THE EFFECT OF VARIOUS DIETARY FATS AND CHOLINE ON THE FATTY ACID COMPOSITION OF LIVER LIPIDS IN THREONINE DEFICIENT RATS

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ABSTRACT

THE EFFECTS OF VARIOUS DIETARY FATS AND CHOLINE ON THE FATTY ACID COMPOSITION OF LIVER LIPIDS IN THREONINE DEFICIENT RATS

by Ivy Mae Woolcock

Weanling rats fed a 9% casein diet supplemented with methionine and tryptophan but not threonine develop fatty livers. The chemical nature of the dietary fat, that is, the degree of saturation, the length of the carbon chain or the presence of unnatural isomers have been proposed as factors which influence the degree to which fat accumulates under these conditions. To test the effect of the unnatural isomers present in chemically hydrogenated fats, as well as the degree of saturation and chain length on liver fat deposition, different dietary fats with varied fatty acid composition were selected and incorporated into low protein, threonine deficient diets. The dietary fats selected included a natural hydrogenated fat (lard), a chemically hydrogenated fat (hydrogenated corn oil), cocoa butter, plus a variety of oils, such as corn, olive, safflower and coconut. These fats were added to the diet at a level of 30%. The level of choline in the basal diet (0.15%) proved to be inadequate for this quantity of fat in the diet and consequently a choline deficiency was induced. In an effort to determine the mechanism of the lipotropic action of choline, choline levels were increased to 1.00% of the diet.

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Animals were fed the low protein, high fat, threonine deficient diets with and without supplemented choline for four weeks. Food consumption, weight gain, liver moisture, fat and nitrogen content were determined. The coefficients of digestibility of the different dietary fats were determined. The fatty acid composition of diet and rat liver lipids were determined for both choline deficient and choline supplemented animals.

Threonine deficient animals fed hydrogenated corn oil (which contains 45% trans isomers) or cocoa butter (which contains 34% stearic acid) did not develop fatty livers. Animals fed natural corn oil, natural lard, olive oil, safflower oil and commercial lard had comparable liver fat levels of approximately 23% dry weight. Of all the dietary fats studied, coconut oil induced the highest liver fat concentration (28%). Choline supplementation significantly lowered liver fat levels for animals fed all dietary fats, but a more pronounced effect was observed in animals fed coconut oil. Analysis of fatty acid composition in livers of choline deficient animals showed an increase in linoleic and oleic acid levels. Upon the addition of choline to the ration, the hepatic concentration of stearic acid and arachidonic acid increased with a concomitant decrease in linoleic and oleic acid levels. This fatty acid pattern was consistent irrespective of the dietary fat fed. A common metabolic pathway was suggested for stearic acid and the trans isomers.

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The data support the concept that an increase in liver fat deposition in choline deficiency is due to interference with transport of fat through the liver.

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Ivy Mae Woolcock

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INTRODUCTION

That fatty livers could be produced experimentally in rats was first shown by Best and Huntsman (1932) by increasing the level of saturated fats in the diet to 40%. The addition of choline to these high fat diets prevented the accumulation of fat in their livers. Best, Huntsman and Ridout (1935) used the term lipotropic to describe substances which "decrease the rate of deposition or accelerated the rate of removal of liver fat."

Ever since the lipotropic action of choline has been demonstrated, extensive investigations have been conducted on the lipotropic action of protein and specific amino acids, choline, vitamins and other dietary factors. This review will be concerned primarily with the effect of the nature of dietary fat on liver fat deposition and the role of choline and the amino acid threonine in controlling liver fat levels. Experiments on the other factors will be cited tangentally because of the complex relationships that exist among the various lipotropic factors.

LIPOTROPIC FACTORS

Protein and amino acids

The fact that choline was not unique in its ability to prevent excessive fat accumulation in the livers of rats fed hypolipotropic diets was shown by Best and Huntsman (1935). These workers pre-fed a hypolipotropic diet of mixed grains and containing 40% of fat for

a period of three weeks. At the end of this period this diet was replaced by sucrose and the feeding period continued for 13 days. By replacing the hypolipotropic diet with sucrose, liver fat levels were elevated from 14% to 23% of the fresh weight of tissue. The addition of choline (75 mg/day) to the sucrose caused the reduction of the liver fat to 5%. When sucrose was supplemented with casein (20% of the diet) liver fat levels were also reduced, but not to the same extent as was observed when choline was supplemented to sucrose. Since casein prevented the increase in liver fat which occurred when sucrose alone was fed, a lipotropic role was postulated for casein. This lipotropic effect of casein was thought to be impurities such as betaines from the protein.

About this same time Channon and Wilkinson (1935) fed rats a 40% fat diet with increasing levels of protein (at the expense of glucose) and observed that as the levels of protein increased, the percent fat in the liver decreases. In this study, rats fed a 5% casein diet developed fatty livers to the extent of 13% on a fresh weight basis. When the level of casein was increased to 20%, liver fat was reduced to 7% and further reduced to 6% when the casein was increased to 50% of the diet. From these results, the authors proposed a lipotropic action for casein on the basis of the amino acid content rather than any impurities it may contain.

Beeston et al (1936) compared the liver fat concentrations of rats fed a 5% casein - 40% fat diet containing various levels of choline, to the concentration of liver fat in rats fed a 30% casein -

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40% fat diet. Livers of rats fed the low protein diet which contained no choline had liver fat levels of 20% (fresh weight basis). The inclusion of 0.1% or 0.2% choline in the low protein diet caused liver fat levels to fall to 9% and 6% respectively. However, livers from rats fed the 30% casein diet contained only 6% fat. From these results the authors postulated a lipotropic activity for casein separate from that of choline. More recent findings by Harper (1953a, 1953b) have confirmed the lipotropic action of increasing levels of protein.

These interesting results with protein led to further investigations in which the lipotropic action of various amino acids was studied. Beeston and Channon (1936) fed rats diets containing 5% casein and 40% fat. Animals developed fatty livers to the extent of 26% of the dry weight of the tissue. When this diet was supplemented with 0.2% or 0.6% cystine, the fat content of the livers was increased to 36% or 37%. When the level of casein was increased to 30%, liver fat accumulation was prevented and the addition of cystine did not increase the level of fat in the liver. The addition of lysine, glutamic acid, aspartic acid, serine, glycine and phenylalanine were ineffective in preventing liver fat accumulation.

This antilipotropic activity of cystine stimulated interest in the sulfur-containing amino acids. In 1937, Tucker and Eckstein found methionine effective in preventing fatty livers in rats consuming choline deficient diets. This lipotropic effect of methionine was attributed to its choline-sparing action. By supplementing low

choline diets with deuterium-labeled methionine, du Vigneaud (1941) demonstrated that the methyl groups of methionine are used for choline synthesis in the rat. A methionine-sparing action of choline was proposed by Engel (1948) and Alexander and Engel (1952).

