THE EFFECTS OF SUPPLEMENTARY THREONINE AND/OR CHOLINE ON ENZYME ACTIVITY AND FAT DEPOSITION IN LIVERS OF ALBINO RATS FED 9% CASEIN DIETS

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Betty Bondurant Brown

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by

Betty Bondurant Brown

AN ABSTRACT

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

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ABSTRACT

Threonine and choline deficiencies in a low protein diet are known to induce different types of fatty livers. The histological difference in these livers indicates that fat is deposited by different routes. In this experiment the amount of fat deposited and enzyme activity were studied to determine if they, too, differed in these two types of fatty livers.

Forty weanling, male, albino rats were divided into four groups and fed a 9% casein diet containing supplements of threonine and/or choline or neither. Food and water were allowed ad libitum throughout the three week experimental period.

The animals were sacrificed by decapitation. Livers were analyzed for fat, nitrogen and water content; and for DPN cytochrome-c reductase and fatty acid oxidase activity.

There was little or no difference in the nitrogen content, amount of fat deposited, or the activity of the DPN cytochrome-c reductase system in the livers of the choline deficient rats as compared with the threonine deficient rats. When the threonine deficiency was superimposed on the choline deficiency there was likewise no difference in fat and nitrogen content of these livers, but the activity of the DPN cytochrome-c reductase system was significantly depressed in this group. Fatty acid oxidase activity was depressed by a choline deficiency whereas the threonine deficient group and the doubly deficient group maintained fatty acid oxidase activity

equivalent to that of the threonine and choline supplemented group.

It was suggested that the chief difference between choline induced and threonine induced fatty livers might be the rate at which adaptation takes place. However, further analysis must be made in order to test this hypothesis.

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INTRODUCTION

INTRODUCTION

In normal animals the lipid content of the liver is approximately five percent of the wet weight of tissue. Under certain conditions, however, the fat content of the liver may rise considerably to a point where the organ is characterized as being a "fatty liver". Starvation, high fat diet, feeding of cholesterol, carbon tetrachloride or phosphorus poisoning, choline and methionine deficiency, and threonine deficiency on a low protein diet are a few of the causes of fatty liver (Fruton and Simmonds 1958). Thus, fatty infiltration of the liver can have a number of apparently unrelated causes, and prevention and cure of the abnormality probably involves a variety of biochemical processes.

In recent years two types of fatty livers have received much attention and subsequent study. It is believed that the role of choline in lipid metabolism is to facilitate fat transport by its effect on phospholipid synthesis and turnover (Fruton and Simmonds 1958). Although threonine has been established as a lipotropic factor on a low protein diet, its role in lipid metabolism is not clear. Several enzyme systems have been studied and various theories advanced as to the mode of action of threonine as a lipotropic agent.

It is known that the lipotropic action of threonine is not observed unless choline or methionine is present in the diet in adequate amounts. Thus, some correlation between

the two lipotropic factors has been sought. This relationship is complex for one very obvious reason -- each substance is involved in other roles besides lipotropism and it is difficult to ascertain which roles take precedence and which are secondary to the lipotropic action, especially when there is a limited supply of one or both of the factors.

Further investigation was stimulated by the fact that fatty livers, induced by the absence of each of these lipotropic factors individually, were histologically different; and, hence, the route of fat deposition was probably different.

Some work has been done in comparing the effect of a threonine deficiency vs a choline deficiency on fat deposition and enzyme activity in the livers of these animals. The purpose of this study was to further investigate and compare those two types of fatty livers re: the fat content and two enzyme systems of the liver -- fatty acid oxidase and DPN cytochrome-c reductase.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The fact that choline is a potent lipotropic factor in the liver was established by Best and co-workers in 1932.

Subsequent studies indicated there were other factors besides choline involved in this lipotropic action. Tucker and Eckstein (1937) and Channon, Manifold and Platt (1938) were the first to report that the deposition of lipids in livers of rats fed low protein rations deficient in choline could be reversed by feeding not only choline but also methionine and cystine. The lipotropic action of methionine was shown to be dependent on the ability of this compound to furnish methyl groups for the synthesis of choline (Tucker and Eckstein 1937).

