive to the quality and quantity of the protein present in the diet (Litwack et al., 1952). In this study the xanthine oxidase activity is extremely labile in threonine deficiency. After two days of feeding the deficient diet, the enzyme activity is so low that differences between the wet and dry treatments could not be measured.

When the animals are fed the threonine-supplemented diet for a week, the enzyme activity does not decrease from "zero time" values, although it does not increase as it would in a normal animal because the diet is still low in protein. However, the animal is in a sufficiently improved nutritional state to better withstand the stress of the deficient diets. When 50% water is added to the diet, no significant decrease in enzyme activity is noted. The decrease obtained by feeding the same diet dry is significant at 5%, and there is no difference between the enzyme activities of the animals fed the threonine supplemented diet and the deficient diet plus water. Thus the addition of moisture

to a low protein, threonine-deficient diet improves the "protein quality".

This increase in the xanthine oxidase activity may be indicative of an increased utilization of the restricted diet protein. Yoshida et al. (1966) suggests that there is an increased incorporation of the limiting amino acid into protein in the livers of animals fed a threonine-deficient diet as compared with control diets. This effect is seen within a very short time, 3.5 to 8 hours. They propose a mechanism responsive to the altered plasma ratio of amino acids induced by an amino acid imbalance which in turn causes a precipitous decrease in food consumption. Since the addition of water to the diet increases the efficiency of protein utilization, indicated by the increased xanthine oxidase activity in rats fed the wet diet, this may more rapidly produce the altered plasma amino acid pattern and the "critical ratio" (see pages 12-15) in the animals fed the wet diet than the same changes occur in those fed the diet dry. Increased protein metabolism in the liver could also explain the elevated liver lipids in the animals in the wet group if more of the most limiting amino acid is used for protein synthesis, leaving less available for the control of fat deposition.

SUMMARY

Within two days after feeding weanling rats a low protein, threonine-deficient diet dry or wet, the liver xanthine oxidase activity is so drastically decreased that any differences caused by the diet treatments are not measureable. When the nutritional status of the animals is improved by feeding a 9% casein diet supplemented with threonine for seven days prior to feeding the deficient diet, differences in enzyme activity due to feeding the imbalanced diet wet are observed. The animals fed the deficient diet dry have a xanthine oxidase activity decreased significantly ($P < 0.01$) from "zero time" values, while no such decrease is caused by feeding the deficient diet wet. Thus the addition of 50% water to a low protein, threonine-deficient diet produces an increase in the efficiency of protein utilization, and hence an improvement in protein quality.

GENERAL DISCUSSION AND SUMMARY

Additions of 20, 50 or 80% moisture to a 9% casein, threonine-deficient diet cause a decrease in weight gain, food and water consumption and Protein Efficiency Ratio in weanling rats. Moreover, liver lipids are elevated in animals fed the deficient diet plus 50% water.

These effects are apparently not caused by chemical interaction between water and the other diet components in the food cup prior to ingestion. When the diet containing 50% moisture is allowed to remain at room temperature for 24 hours and then freeze-dried before feeding, liver lipids are not elevated as observed when the diets are fed wet.

The addition of water to the threonine-deficient diet increases the efficiency of utilization of the dietary amino acids as measured by the xanthine oxidase activity of the liver after four days of feeding the diet. This may appear to contradict the data obtained in Part I, in which the PER was decreased with increasing water in the diet. However, in the imbalanced animals receiving the wet diet, two different mechanisms are apparently in operation to

maintain homeostasis. Initially the efficiency of utilization of the amino acids is increased in the animals fed the deficient diet wet as compared with those fed the same diet dry, using xanthine oxidase activity of the liver as the criterion. However, this initial increase in the efficiency of protein utilization is followed by a decrease in the food intake in response to the altered plasma amino acid pattern (Yoshida et al., 1966). This pattern is more rapidly distorted towards the "critical ratio" (see pages 12-15) in the rats fed the wet diet than is the case with those fed the dry diet. Thus the increased availability of the amino acids in the imbalanced diet induced by the addition of water serves only to aggravate the situation caused by the imbalance, and over the two week experimental period results in a significantly decreased food consumption, weight gain and PER.

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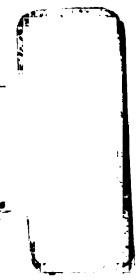

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