Amino acid balance

The idea that factors other than the methionine content of a protein may be lipotropic led other workers to investigate the activity of various amino acids. In 1949 Singal et al showed that the addition of threonine to a 9% casein diet supplemented with cystine, choline and tryptophan resulted in a decrease of liver lipids in rats from 16.0% to 6.6%. When animals were pair-fed, liver fat levels were 14.4% and 5.9% respectively. In a subsequent experiment these workers (1953) produced fatty livers on both threonine and lysine-deficient diets, by using either a 9% casein diet or a mixture of amino acids simulating a 9% casein diet. About that same time Harper (1953) reported that liver fat levels produced in rats by feeding a 9% casein diet could be reduced by supplementing the diets with threonine, gelatin or casein. In subsequent studies, Harper et al (1954 a, 1954 b) demonstrated that the lipotropic effect of methionine was mediated through the synthesis of choline. However, when methionine and choline were adequate in the low casein diet threonine still exerted a lipotropic effect thus demonstrating a distinct and separate effect of threonine. Thus, threonine prevented extensive accumulation of liver fat which occurred when protein is provided in inadequate amounts.

Results of histological studies gave further evidence that deficiencies of amino acids other than methionine cause a defect in hepatic lipid metabolism which is different from that caused by choline deficiency. Best et al (1955) have shown that in contrast to the centralobular distribution of fat observed in the livers of choline deficient rats, protein or amino acid deficiencies cause a more periportal distribution of liver fats. Shil and Stewart (1954) demonstrated the difference between these two types of fatty livers in a series of experiments in which they fed various amounts of corn and casein to rats with and without additional choline. The imbalanced corn protein caused a periportal distribution of liver fat in the lobules, while the choline deficiency resulted in the centralobular distribution of liver lipid.

The fact that factors other than the absolute amount of amino acids in a protein can affect the lipotropic activity was shown by Harper et al (1954) giving rise to the concept of amino acid imbalance. Feeding rats a 9% casein diet supplemented with choline and tryptophan resulted in normal liver fat levels. The addition of 0.1% methionine resulted in liver fat accumulation. This could be reduced by supplementing threonine to the diet, or by pair-feeding the methionine-supplemented rats with a control group. The addition of methionine caused increased protein synthesis, thus precipitating the threonine deficiency. The authors concluded that the proportion as well as the absolute amounts of amino acids in a protein affects the deposition of fat in a low protein diet. Fatty livers thus may be produced by supplementing a low protein diet with the most limiting amino acid or

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acids (Harper 1954 and Winjie 1954) thus causing a deficiency of the second most limiting amino acid, usually threonine. A second type of imbalance may be created by supplementing the diet with the second most limiting amino acid or acids in the diet. Deshpande, Harper and Elvehjem (1958) fed rats 6% fibrin diets, in an attempt to determine the fate of the amino acids that were not used for protein synthesis. They found that four amino acids, leucine, isoleucine, valine and histidine were all about equal and first limiting for growth. Addition of 0.4% methionine and 0.6% phenylalanine (second most limiting amino acids) to 6% fibrin diets caused a growth retardation that was reversed with the addition of all four of the most limiting amino acids. These findings were later confirmed by Morrison and Harper (1960) who used an 8% casein diet and L-cystine or DL-methionine and added separately a series of amino acids in attempts to produce a growth retardation. Those diets that were supplemented with threonine, the second most limiting amino acid, showed growth depression. These experiments confirmed the earlier concept demonstrated by Litwack, Hankes and Elvehjem (1952), that one amino acid might be limiting for growth and another for fat deposition. They fed rats a 9% casein diet supplemented with either tryptophan or threonine. Under these dietary conditions tryptophan was found to be the most limiting for growth and threonine the most limiting for liver fat deposition. This complicated picture emphasized the relationship between protein synthesis and liver fat deposition.

Relationship of methyl groups to choline synthesis

At about the same time as the lipotropic activity of methionine was demonstrated by Tucker and Eckstein (1937), it was demonstrated by

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Womack, Kemmerer and Rose (1937) that methionine is an indispensable amino acid. These observations led to intensive investigations of the metabolism of the sulfur-containing amino acids during which it was found that homocystine could replace methionine in the diet of young rats as long as choline was provided (Du Vigneaud et al 1939). This suggested the methyl group of choline might be transferred to homocystine. It was evident from Du Vigneaud's studies in 1941 that the reverse process, the transfer of the methyl group of methionine to a choline precursor, occurred. This led to further investigations of the metabolism of methyl groups.

Borsook and Dubnoff (1947) reported that there were two types of methylation reactions; one in which methionine in an active form serves as the methyl donor and guanidinoacetic acid or nicotinamide serves as acceptors, and the other in which betaine serves as methyl donor and homocytine or homocysteine as acceptors. Since both methionine and betaine serve as precursors of choline in vivo, it has been assumed that the methyl groups of these compounds can be transferred to acceptors such as ethanolamine, monomethyl-ethanolamine or dimethyl-ethanolamine in the animal body. Thus the lipotropic activities of methionine and betaine have been accounted for by their role in providing methyl groups for the synthesis of choline, which is the compound that is required for the control of fat deposition.

Choline

The fact that choline, and not the methyl groups of choline, is the lipotropic agent was established by Welch (1937), and Welch and Landau (1942). They fed rats a high fat, low choline ration and supplemented with arseno-choline to determine whether the intact choline molecule or only the labile methyl group of choline was

responsible for the lipotropic effect. The lipotropic effect of arseno-choline was approximately equivalent to that of choline-chloride. Lecithins isolated from the liver after three weeks' treatment contained significant amounts of arsenic. Since arseno-choline contains no labile methyl group, the lipotropic activity of choline resides in the intact molecule.

The observation that choline is an essential component of phospholipids led early investigators to assume that liver fat deposition was the result of faulty phospholipid metabolism. As early as 1939, Perlman and Chaikoff began a series of studies on phospholipid metabolism in the liver using radioactive phosphorous (Perlman and Chaikoff 1939a; Perlman and Chaikoff 1939b; Perlman, Stillman and Chaikoff 1940). The administration of choline to rats which had been maintained on high fat, low protein diets for three days to three weeks increased the rate of phospholipid synthesis and the rate of removal of phospholipids from the liver. Cholesterol on the other hand, depressed the stimulation of phospholipid turnover by choline. Methionine, cystine, and cysteine had similar effects as choline in phospholipid turnover.

Artom and Fishman also conducted an extensive series of studies of the effects of various levels of protein, fat and choline in the intact rat (Artom and Fishman 1943a; 1943b; 1943c; Fishman et al 1946). On low protein, low choline diets, while total liver lipids were increased, phospholipids were decreased, chiefly in the lecithin fraction. Although choline supplementation always reduced total liver lipids, its effect on raising lecithin levels to normal varied under different

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experimental conditions. In weanling rats on a 10% casein 10% fat diet addition of choline in the diet increased lecithin synthesis. The addition of choline to similar diets fed to adult rats did not raise the levels of lecithin. When the level of fat was raised to 20%, the addition of choline caused an increased phospholipid synthesis in adult animals. The authors suggested that under the conditions of the experiment the level of lecithin in the liver is dependent on the dietary supply of both choline and fat.