Several groups of workers (Channon, et al 1938, 1940; Best and Ridout 1940; Tucker and Eckstein 1938) have suggested that some proteins are more effective in reducing the liver lipids which accumulate during choline deficiencies than would be expected from their methionine and cystine content. This led to many attempts to demonstrate a lipotropic action for other amino acids and protein, and consequently, much controversy. Tucker, Treadwell and Eckstein (1940) maintained that the lipotropic effects of protein could be accounted for solely by the cystine: methionine balance. In support of this theory Beeston and Platt (1939), Setton and Grail (1942), Channon, Mills and Platt (1943) and Eckstein

(1952) failed to produce evidence that specific amino acids other than methionine and cystine influence the deposition of fat in the livers of animals receiving diets deficient in choline.

Other workers (Dick et al 1952) suggested that some fatty livers may reflect a nonspecific effect of protein deficiency rather than a specific lack of any one essential amino acid. According to this theory, inadequate dietary protein might lead to a faulty production of protein in the cells of the liver or to faulty formation of the lipotropic complexes characteristic of this tissue.

Evidence began to accumulate which indicated that fatty livers could be produced by a deficiency of an amino acid other than methionine or cystine. Singal et al (1953a) and Harper et al (1954a) agreed that the secondary lipotropic effect of protein (the sparing of choline by methionine being the primary) is not a choline sparing action, but results from the provision of certain amino acids; i.e. threonine, a deficiency of which causes fat to accumulate in the liver. This effect was observed only when the diet contained either choline or methionine in amounts approaching what is considered to be the requirement.

Harper et al (1954b) reported that the amount of fat deposited in the livers of young rats receiving choline was dependent on the interaction of several factors. They considered threonine to be of major importance in low casein

diets but warned that when proteins of different amino acid composition were fed the proper balance of other amino acids must be kept in mind.

This observation confirmed that made by Singal *et al* (1949) while studying niacin-tryptophan relationships. in rats fed a 9% casein ration containing choline. They found that under these conditions liver lipids developed to the extent of 16.0% (dry wt.) whereas the addition of threonine to the diet reduced the liver lipids to 5.1% (dry wt.). However, the addition of threonine also created an amino acid imbalance which markedly decreased growth. To correct this imbalance, niacin or tryptophan was added to the diet and the rats grew at a more rapid rate. Under these conditions liver lipids were 14.4% in the absence of threonine and 5.9% when threonine was supplemented to the diet.

Since the amount of fat deposited in the livers of rats receiving choline decreased slightly as the amount of dietary fat increased, Harper *et al* (1954b) concluded that the liver fat arose from the conversion of carbohydrate or protein to fat. Further evidence supporting this conclusion was obtained from the observation that high fat deposits occurred in animals on a fat free diet (Harper *et.al* 1954b).

The specific lipotropic effect of threonine was conclusively established by several workers. By increasing the choline content of the basal ration (9% casein) threefold and raising the methionine to 0.3%, Harper and co-workers

(1953) observed some reduction of liver fat, but not to the extent which was obtained with the addition of just 0.18% DL threonine. They were also able to demonstrate a reversable effect of threonine by withdrawing it from the diet after two weeks of supplementation and observing a subsequent increase in liver fat. Inclusion of threonine for two weeks after the rats were fed the deficient diet two weeks reduced the accumulation of liver fat.

The effects of choline, methionine and additional protein on fat deposition in the livers of rats fed low protein diets were studied by Harper et al (1954a). They, too, found that neither choline nor methionine in amounts sufficient to meet the stated requirement of the rat for growth and prevention of fat infiltration prevented some excess of fat from accumulating in the liver. Only when either the protein or threonine content of the diet was increased was the fat content of the liver reduced to what is considered the normal range. These results were in agreement with those obtained by Singal et al (1953b) and Nino-Herrera and co-workers (1954).