Experiments on hepatectomized dogs by Chaikoff and associates in 1943, suggest that the liver is the main source of phospholipids in the plasma. Inorganic P^{32} was injected into normal and hepatectomized dogs and the recovery of P^{32} compared in these animals. Excision of the liver reduced the recovery of phospholipid P^{32} of the plasma to very small quantities. However, synthesis of phospholipid in the kidney and small intestines was not reduced by excision of the liver. The authors concluded that the primary source of plasma phospholipid is the liver with the kidney and intestines contributing little to maintaining plasma levels of this compound.

Efforts have been made to identify the mechanism by which choline exerts its lipotropic effect. However, no specific role has yet been well-defined, although several theories have been presented. The possibility that fatty livers resulting from choline deficiency are caused by a failure in the fat-transport mechanism has long been suggested. Barret, Best and Ridout (1938), Stetten and Grail (1943), and Stetten and Salcedo (1944) studied the source of fat which accumulated in livers when animals were maintained on a diet poor in

lipotropic factors, by the use of deuterium as an indicator. From their findings it was shown that choline promotes the mobilization of fat from the liver to the fat depots. The mechanism of this transport, however, was not defined.

The early assumption that choline may exert its lipotropic effect by stimulating the removal of fatty acids, in a sense that they are incorporated into the phospholipid molecule, has almost been totally rejected. Studies by Entenman et al (1946) and Bloom et al (1951) showed that labeled fatty acids were incorporated primarily into triglyceride rather than into phospholipid of either the plasma or the lymph; hence phospholipid was not the major pathway for fatty acid transport in either medium. However, the lipotropic action of choline is still considered to be related to phospholipid synthesis.

Since phospholipids are integral constituents of lipoprotein (Macheboeuf 1953) and since choline is an integral constituent of phospholipid, the importance of choline in lipoprotein metabolism is established. Beveridge (1956) and Harper (1958) have suggested that choline appears to be involved in fat transport via the synthesis of phospholipid in the liver (Fishler et al 1943) and the corresponding synthesis of serum lipoprotein. In support of this assumption several groups have noted decreases in various serum lipid and lipoprotein fractions in animals deficient in choline (Ridout et al 1954; Wilgram et al 1955; Mookejea 1965; Haines 1966; Lombardi et al 1966).

Other workers have suggested that choline facilitates the oxidation of fatty acids. Artom (1953) studied the rate at which various liver preparations from rats fed different diets oxidized labeled

stearate or palmitate. Liver tissue from rats on a low protein diet, containing added guanidinoacetic acid, given to deplete further the animals' store of labile methyl groups, was less effective in oxidizing the fatty acid substrates than the liver tissue from rats on a stock diet. The ability to oxidize these fatty acids at a normal rate was generally restored by supplementation of the low protein diet with choline or by injection of massive doses of choline shortly before removal of the liver. Neither the addition *in vitro* of choline nor any of the derivaties,betaine or betaine aldehyde or phosphoryl-choline, was effective in stimulating the oxidation of fatty acid. On the basis of these findings, Artom suggested that the lipotropic effect of choline might be due largely to the increase in the rate of fatty acid oxidation in the liver because of the action of certain substances, probably phospholipids, which are formed from choline.

Further evidence in support of a role for phospholipids in fat oxidation has been reported by Zilversmit and Diluzio (1952). These authors assumed that since fat metabolism is going on rapidly in the diabetic dog, phospholipid synthesis should be increased if these compounds are involved in fat oxidation. They found phospholipid synthesis was markedly increased in the liver and increases of smaller magnitude were noted in the small intestines and kidney.

Kennedy *et al* (1949) have found that liver mitochondria have certain enzyme systems which are very high in phospholipid content for many oxidative processes including fat oxidation. This they

suggested may support the theory that phospholipids play some role in facilitating fat oxidation.

Evidence has also been obtained that fatty acid synthesis is stimulated during choline and threonine deficiencies. Yoshida and Harper (1960) fed weanling rats a low protein diet deficient in choline and threonine. Acetate-1-C^{14} or palmitate-1-C^{14} was injected intraperitoneally and their incorporation into liver and carcass fat, along with their conversion into respiratory carbon dioxide studied. Stimulation of fat synthesis accompanied the fat accumulation in the livers and carcasses of rats fed a low protein diet deficient in threonine. The effect of choline deficiency was less clear-cut. Although increased fat synthesis was observed in the liver, no increased synthesis was observed in the carcasses. The authors suggested that choline deficiency stimulates the synthesis of neutral fat in the liver.

Dietary fats

In the past, much attention has been focused on the effect of dietary fats on the deposition of fat in the liver. Two distinct effects so far have been noted; (1) an essential fatty acid deficiency which produced increased fat accumulation in the liver in the presence of choline, and (2) different types of fatty acids affecting the extent of liver fat accumulation induced by deficiencies of lipotropic factors.

1. Essential fatty acid deficiency

An accumulation of fat in the livers of rats fed diets deficient in essential fatty acids has been observed by Engel (1942). Rats were

fed diets with and without corn oil and receiving daily supplements of 10 mg choline chloride. Liver fat levels in the fat-free group were twice as high as those in the control animals after each of the 8, 10, 12, and 20 week periods. The authors concluded that the essential fatty acids are necessary in the diet for choline to function properly as a lipotropic agent.

The concept that the addition of saturated fat to the diet promotes an essential fatty acid deficiency has been demonstrated by several workers. Peifer and Holman (1959), fed weanling rats diets containing completely hydrogenated coconut oil with and without 1% corn oil or 0.5% ethyl linoleate as essential fatty acid (EFA) supplements. When the EFA-deficient diet was fed, growth was inhibited and caloric efficiencies reduced. Intakes of EFA-supplemented diet allowed greater growth and increased to normal the caloric efficiency of the rats. EFA-deficient rats fed different levels of hydrogenated coconut oil (HCO) had equally high concentrations of non-essential trienoic acids in the heart and liver tissues. Only when the level of HCO was raised to 24.5% in the diet, did the trienoic acids accumulate in the heart and liver tissues of EFA-supplemented rats. These studies demonstrated that essential fatty acids are required for the proper utilization of fat calories. They found also that the high ratios of saturated fat: EFA, promote the onset of EFA-deficiency symptoms in the rat. Barnes et al (1959), from studies involving the essential fatty acids and coprophagy in the rat, found increased cholesterol accumulation in the livers of EFA-deficient animals. They believed that total fat accumulation is a

later manifestation of essential fatty acid deficiency than cholesterol accumulation in the liver.

2. Saturation versus unsaturation of fatty acids

Other workers have correlated infiltration of the liver with the nature of the dietary fat fed. The degree of saturation as well as the length of the carbon chain of the fatty acid component has been investigated. In an attempt to correlate chemical composition and physical properties of a dietary fat with liver fat accumulation during choline deficiency, Channon and Wilkinson (1936) fed weanling rats a diet containing a variety of dietary fats with 5% casein and 40% fat. The highest liver fat levels were produced by rats fed butterfat while those fed cod liver oil had the lowest levels (30.7% vs 7.2%) when calculated on a wet weight basis. Since butterfat contains a large proportion of long chain saturated fatty acids as compared to cod liver oil, the authors concluded that liver fat deposition depended directly on the intake of C_{14} -C_{18} saturated fatty acids. The addition of choline prevented fat deposition; hence, it was assumed that choline participates in a process of desaturation. A subsequent experiment reported by Channon et al (1942) confirmed the assumption that with a choline deficient diet the extent of fatty infiltration is related to the proportion of the saturated fatty acids from carbon fourteen through carbon eighteen.