That the lipotropic action of threonine was not the result of increased food intake was demonstrated in a paired feeding experiment by Singal et al (1949). The liver lipids of rats pair-fed a casein ration supplemented with tryptophan was 14.4% compared to 5.9% when threonine was included. They also found that the livers of rats on an amino acid diet simulating the 9% casein ration supplemented

with tryptophan contained 17.0% lipids, and supplementary threonine reduced the lipids to 5.3%. When a diet completely free of threonine was fed, the rats lost weight and the liver lipids were only slightly above normal.

After threonine was proven to be a lipotropic factor in low protein diets, much interest was directed toward determining the mode of action of threonine. The accumulation of abnormal amounts of liver lipids in choline deficiency is usually explained as a failure of fat transport. In the threonine deficient animal in which the level of dietary fat is low, the accumulation of abnormal amounts of liver lipids is difficult to explain.

A study of the biochemical changes in the livers by Sidransky and Farber (1958b) revealed that the liver protein content of animals on a threonine free diet was the same as -- or slightly greater than -- that of the control animals. Protein synthesis in the liver as measured by radioactive amino acid incorporation into liver protein was also not decreased in the rats on the threonine free diet, and in three experiments increased amino acid incorporation was observed. They point out that the failure to find a general inhibition of protein synthesis with a threonine free diet does not rule out selective inhibition of the synthesis or turnover of one or more liver enzymes in these animals. Dick et al (1952) and Singal et al (1953a,b) also indicated the possibility that the liver fat was due to reduced enzyme

function.

Singal et al (1953b) found that a threonine deficiency depresses the rate of synthesis of phospholipids and nucleoprotein phosphorus fractions of the livers, while supplementary threonine stimulated the turnover to near normal values. They stated that this could be effected through a reduction in the amount of enzymes due to an inadequate supply of amino acids to be synthesized into enzymes, or an impairment of the system by which these enzymes are elaborated. In regard to the latter, Spiegelman and Kamen (1946) have suggested that nucleoprotein may be "specific energy donators" which make possible reactions leading to protein and enzyme synthesis.

A study of the effects of feeding a protein free diet on the activities of oxidative enzymes was reported by Wainio et al (1953). They found that DPN cytochrome-c reductase decreased in unit activity and total activity when the depleted animals and their pair-fed controls were compared. A general decrease in activity per unit of nitrogen indicated that the enzyme proteins were lost more rapidly than was total protein.

Pilsum, Speyer and Samuels (1957) while studying the effects of protein and amino acid deficiencies on several enzyme systems concluded that the enzymes studied seemed to divide into two groups: those which change with the protein of the organ irrespective of the cause of protein alteration,

and those which decrease with the absence of a given amino acid in the diet irrespective of the total protein of the organ. Their results indicated (a) the missing amino acids do not play a role in the structure of the first group while they are vital to the formation of the second; (b) the turnover of the second group is more rapid and, therefore, interference with synthesis would appear earlier; or (c) the system synthesizing the first group have priority over the second in the utilization of the limited amount of the deficient amino acid in circulation. The effects of the amino acid deficiency appear to depend upon the relative "priority" of the particular protein for the available amino acid supply as a whole.

Arata (1959) observed profound disturbances in the metabolism of adenosinetriphosphate (ATP) and diphosphopyridine nucleotide (DPN) with a concommitant disruption of the ATP — and DPN -- dependent enzyme systems in fatty livers induced by a threonine deficiency. She states that the fat accumulation is probably due to a loss in the ability of that organ to oxidize fatty acids, especially since ATP is necessary for activation of fatty acid oxidation and DPN is an obligatory hydrogen acceptor in the fatty acid cycle (Van Baalen and Gurin, 1953).

A similar observation was made by Dianzani (1955, 1957) who studied the ATP and DPN content of fatty livers induced by CCl_4 poisoning and choline deficiency.

As a result of the many theories involving enzyme function as affected by threonine deficiency, much research has been undertaken in that area. Harper et al (1953) observed that the addition of threonine to a 9% casein diet resulted in a reduced deposition of fat in the liver and a concommitant increase in the activities of certain soluble liver enzymes and in endogenous oxidation and decarboxylation. A decrease in the activity of certain mitochondrial enzymes in the liver was also observed.

Arata and co-workers (1954) observed that the inclusion of 0.36% threonine to a basal diet (9% casein supplemented with choline, tryptophan and methionine) caused a significant increase in endogenous oxidation and decarboxylation. In their experiments, there was no significant effect of threonine on the activities of the mitochondrial enzymes (succinic, choline and pyruvic oxidases). These latter findings were not consistant with those of Harper et al (1953). It was also observed that the accumulation of fat in the liver does not, in itself, cause the depression of the endogenous respiration and the two soluble enzymes observed.

In a later study (Arata et al 1956) of the pyridine nucleotide-linked enzyme systems, they concluded that both a defect in DPN production and the improper metabolism of endogenous DPN in the liver are major factors in liver fat accumulation in a partial threonine deficiency.

Few reports are included in the literature of a

comparison of fatty livers induced by a threonine deficiency with those caused by a deficiency of choline. Arata and co-workers (1954) observed, when choline was omitted from the basal ration and threonine added, a significant increase in endogenous oxidation and decarboxylation and in the activities of two soluble enzyme systems (tyrosine oxidase and xanthine oxidase). However, the fat content of the liver was not lowered when threonine was supplemented. Singal and co-workers (1953a) also observed that when choline was omitted from the basal diet, supplementation with threonine caused no reduction in the level of fat in the liver.

A comparison of the type of fat deposition in threonine vs choline deficient diets was made by Sidransky and Farber (1958a). While studying the morphologic changes in immature rats fed a threonine free diet, they found that the lobular distribution of the liver lipid differs with the different types of fatty liver. In choline deficiency, the fat appears about the central vein and then progressively involves the remainder of the lobule. In contrast to this, when fatty livers are induced by protein and amino acid deficiencies, a periportal lipid distribution is found in the liver.

This investigation was undertaken to study further the similarities and dissimilarities between the choline induced vs threonine induced fatty livers. Two enzyme systems -- fatty acid oxidase and DPN cytochrome-c reductase -- were chosen for study and the results are presented in this paper.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Forty male weanling rats of the Sprague-Dawley strain weighing from 41 to 52 grams were divided into four groups of ten animals each and were maintained in individual screen-bottom cages in an air conditioned room. A nine percent casein diet supplemented with 0.15% choline and/or 0.36% DL threonine was fed ad libitum throughout the experimental period of three weeks. The composition of these diets is given in Table I. Weights of the animals were taken every four or five days and prior to sacrificing them.

At the termination of the experimental period, the rats were stunned by a sharp blow on the head, decapitated, and the livers excised for the determination of fatty acid oxidase, DPN cytochrome-c reductase, fat and nitrogen. Immediately after removal, the livers were chilled by immersion in ice and then blotted free of moisture. Weighed portions (2 to 3 gms.) of each liver were homogenized with two volumes of ice cold 0.039M sodium potassium phosphate buffer (pH 7.3) using a Potter-Elvehjem type homogenizer. The chilled liver homogenates were pipetted into previously prepared, chilled Warburg flasks. In the determination of both enzyme systems, carbon dioxide was adsorbed on pieces of filter paper saturated with 0.2 ml. 10% KOH in the center well of each flask. The flasks were mounted on manometers and immersed in a constant temperature water bath maintained at 25°C. A five minute temperature equilibration period was

allowed for all flasks before the manometers were adjusted to 15 and the systems closed. Thereafter, readings were taken at five minute intervals for thirty minutes. Calculations were based on 15 minutes for fatty acid oxidase and 20 minutes for DPN cytochrome-c reductase; the activity of the respective enzymes decreased after this interval of time. Results are expressed as ul O_2/hr/unit weight fresh liver tissue and ul O_2/hr/unit weight of nitrogen.