These findings were contrary to results reported by Stetten and Salcedo (1945). Feeding the ethyl esters of the saturated fatty acids with chain lengths ranging from four to eighteen, to choline deficient rats, Stetten and Salcedo (1945) found that as the chain length of the

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dietary fatty acids decreases from eighteen through fourteen, liver fat accumulation increases.

Harper, Monson, Benton and Winjie (1954) also found a significant difference in liver fat deposition when butterfat instead of corn oil was fed to rats on a low protein diet, deficient in choline and threonine. This finding was the same whether the level of fat in the diet was either 5% or 20%.

Later studies by Benton et al (1956) confirmed the earlier findings of Channon (1942), that liver fat deposition was influenced by the amount of saturated fat in a low protein diet deficient in threonine and choline. When different dietary fats were fed at a level of 20% in a low protein diet (9% casein), the level of liver fat in the rats was high when butterfat or lard was fed and was low when corn oil or margarine was fed. This effect was increased when the level of protein in the diet was decreased and when choline was omitted from the diet. The fatty acids of butter, isolated and fed as glycerides produced liver fat levels equivalent to those obtained for butterfat. However, the solid fatty acid fraction of butter caused a much greater accumulation of liver fat than did the liquid fatty acid fraction.

In a subsequent study Benton et al (1957) reported that rats fed diets containing 30% butterfat had a higher requirement for choline than those receiving a similar diet containing 30% corn oil. They concluded that the requirement of choline varies with the type of dietary fat fed.

In agreement with previous reports of Channon et al (1942) and Benton et al (1956), are the studies reported by Iwamoto et al (1963). These authors contend that the degree of unsaturation of the dietary fat appears to be primarily responsible for the differences in fat content of livers of animals fed choline deficient diets. They fed weanling rats 13 different fats or mixtures of fats each included in a choline deficient diet at three levels (10%, 20%, 40%) with 12% casein. The dietary fats included coconut oil, olive oil, and safflower oil. Maximum liver lipid concentration was reached when coconut oil alone, or when a mixture composed of two-thirds coconut oil and one-third olive oil was fed. The results further confirmed those reported earlier by Harper (1954), that the amount of liver lipid was not dependent to a significant degree upon the amount of fat in the diet. The liver fat levels were not significantly different when the three levels of dietary fat were fed.

Hill, Linazasoro, Chevallier and Chaikoff (1957) investigated the influence of dietary fats on hepatic lipogenesis. They found that the capacity of the liver to incorporate the C^{14} of C^{14}-glucose and C^{14}-acetate into fatty acids is related to the amount of fatty acids ingested by the rat. Rats were fed for three days synthetic diets containing 0.1, 2.5, 5, 10, and 15% fat of either lard, corn oil, vegetable oil, or hydrogenated vegetable oil. Livers of rats severely depleted of fat had the highest capacity for converting acetate carbon to fatty acids. All fats tested were effective in reducing hepatic lipogenesis. The authors postulated the existence of a homeostatic mechanism in the regulation of hepatic lipogenesis.

a. Unnatural isomers

The possibility that trans isomers have lipotropic activity has been suggested by Morris, Arata, and Cederquist (1965). When male weanling rats were fed a 9% casein diet deficient in threonine and choline and containing corn oil as the fat source (30% of diet), liver fat accumulates to very high levels. Replacing corn oil in the diet with hydrogenated vegetable oil significantly reduced liver fat levels. When the corn oil was hydrogenated to an iodine value of 74 and fed to weanling rats under similar experimental conditions, the liver fat level was reduced to values within normal range. Since the two dietary fats (hydrogenated corn oil and un-hydrogenated corn oil) have similar carbon chain lengths and hydrogenated corn oil contains 45% trans acids, the lipotropic action of hydrogenated corn oil might reside in the trans acids. The authors postulated that these isomers may mediate a metabolic path different from that taken by natural fats and oils.

Much interest has now been directed towards investigating the effect on fat metabolism of these unnatural isomers that are present in chemically hydrogenated fats. These occur during the hydrogenation of unsaturated fatty acids. These are present not only as trans isomers but also as positional isomers. The metabolism of the trans isomers of oleic and linoleic acids has been well investigated by a number of workers. Mattson (1960), fed weanling rats that had been on fat-free diets, 50 or 100 mg/day of various purified trans acids either alone or in combination with cis-cis linoleate. He found that trans acids had neither essential fatty acid activity, nor did they possess an anti-essential fatty acid activity. No adverse effects

were observed other than those of essential fatty acid deficiency which indicated that the trans isomer is not toxic to rats within the range of the amount fed.

Clement and Clement (1965) fed elaidic acid to rats either as the free acid or as a triglyceride. The intestinal mucosa and lymph triglycerides were isolated and their structure determined by pancreatic lipase. The elaidic acid level was determined by GLC by the use of capillary columns. The results showed a marked tendency for elaidic acid to be located at the external positions of the triglyceride molecule both in the lymph and mucosa.

The metabolism of trans isomers has been extensively studied by Coots (1964). He compared the metabolism of elaidic acid with that of palmitic acid, stearic acid and oleic acid by the use of labeled fatty acids. 1-C^{14}-fatty acids were methylated and the mono- and di-glycerides prepared and transesterified with soybean oil. These were incorporated into a liquid diet and fed to weanling rats at a level of 27% in the diet. He found that 66-70% C^{14} appeared as $C^{14}O_2$ over a 51-hour period for palmitic acid, oleic acid and elaidic acid. Less than 3% of the C^{14} appeared in the feces and about 30% in the carcass. Between 5-17 hours the palmitic, oleic and elaidic acids were incorporated into phospholipids. The distribution of elaidic acid in phospholipids resembled that of stearic acid more than that of palmitic and oleic acids respectively. These findings demonstrated that elaidic acid is efficiently absorbed, metabolized and incorporated into body tissues.

b. Fatty acid composition

Recently, emphasis has been focused on investigating the fatty acid composition of the liver and the plasma, in an attempt to obtain a clue concerning the mode by which fat accumulates in the liver. To determine the chemical composition of the fatty liver produced in threonine and lysine-deficient rats, Viviani et al (1964) assayed the lipids and the fatty acid composition of total lipid, neutral fat and phospholipids of livers of both normal and deficient animals. The animals were fed low protein rice diets with and without supplements of lysine and threonine. The absolute amounts of linoleic acid, palmitic and oleic acids in fatty livers were increased. More linoleic acid and less $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$ in percentage of fatty acids were found in total liver lipids in lysine and threonine deficient rats. In the neutral fat fraction there was an increase in the percentage of linoleic acid. There was also a decrease in the percentage of $C_{20:5}$ $C_{22:5}$ and $C_{22:6}$ in the phospholipid fraction. The authors postulated a control by lysine and threonine on polyunsaturated fatty acid metabolism.