The substrate for DPN cytochrome-c reductase (0.2 ml. of 0.5% DPN) was tipped into the main compartment of the flask from the side arm following temperature equilibration. In the fatty acid oxidase system, the octonoate was pipetted directly into the main compartment.

The fatty acid oxidase activity of the liver homogenates was determined by the method of Colowick and Kaplan (1955) with 0.6 ml. sodium potassium phosphate buffer (pH 7.3), 0.3 ml. sodium octanoate and distilled water to make 3.2 ml. A control flask contained the same components except that the substrate was replaced by water.

DPN Cytochrome-c reductase activity of the liver homogenates was determined by the method of Potter (Umbreit et al 1951) with 0.4 ml. of 3×10^{-4} M cytochrome-c and buffer to make a total volume of 3.2 ml. Crude malic dehydrogenase was isolated from fresh pig liver according to the method of Potter (1946). The control flask was treated the same except 0.2 ml. of 0.5% DPN was replaced

with distilled water.

The remaining portions of the livers were weighed, homogenized with water and dried for 12 hours at 90°C. The dried liver homogenates were cooled, weighed and ground to a powder. Fat content was determined by ether extraction of weighed samples (appx. 1 gm.) in a Goldfisch fat extractor. The fat content of these samples was expressed as percent of dry weight of tissue.

Nitrogen was determined on the dried fat free liver by the macro Kjeldahl method. Kjeldahls were run in duplicate on 0.350 gm. samples; copper sulfate was used as a catalyst. Percent nitrogen was calculated on the basis of dry and fresh weight of the sample.

TABLE I.

COMPOSITION OF DIETS

INGREDIENTS	DIET			
	I (gm)	II (gm)	III (gm)	IV (gm)
Sucrose	80.80	81.20	81.00	81.40
Casein	9.00	9.00	9.00	9.00
DL Methionine	0.30	0.30	0.30	0.30
DL Tryptophan	0.10	0.10	0.10	0.10
Corn Oil[1]	5.00	5.00	5.00	5.00
Salts W[2]	4.00	4.00	4.00	4.00
Vitamin mix[3]	0.25	0.25	0.25	0.25
Choline	0.15	0.15	----	----
DL Threonine	0.36	----	0.36	----

1. Containing 7.5 mg. tocopherol, 0.38 mg. menadione.
2. Obtained from National Biochemicals Corporation.
3. Containing in milligrams per 100 grams ration: thiamin hydrochloride 0.5, riboflavin 0.5, niacin 1.0, calcium pantothenate 0.25, pyridoxine hydrochloride 0.25, biotin 0.01, folic acid 0.02, vitamin B_{12} 0.002, inositol 10.0, vitamin A 10.0, vitamin D 0.18, and sucrose to make 0.25 gm. of mix.

RESULTS

RESULTS

The results from this study are summarized in Tables II, III and IV.

The average weight gain in grams per week and the average percent liver fat (dry weight) for the four groups of animals are presented in Table II. The animals fed a 9% casein diet supplemented with threonine (Group III) or threonine and choline (Group I) showed significantly higher growth rates than did the animals fed diets which did not contain additional threonine (Groups II and IV). The inclusion of choline in the diet had no effect on the growth rate regardless of whether or not threonine was added. Group I (+ threonine and + choline) was not significantly different from Group III (+ threonine) nor was Group II (+ choline) significantly different from Group IV (no supplement), according to the standard error of the mean.

When neither threonine nor choline were supplemented to the 9% casein diet (Group IV), an abnormally high quantity of fat was deposited in the livers of the rats. The addition of either choline (Group II) or threonine (Group III) to this diet was ineffective in reducing the amount of liver fat. However, when both of these compounds were added to the 9% casein ration (Group I), the liver fat was significantly depressed. Although the standard error of the mean indicated a slight difference in the extent to which threonine was

effective in reducing liver fat in Group III as compared with Groups II and IV, in view of the variation and overlapping of the values within the groups, the author did not consider this difference significant.