Around that same time Tesluk and Stewart (1964) were engaged in studies investigating the mechanism involved in the production of fatty livers in rats by the use of different dietary methods. Rats were fed diets which caused central (choline deficient), midzonal (Flectol H), and peripheral (protein deficient) accumulation of fat. The fatty acid composition of liver, depot and dietary fat was determined. In all experiments the triglyceride levels of plasma were

reduced proportionate to the degree of fatty change in the liver. The fatty acid composition of the body fat remained essentially unchanged. However, there were changes in the fatty acid composition of the liver. There was a reduction in palmitic and stearic acid levels with concomitant increases in oleic and linoleic acid levels. Irrespective of the diet fed the fatty acid pattern was the same in experimental animals. The authors concluded that the increase in hepatic fat is due to an interference with transport of fat through the liver rather than to partial interference with fat oxidation.

An impaired mobilization of triglyceride from the liver due to a decrease in lipoprotein synthesis was suggested by Tinoco et al (1964a, 1964b). They studied the fatty acid composition of the liver and of the plasma of choline deficient rats, both males and females. Differences in the fatty acid composition of choline deficient and choline supplemented rat livers were pronounced. There were also differences between the sexes.

Contrary to these findings are those reported by Glende and Cornatzer (1965). They studied the fatty acid patterns of the major lipids involved in liver lipid metabolism. This was done in order to investigate whether the fatty acids of these compounds are involved in the abnormal lipid metabolism taking place during choline deficiency. They found no significant alteration of liver lipid fatty acids between animals fed rations with and without choline supplements.

More recently, Chalvardjian (1966) fed weanling rats diets with and without supplemented choline, fat-free and containing fat at the

level of 4% and 30% of the diet for two weeks. The fatty acid pattern of the major lipid fractions of liver, serum and adipose tissue was determined. He found no quantitative similarities in the fatty acid pattern between hepatic triglycerides on the one hand and adipose tissue, serum triglycerides and dietary fat on the other. In addition, the level of the dietary fat had a more marked effect on altering the fatty acid composition of tissues, than the level of choline in the diet. A relative preponderance of 16-carbon fatty acids occurred in the hepatic triglycerides of choline deficient animals fed the fat-free and low fat diets. This finding was taken by the author to mean a direct effect of choline deficiency on the mitochondria causing an interference with elongation of fatty acids.

GENERAL METHODOLOGY

Male weanling rats of the Sprague-Dawley strain, were used in all experiments. Their initial weights ranged from 41-62 grams. They were separated into groups, which did not differ in their average initial weights within each experiment by more than 0.5 grams. They were housed in individual cages with raised screen bottoms in a temperature controlled room.

These groups were offered the various diets and water ad libitum except on one occasion, when the pair feeding technique was used. Total food consumption was determined weekly, and the average weight gain for the four week period determined. The Feed Efficiency of each group was calculated as:

$$\frac{\text{Average body weight increase(g)}}{\text{Average food consumption (g)}}$$

The percentage composition of the basal diet was as follows: casein, 9; sucrose, 25; salt's W[1],4; choline chloride, 0.15; DL-methionine, 0.30; DL-tryptophan, 0.10; corn oil[2], 30; alphacel, 31.20; vitamins 0.25. Vitamins were included in sucrose to provide in mg per 100 grams diet: Vitamin A concentrate 0.4 (500,000 I.U./gram); calciferol 0.0383 (40,000,000 I.U./gram);

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[1] Wesson, L. G. 1932: A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. Science, 75:339. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[2] Containing 75 mg of α-tocopherol per kilogram diet. In those experiments where a fat other than corn oil was used in compounding the diet, 10 ml. of corn oil (containing 75 mg α-tocopherol) were added to the fat for every Kg. of diet prepared.

thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1.0; pyridoxine, 0.25; Ca pantothenate, 2.0; inositol, 10.0; folic acid, 0.02; Vitamin B_{12}, (0.1% trituration) 2.0; biotin, 0.01; para-amino benzoic acid, 1.0; menadione, 0.38.

Feces were collected from all animals for the last four days of the experiment and the total weight for each group determined. The total quantity from each animal was homogenized in a mortar, and two gram samples of the ground material brought to constant weight in a vacuum oven at 50°C. Fat determinations were made on the dried feces on 1.5 gram samples by continuous chloroform-methanol (2:1 v/v) extraction for three hours on a Goldfisch apparatus. Total fecal fat for the four day period was determined.

At the end of the four week experimental period, the animals were killed by a sharp blow on the head and decapitated. The livers were excised, weighed and homogenized with distilled water in a Potter-Elvehjem homogenizer.[3] The homogenates were quantitatively transferred to weighed evaporating dishes and dried in a forced air drying oven at 85°C for twelve hours. They were transferred to a vacuum oven and were brought to constant weight at 50°C. Moisture determinations were made from the weights of the wet and dried livers. The dried livers were ground in a Wiley mill. Fat was determined by subjecting one gram sample of the dried ground liver to continuous ether extraction for three hours on a Goldfisch

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[3] V.R. Potter and C. A. Elvehjem: J. Biol. Chem. 114, 499, 1936.

apparatus, and then weighing the ether soluble material. The concentration of fat in the tissue was expressed as per cent dry weight. The percentage nitrogen was determined on 0.1 gram sample of the liver residue, by the micro-Kjeldahl method and calculated as fresh weight of tissue. Standard error of the means was calculated for all data, and Student's t-test was used as a measure of significance. Unless otherwise stated, a probability of 0.05 or less, will be used as a measure of significance.

PART I

THE EFFECTS OF NATURAL VERSUS CHEMICALLY TREATED LARD ON LIVER FAT LEVELS OF THREONINE DEFICIENT RATS

INTRODUCTION

When weanling rats are fed a 9% casein diet containing sucrose as the carbohydrate source and supplemented with methionine and tryptophan, but not threonine excess fat accumulates in the liver (83,52). The addition of either protein or threonine to the diet causes a considerable decrease in liver fat deposition. Singal and his associates (85), using diets containing crystalline amino acids as the only source of nitrogen, have shown that the amount of fat accumulating in the liver varies with the amount of threonine in the diet.

Several factors have been shown to influence liver fat accumulation in threonine deficiency. Harper and associates (51,50), have shown that in addition to the amount of protein or tryptophan, the type of fat in the diet modifies the effect of supplemented threonine in reducing liver fat levels. In that study, corn oil was increased in threonine deficient diets, from 5% to 20% without causing a concomitant increase in liver fat. However, when butterfat replaced corn oil in the diet containing 20% fat liver fat values were significantly higher. When threonine was added to these high fat diets, the liver fat levels of the corn oil group were reduced to a greater extent than were those of the butterfat fed group, although the total amount of liver fat was reduced in each case. Thus, the nature of the fat source in the diet appeared to influence the effect of a threonine deficiency on fat metabolism.