The nitrogen content of the liver was calculated on the basis of wet weight of tissue. These data are summarized in Table III. Data for the percent nitrogen on the basis of dry weight are recorded in the Appendix (Table V). When the unsupplemented diet (Group IV) was fed to the animals, the nitrogen content of the livers was slightly less than when choline and threonine were supplemented (Group I). This difference indicated by the standard error of the mean was so slight it was not considered significant by the author upon considering the differences within the groups. There were no significant differences in liver nitrogen between Groups II, III and IV.

Although the nitrogen content of the livers remained fairly constant from one group to another, the amount of moisture in these tissues varied to a measurable degree (Table III). In fact, a comparison of the moisture content with the percent fat in the livers (Table II) revealed an inverse relationship; i.e., the higher percent fat, the lower the percent water, etc.

Enzyme activity data are reported as ul.O_2/ hr./ unit weight of fresh liver tissue in Table IV. Enzyme activity expressed as ul.O_2/ hr./ unit weight of nitrogen may be found in the Appendix (Table VI). The former unit of measure is used in this report because fluctuations in the

nitrogen content of the livers did not follow fluctuations in enzyme activity.

DPN cytochrome-c reductase activity was greatly depressed in the livers of animals receiving no supplementary threonine or choline (Group IV) as compared with the supplemented group (I). The addition of either choline (Group II) or threonine (Group III) to the 9% casein diet resulted in an increase in the activity of this enzyme to equal that of the supplemented control (Group I). The removal of threonine or choline from the diet did not alter the DPN cytochrome-c reductase activity; only when both threonine and choline were omitted was the enzyme activity reduced to a significant degree.

Fatty acid oxidase activity in the livers of animals fed a diet containing no supplementary choline (Group III) was depressed to approximately 30% of the activity of the remaining three groups of animals. When threonine was not added to the diet, no significant effect on the activity of this enzyme was observed regardless of whether or not choline was present in the diet (Groups II and IV).

TABLE II.

Growth Rate and Liver Fat of Rats Fed 9% Casein Diet

GROUP	SUPPLEMENT	WEIGHT GAIN (gm./wk.)	FAT gm./100 gm. LIVER (dry weight)
I	0.15% choline 0.36% DL threonine	32.3 ± 1.3*	11.4 ± 0.6*
II	0.15% choline	20.7 ± 1.1	22.3 ± 1.7
III	0.36% DL threonine	30.4 ± 1.4	18.2 ± 1.3
IV	no supplement	20.9 ± 1.5	24.7 ± 1.7

*Standard error of mean

TABLE III

Nitrogen and Water Content of Livers From Rats Fed 9% Casein Diets

GROUP	SUPPLEMENT	N/100 gm. LIVER (wet weight)	H_2O gm./100 gm. LIVER
I	0.15% choline 0.36% DL threonine	2.7 ± 0.2*	74.9 ± 0.7*
II	0.15% choline	2.2 ± 0.1	71.3 ± 0.6
III	0.36% DL threonine	2.3 ± 0.1	73.1 ± 0.7
IV	no supplement	2.1 ± 0.1	70.3 ± 1.1

*Standard error of mean

TABLE IV.

Activities of Two Enzyme Systems in Liver Tissue of Rats Fed 9% Casein Diets

GROUP	SUPPLEMENT	DPN Cytochrome-c Reductase ul.O_2/hr./10 mg. LIVER	Fatty Acid Oxidase ul.O_2/hr./gm. LIVER
I	0.15% choline 0.36% DL threonine	177 ± 6	253 ± 64
II	0.15% choline	167 ± 8	374 ± 85
III	0.36% DL threonine	161 ± 14	103 ± 39
IV	no supplement	115 ± 9	328 ± 52

DISCUSSION

DISCUSSION

In this experiment, threonine was the limiting factor for growth. Maximal growth rates (approx. 30 gm./ week) were attained only when threonine was present in the ration; the presence or absence of choline had no effect on the growth rate. Some correlation was noted between the rate at which the animals grew and the amount of nitrogen present in 100 gm. of dry weight of liver tissue. Livers from rats growing at a less rapid rate contained less nitrogen than did those from rats growing more rapidly, as would be expected. This correlation was not observed when nitrogen was calculated on the basis of fresh weight of tissue.