Various factors, such as the degree of saturation of the component fatty acids, the length of the carbon chain, and the presence of unnatural isomers in a dietary fat may influence the level of liver fat accumulation in the threonine deficient state. Channon et al (26,27) in an attempt to study the effect of the degree of saturation of a dietary fat on liver fat deposition, fed different dietary fats to adult rats at a level of 40% of a diet containing 5% casein and deficient in choline. They found the highest liver fat values for those fed butterfat, and the lowest in rats fed olive oil, with those fed coconut oil intermediate. By comparing the amounts of the different fatty acids in these fats they concluded that the increased infiltration of fat into the liver, was proportional to the percentage of saturated (carbon 14-carbon 18) acids in the dietary fat. These findings were later confirmed by Benton et al (12) who reported higher liver fat values when 20% of butterfat or lard served as the fat source than when 20% corn oil or margarine was used in threonine deficient diets. Although addition of threonine reduced liver fat levels in each case, the liver fat levels for groups fed butterfat and lard were consistently higher than for those fed corn oil or margarine. They concluded as did Channon et al (26,27) that the solid long chain fatty acid composition of the dietary fat determines the severity of liver fat deposition.

Stetten and Salcedo (86), in an attempt to determine the effect of the fatty acid chain length on liver fat deposition, compared the effects of the ethyl esters of the saturated fatty acids in the

diets of choline deficient rats. The chain length was found to be important, but contrary to the findings of Channon et al (26,27), and Benton et al (12), ethyl myristate produced livers with more fat than either the longer or shorter chain acids.

In addition to the degree of saturation and chain length of a fatty acid, the degree of hydrogenation of a dietary fat has been shown to influence the amount of fat that accumulates in the livers of rats fed threonine deficient diets. Morris et al (74) fed weanling rats a 9% casein diet containing different dietary fats 30% of the diet with and without additional threonine. When corn oil was used as the fat source in a threonine deficient diet liver fat accumulated to 22.5% of the dry weight of the liver. The substitution of corn oil with either cotton seed oil or hydrogenated vegetable oil reduced liver fat levels significantly (22.5% vs 17.9% and 17.6% respectively). When corn oil was hydrogenated to the same iodine number as the hydrogenated vegetable oil, and subsequently fed under the same conditions as the corn oil control the concentration of liver fat was reduced to 13.5%. Since chemically hydrogenated fats appeared to be more protective against the appearance of fatty livers associated with threonine deficiency, Morris et al (74) postulated that the presence of unnatural isomers which occur during the hydrogenation process could mediate a different metabolic path for chemically hydrogenated fats as compared with natural fats.

To test the hypothesis, that unnatural isomers that occur during the hydrogenation process could mediate a different metabolic path for hydrogenated fats, the following experiment was designed in which corn oil, (native and hydrogenated) was compared with a natural hydrogenated fat (lard), which had not been treated chemically and with a commercial lard which had been altered chemically to a limited extent. The two lards were compared with corn oil with respect to their ability to lower liver fat levels associated with a threonine deficiency. Natural lard, like hydrogenated corn oil, is solid at room temperature, but should be free of unnatural isomers which are present in hydrogenated corn oil, since it had not been subjected to chemical hydrogenation processes. Commercial lard, on the other hand, is subjected to a process of interesterification or molecular re-arrangement wherein the fatty acids on the glycerol moiety are re-distributed in order to improve its plastic range. This process does not change the degree of unsaturation or isomeric state of the fatty acids but results in a considerably wider temperature range. Coupled with this treatment, commercial lard is usually partially hydrogenated, deodorized and supplemented with anti-oxidants and monoglycerides. In view of this fact, both natural and commercial lards were incorporated into the experimental design as an internal control. These two dietary fats, (natural and commercial lard) are comparable in their fatty acid profile, except for the degree of unsaturation and for the possible presence of unnatural isomers in commercial lard, and therefore should show differences in their liver fat levels in

threonine deficient rats, if this hypothesis holds. Coupled with this comparison is that between natural lard, a saturated, trans isomer-free fat, and hydrogenated corn oil, a saturated trans isomer-containing fat. By comparing the response of threonine deficient animals to these various dietary fat patterns, some significant clues concerning the major parameters of the fat metabolism pathway in deficient animals might emerge.

The Digestibility Coefficient of each dietary fat was also measured.

METHODS

Four groups of 10, 10, 9, 10, animals were fed basal diet[1] or the basal diet with the fat source substituted by hydrogenated corn oil[2], natural lard[3], or commercial lard.

Data from a pilot study, using similar diets, suggested that groups fed corn oil, natural lard and commercial lard, ate significantly more than the group fed hydrogenated corn oil. To ensure a more equitable balance of calories among the four groups, a pair-feeding technique was used in which the group fed hydrogenated corn oil served as the control group.

Food consumption and weight gain were recorded and Feed Efficiency Ratio for the four groups calculated. Total liver lipids, moisture and nitrogen content were determined for all groups studied. Feces were collected, and fat determinations made on the dried matter. These procedures are described in section entitled General Methodology.

A quantitative determination of the trans double bonds was made on each diet fat by infrared spectrophotometry by the method of Firestone and Villademar (39). Since trans double bonds exhibit an absorption band with a maximum at about 10.33μ, arising from a C-H deformation about the trans double bond, these can be quantitatively

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1 The composition of the basal diet is given on page 24.

2 Hydrogenated by Proctor and Gamble; Cincinnati, Ohio to an iodine value of 74.

3 Refined lard, no hydrogenation or anti-oxidant added, prepared by Hormel & Co., Austin, Minnesota.

determined since the cis double bonds do not absorb at that wave length. To determine the quantity of trans isomer present, approximately 200 mg of each diet fat was quantitatively methylated by the method of McGinnis and Dugan (70). The sample was transferred to a tared 10 ml volumetric flask, diluted to volume and compared with a standard. A methyl elaidate standard[1] solution was prepared in order to obtain transmittances between 20% and 70% at 10.33μ(200 mg were dissolved in 10 ml carbon disulfide). Various dilutions were made 1.5%; 1%; 0.5%; 0.25%; and a standard curve was obtained by plotting absorbance at 10.33μ against concentration and extrapolating to 0. By use of the standard curve the total quantity of trans isomers in each sample was determined. A Perkin Elmer model 337 Infrared Spectrophotometer with a 1 mm KCL cell was used for the determination of trans isomers.

The fatty acid composition of the four diet fats was determined by gas liquid chromatography. Methyl esters of the fatty acids were prepared by the method of McGinnis and Dugan (70). The fatty acid methyl esters were separated by gas liquid chromatography[2]. The stationary phase packed in a 6-foot by 3 mm (ID) U-tube glass column, consisted of 5% Diethylene Glycol Succinate coated on 80-100 mesh Diatoport S. A flame ionization detector was used. Oven temperature was programmed from 160-220° at 5°/min for all fatty acid operations. Detector and Injection port temperatures were 230° and 220°C

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1 Applied Science Laboratories Inc., State College, Pennsylvania.

2 Model 400 Gas Chromatograph, F & M Scientific Corporation, Avondale, Pennsylvania.

respectively. Helium was used as the carrier gas at an inlet pressure of 40 psi. All determinations were made in duplicate.

The amount of each fatty acid was quantitated by multiplying the peak height by its width at half-height. The results are expressed as percentage of the total fatty acids of each dietary fat. For identification of dietary fatty acids, the retention times of the methyl esters were determined and compared with methyl ester standards.[1]

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[1] Applied Science Laboratories Inc., State College, Pennsylvania.