The fact that the addition of choline alone or threonine alone to a 9% casein diet was insufficient to reduce liver fat has also been observed by other workers (Singal *et al* 1953b, Harper *et al* 1954b, and Nino Herrera *et al* 1954). Only when both of these factors are present in adequate quantities is liver fat reduced to a more normal value. It has been shown that the threonine induced fatty liver is histologically different from the choline induced fatty liver (Sidransky and Farber 1958a). Thus, in these two types of fatty livers, the fat must be deposited *via* different routes. However, under the conditions of this experiment, the two types were not additive since the fat deposited in the liver was no greater in quantity when both

choline and threonine were omitted from the diet than it was when either threonine or choline was omitted.

It was somewhat puzzling to observe that either choline or threonine was equally effective in increasing the activity of DPN cytochrome-c reductase to a level comparable with the positive control group. These data are in conflict with those of Arata et al (1959) who observed a significant depression in the activity of this enzyme system when threonine was not added to a 9% casein ration containing choline. A possible explanation for this disagreement is the fact that, in this study, the activity of DPN cytochrome-c reductase was determined at 25°C, while the optimum temperature for this enzyme system is 37°C (Umbriet et al 1951). The lower temperature was chosen in this study to make possible the determination of DPN cytochrome-c reductase and fatty acid oxidase simultaneously. It was found that the DPN cytochrome-c reductase system was active at 25°C, however, the activity was not maximal. As a result, small differences which might have existed between experimental groups might have been obscured. In any case, the activity of DPN cytochrome-c reductase did not parallel liver fat levels.

The activity of the fatty acid oxidase system appeared to be independent of the quantity of threonine available to the animals. There was no significant difference in the uptake of O_2 whether threonine was supplemented to the ration or not. These data do not agree with those of Arata et al

(1959) who observed that fatty acid oxidase activity in the livers of rats fed a threonine deficient ration was significantly lower than that in the livers of animals receiving threonine supplements.

However, Arata et al observed a tendency of this enzyme system to adapt to the deficiency of threonine, although their study was not of sufficient duration to measure the extent of this adaptation. In a previous paper, Carroll et al (1959) observed varying degrees of adaptation depending upon the enzyme system studied. If fatty acid oxidase is one of the systems which undergoes complete adaptation (as is the case with malic dehydrogenase), it is entirely possible that this system had already adapted in the study reported here.

This possibility illustrates the hazard involved in selecting a single time interval for analytical determinations in a syndrome which changes with time. Since both the liver fat and the enzyme systems tend to describe hyperbolic curves when plotted against time, the time of maximum differences is difficult to predict for a single analysis.

Though no effect on fatty acid oxidase activity was observed when threonine was removed from the supplemented control ration, a marked effect was noted when choline was removed from this diet. If one assumes that the fatty acid oxidase system has already adapted in the animals fed the threonine-deficient diet, then the inhibition observed in this system in animals fed a choline-deficient diet suggests

another interesting possibility. It is possible that the deficiency of choline places a greater stress on the rat than does a deficiency of threonine -- at least with respect to the adaptation of fatty acid oxidase. It may be that the albino rat cannot adapt to a choline deficiency, or that if such adaptation can take place its appearance is much more prolonged than it is when the diet is deficient in threonine.

The observation that the inhibition of the fatty acid oxidase system does not persist when a threonine deficiency is superimposed on a choline deficiency (Group IV) is puzzling. However, the rats fed the doubly-deficient diet (Group IV) grew less rapidly than those fed the choline-deficient diet (Group III). Therefore, it is possible that two factors were operating: (1) the slower rate of growth created less stress in the unsupplemented rats, allowing them to adapt more easily and (2) the slower growth rate in this group (IV) and the concommitant lower requirement for choline for purposes of growth would allow more of this compound to be used to maintain the activity of the fatty acid oxidase system.

The only significant differences in the two types of fatty livers produced in this experiment were reflected in the growth rate and the fatty acid oxidase activity of the livers. The threonine deficiency affected the growth rate adversly, but had no effect on the activity of either enzyme system studied, as compared to the control group (I). The rats fed the choline deficient diet (Group III) grew at a rate

comparable to the control group (I), but a significant depression in fatty acid oxidase activity was observed.

Thus, few biochemical differences were observed between these two types of fatty livers. Moreover, the differences which were noted might have been more qualitative than quantitative. It is possible that the primary difference between choline induced and threonine induced fatty livers is the rate at which adaptation takes place. This study should be repeated with more frequent analyses in order to test this hypothesis.

SUMMARY

SUMMARY

Forty, male, weanling albino rats were fed a 9% casein diet supplemented with choline and/or threonine. The control animals were fed the 9% casein ration plus choline and plus threonine. Food and water were allowed *ad libitum* for a period of three weeks. Records of weight gain were kept.

At the end of the experimental feeding period, the rats were killed and the livers were analyzed for fat, nitrogen, moisture, DPN cytochrome-c reductase and fatty acid oxidase activity.

Supplementary threonine was found to have significantly increased the growth rate, regardless of the presence or absence of the choline supplement. When threonine was omitted from the diet, the growth rate was only 65% of the threonine supplemented animals.

A deficiency of threonine, or choline, or both, resulted in an abnormally high deposition of fat in the livers of rats. When both threonine and choline were added to the diet, liver fat levels were reduced to normal. This effect was not observed when threonine or choline alone was supplemented. These data are in agreement with several published reports.

The nitrogen content of the livers remained fairly constant between groups, but the amount of moisture present

varied inversely with the fat content (dry weight).

When neither threonine nor choline were added to the diet, the activity of DPN cytochrome-c reductase was decreased. The addition of either threonine or choline was equally effective in increasing the activity of DPN cytochrome-c reductase to the level of the control group.

The presence or absence of threonine appeared to have no effect on the fatty acid oxidase activity in the liver. The removal of choline from the supplemented control ration, however, resulted in a marked decrease in the activity of the enzyme system; while the removal of both choline and threonine had no effect. The possibility of enzyme adaptation and the possible effect of the growth rate on these data are explained and discussed at length.

Under the conditions of this experiment, the two types of fatty livers produced were comparable in fat content, nitrogen content and DPN cytochrome-c reductase activity. The growth rate of the animals deficient in choline was near that of the supplemented control animals, but the fatty acid oxidase activity was greatly depressed on this diet. On the other hand, a deficiency of threonine had the reverse effect on these two factors. A double deficiency (threonine and choline) resulted in decreased growth rate and depressed DPN cytochrome-c reductase activity. In this group, the fatty acid oxidase activity was comparable to that of the control group.

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APPENDIX

TABLE V.

Liver Nitrogen of Rats fed 9% Casein Diets
Calculated on Basis of Dry Weight of Liver

GROUP	SUPPLEMENT	N gm./100 gm. LIVER (dry weight)
I	0.15% choline 0.36% DL threonine	10.8 ± 0.3*
II	0.15% choline	7.7 ± 0.3
III	0.36% DL threonine	8.7 ± 0.2
IV	No supplement	7.4 ± 0.4

*Standard error of the mean

TABLE VI.

Activities of Two Enzyme Systems in Livers of Rats Fed 9% Casein Diets, Calculated on Basis of Nitrogen Content

GROUP	SUPPLEMENT	DPN Cytochrome-c reductase ul.O_2/hr./mg. N	Fatty Acid Oxidase ul.O_2/hr./10 mg. N
I	0.15% Choline 0.36% DL threonine	656 ± 27*	73 ± 20*
II	0.15% choline	767 ± 42	177 ± 40
III	0.36% DL threonine	708 ± 68	61 ± 21
IV	No supplement	539 ± 42	160 ± 30

*Standard error of the mean.