RESULTS

Food intake, weight gain and feed efficiency ratios for rats fed a 9% casein diet and containing four different sources of dietary fats are summarized in Table 1. There was no significant difference in food consumption (animals were pair-fed) and correspondingly no significant difference in growth patterns. Consequently, the feed efficiency ratios were the same. The physical appearance of rats in all groups studied, was identical.

The most pronounced effect of the different dietary fats was shown in the liver composition of rats fed a 9% casein diet (Table 11). There was a significant increase in the moisture content and a concomitant decrease in the fat content ($P<0.01$) of livers of rats when hydrogenated corn oil replaced corn oil as the fat source. No significant difference was observed in these parameters when either natural lard or commercial lard replaced corn oil in the diet. Although liver fat levels for rats fed natural lard appeared slightly less than for those fed commercial lard (26.9% vs. 30%) the difference was not statistically significant.

When hydrogenated corn oil was used as the diet fat in a 9% casein diet, liver nitrogen was not significantly different from the group fed corn oil (Table 11) despite the fact that the concentration of liver lipid was markedly different in these two groups. Neither was there any significant difference in liver nitrogen between the group fed natural lard and that fed commercial lard.

Summarized in Table III, are the fecal fat values and the coefficient of digestibility of dietary fats fed to rats on a 9% casein diet. Rats fed hydrogenated corn oil had a significantly higher fecal fat concentration ($P<0.01$) than rats fed corn oil when calculated on a percentage basis as well as when the total amount of fat excreted was determined. There was no significant difference in the fecal fat concentration when natural lard replaced corn oil in the diet. However, when commercial lard replaced corn oil, there was a significant difference ($P<0.01$), between these two values when total amount were calculated for each group. The total amount of fat excreted by rats fed commercial lard was lower than that obtained when hydrogenated corn oil served as the fat source. Consequently, the coefficient of digestibility of hydrogenated corn oil was significantly lower ($P<0.01$) than that of commercial lard and much lower than that of corn oil (Table III). There was no significant difference between the coefficient of digestibility of corn oil and that of natural lard, however, the failure to establish significance between these two groups is likely a mathematical artifact. If the coefficient of digestibility of commercial lard is significantly different from that of hydrogenated corn oil, and if no significant difference exists between natural and commercial lards, then natural lard must be significantly different from hydrogenated corn oil. Inability to establish significance by Student t-test is probably due to a much higher standard error in the digestibility figure for natural lard than for commercial lard.

The fatty acid composition of the dietary fats used in the experiment is summarized in Table IV and is expressed as the per cent of the total fatty acids in each dietary fat. Of the fats studied, corn oil contains the highest proportion of linoleic acid and the lowest of stearic acid,while hydrogenated corn oil has a very high percentage of oleic acid (including the unnatural isomers which occur during hydrogenation process) and a much lower level of linoleic acid, than does corn oil. The fatty acid composition of natural lard differs only slightly from that of commercial lard. Commercial lard is slightly higher in palmitic and stearic acid content and is lower in oleic and linoleic acids.

Table V summarizes the percentage of trans double bonds of the methyl esters of the dietary fats fed to rats consuming a 9% casein sucrose diet. No measurable amount of trans component was detected in either corn oil, natural lard, or commercial lard, although a high percentage (45% of the methyl esters) was observed in hydrogenated corn oil.

DISCUSSION

Feeding hydrogenated corn oil as the fat source to weanling rats consuming a 9% casein-sucrose diet supplemented with methionine and tryptophan but not threonine significantly decreased liver fat levels compared with the same diet containing corn oil. A significant inverse relationship seems to exist between liver fat and moisture concentration in the liver; the fat infiltrates the liver at the expense of the moisture content of the cell, while the nitrogen content remains reasonably constant.

Results from this study support the theory of Morris et al (74) and others (26,12,58) that the degree of liver fat accumulation depends to an extent on the nature of the fatty acid component of the dietary fat in a threonine imbalanced diet, when fed to weanling rats. Hydrogenated corn oil significantly reduced liver fat levels to almost one-third the value of that obtained when fed corn oil that is not hydrogenated.

Natural lard, like hydrogenated corn oil, was solid at room temperature. However, substitution of the former for corn oil did not reduce liver fat levels as did substitution of hydrogenated corn oil for corn oil in threonine imbalanced diets. This observation tends to support the suggestion of Morris et al (74) that the presence of isomers in chemically hydrogenated fats may be responsible for the 'lipotropic effect' of hydrogenated vegetable oils and hydrogenated corn oil.

Since commercial lard does sustain chemical alteration in processing, one would expect a decrease in liver fat accumulation similar to the effect of hydrogenated corn oil. This effect failed to materialize. However, the degree of hydrogenation of commercial lard is apparently very low as evidenced by the absence of trans isomers. As a result, the data in this study do not negate the possibility that unnatural isomers which occur during chemical hydrogenation may be more protective against fatty livers associated with a threonine deficiency. Of the four dietary fats studied, only hydrogenated corn oil has appreciable quantities of trans isomers present, and since this fat alone significantly reduced fatty livers associated with threonine deficiency these unnatural isomers may in some way have a marked effect on liver fat utilization.

The liver fat values for rats fed natural lard or commercial lard do not support the contention of Channon et al (26,27) or Benton et al (13) that fat deposition is proportional to the amount of the saturated fatty acids supplied by the dietary fat, or of Iwamoto et al (58), who contend that the deposition of liver lipids is inversely related to the degree of unsaturation of the dietary oils. Since there was no significant difference between the liver fat values of rats fed corn oil with an iodine value of 123, and that of rats fed lard with an iodine value of 55, and since hydrogenated corn oil with an iodine value of 74, produced the lowest liver fat values, the degree of saturation was obviously not a major factor in the accumulation of fat in threonine deficient rats under the conditions used in this

experiment. This statement is more in agreement with that of Best et al (19) who concluded that wide differences in the degree of unsaturation, produced only minor differences in the degree of fatty liver produced in choline deficient rats, fed a number of different dietary fats.

The somewhat lower coefficient of digestibility for natural and commercial lard and the more marked decrease in digestibility of hydrogenated corn oil could be due to the presence of tristearin (which is poorly absorbed) in these fats. Since these fats are hydrogenated to a greater degree, they are more likely to contain a saturated triglyceride than is corn oil. Corn oil with the lowest amount of stearic acid and the highest iodine value was therefore less likely to contain tristearin and thus was best absorbed. Although the absorbability of a dietary fat is presumably determined by its melting point (13) more recent findings of Mattson (69) have demonstrated that the coefficient of absorbability of a fat is inversely proportional to its content of simple saturated triglyceride having a chain length of eighteen carbon atoms or greater, and is influenced by the amount of such saturated fatty acids only when present as saturated triglycerides. Johnston et al (59) and more recently Coots (39) have demonstrated that the trans isomers of fatty acids are efficiently absorbed therefore these isomers present in hydrogenated corn oil would not account for the lower coefficient of digestibility of this fat.

The lower digestibility of hydrogenated corn oil might be cited in explanation of the decrease in liver fat values in this group (9.7% vs 31.0%). However, a simple calculation tends to negate this suggestion. Animals fed hydrogenated corn oil consumed approximately 11.7 grams of food daily as opposed to 11.2 grams per day for those fed corn oil. The percentage of fat in the diet is at the 30% level, therefore with a digestibility coefficient of 85% for hydrogenated corn oil, each animal would absorb approximately 3.0 grams of fat per day as opposed to 3.2 grams daily for those fed corn oil with a digestibility coefficient of 94%. Since the difference between these values is very small, the digestibility of hydrogenated corn oil, although lower than that of corn oil could not entirely account for the significantly low deposition of liver fat obtained in threonine deficient rats. In further support of this statement the lower digestibility of hydrogenated corn oil decreases the amount of fat entering the animal to the equivalent of a diet containing approximately 25% of fat. In previous experiments (51,50)feeding threonine deficient diets containing as low as 5% of fat, still induced fatty livers in rats. Animals fed hydrogenated corn oil as the fat source in threonine deficient diets do not develop fatty livers. The addition of threonine (74) to this diet did not significantly alter the liver fat levels, therefore these unnatural isomers which occur in hydrogenation process might be closely related to the function of threonine in hepatic fat utilization.

The possibility that different dietary fats may have different metabolic routes, some going directly to the adipose tissue, with others going first to the liver for chemical alteration has been proposed. Another issue to be considered is the relationship of fat metabolism and dietary choline under these conditions. The high fat diets (30%) used in these experiments increase the animal's requirement for choline as proposed by Griffiths (70) who found an increased choline requirement with increased fat in the diet. Increasing the level of choline from 0.15% to 0.50% in low protein, high fat, threonine deficient diets, lowered liver fat levels for both corn oil and olive oil fed groups (Morris[1]), but a significant difference in liver fat still remained between these two groups with corn oil fed animals having the lower (17.3% vs 21.6%) liver fat level. Since corn oil is very high in linoleic acid, and olive oil in oleic acid, the effect of choline may vary with the fatty acid composition of a dietary fat. To test the effect of choline in lowering liver fat levels in low protein, high fat, threonine deficient diets, a subsequent experiment was designed in which different dietary fats chosen to provide a varied fatty acid composition was fed to weanling rats with two levels of choline, (0.15% and 1.00%)in the diet.

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1 Unpublished data.

Table I. Food intake, weight gain and feed efficiency ratio of rats fed a 9% casein diet containing four different sources of dietary fats.

Group #	Diet[1]	# of animals/group	Food intake g/week	Wt. gain g/week	Feed eff.
1	corn oil	10	78.3 ± 1.7[2]	22.0 ± 0.5[2]	0.28
2	hydrog. corn oil[3]	10	82.2 ± 2.5	23.2 ± 0.9	0.28
3	natural lard[4]	9	79.4 ± 2.4	22.9 ± 1.0	0.29
4	commercial lard	10	82.0 ± 2.4	23.6 ± 0.9	0.29

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1 Fat content of all diets = 30% w/w. Length of experimental period = 4 weeks.

2 Standard error of the mean.

3 Corn oil was hydrogenated to an iodine value of 74 by Proctor and Gamble Co., Cincinnati.

4 Refined lard, no hydrogenation or anti-oxidant added, prepared by Hormel & Co., Austin, Minnesota.

Table II. Liver composition of rats fed 9% casein diets containing four different sources of dietary fats.

Group #	Diet[1]	Moisture %	Nitrogen % wet wt.	Fat % dry wt.
1	corn oil	66.6 ± 0.6[2]	2.55 ± 0.08[2]	31.0 ± 1.5[2]
2	hydrog. corn oil[3]	72.5 ± 0.4[5]	2.82 ± 0.11	9.7 ± 0.7[5]
3	natural lard[4]	68.7 ± 1.1	2.65 ± 0.07	26.9 ± 2.6
4	commercial lard	66.4 ± 0.6	2.74 ± 0.08	30.0 ± 1.5

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1 Fat content of all diets = 30% w/w, length of experimental period = 4 weeks.

2 Standard error of the mean.

3 Corn oil hydrogenated to an iodine value of 74 by Proctor and Gamble Co., Cincinnati.

4 Refined lard, no hydrogenation or anti-oxidant added, prepared by Hormel & Co., Austin, Minnesota.

5 Significant difference from corn oil control ($P<0.01$).

Table III. Fecal fats and coefficients of digestibility in rats fed a 9% casein diet containing four different sources of dietary fats.

Group #	Diet[1]	# of animals/group	Fecal Fat		Coeff. of Dig.
			% dry weight	Total amt. excreted[5] gm. dry wt.	
1	corn oil	5	8.05± 0.31[2]	0.86 ±0.08[2]	94.4 ±0.4[2]
2	hydrog. corn oil[3]	5	14.45± 0.52[6]	2.51 ±0.21[6]	84.7 ±1.6[6]
3	natural lard[4]	5	9.04± 0.57	1.22 ±0.21	92.6 ±0.8
4	commercial lard	5	10.43± 0.43	1.54 ±0.11[6]	91.0 ±0.3[6]

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1 Fat content of all diets = 30% w/w; length of experimental period = 4 weeks.

2 Standard error of the mean.

3 Corn oil hydrogenated to an iodine value of 74 by Proctor and Gamble Co.; Cincinnati.

4 Refined lard, no hydrogenation or anti-oxidant added, prepared by Hormel & Co. Austin, Minnesota.

5 Total fat excreted in 4 day period, corrected for endogenous fat (determined in rats fed fat-free diet).

6 Significant difference from corn oil control ($P \leq 0.01$).

Table IV. Fatty acid composition of dietary fats fed to rats on a 9% casein diet (/100 grams of total fatty acids).

Diet[1]	Palmitic acid $C_{16:0}$	Stearic acid $C_{18:0}$	Oleic acid $C_{18:1}$	Linoleic acid $C_{18:2}$	Other
corn oil	13.9	2.2	27.8	56.2	0
hydrog. corn oil[2]	12.2	11.9	69.6	6.3	0
natural lard[3]	24.1	10.4	47.2	13.0	5.3
commercial lard	29.8	13.6	40.8	10.1	6.1

Table V. Amount of trans double bonds in dietary fats fed to rats on a 9% casein diet.

Diet[1]	Trans double bonds %
corn oil	0
hydrogenated corn oil[2]	45
natural lard[3]	0
commercial lard	0

1 Fat content of all diets = 30% w/w, length of experimental period = 4 weeks.

2 Corn oil hydrogenated to an iodine value of 74 by Proctor and Gamble Co., Cincinnati.

3 Refined lard, no hydrogenation or anti-oxidant added, prepared by Hormel & Co., Austin, Minnesota.

PART II

THE ROLE OF CHOLINE IN LIVER FAT ACCUMULATION IN THREONINE DEFICIENT RATS FED VARIOUS DIETARY FATS

INTRODUCTION

The lipotropic properties of choline were first demonstrated by Best and Huntsman (16) who showed that the fatty infiltration of livers from rats fed diets high in saturated fats could be prevented by choline supplementation. Ever since that time, many workers have investigated fatty liver induced in experimental animals by feeding diets deficient in choline in an attempt to elucidate the mode by which choline exerts this effect. However, although much light has been thrown on the transport of fatty acids in the body, the exact mode of action of choline is still not well defined (5, 102, 75) and remains a complicated problem which is apparent from the many conflicting theories concerning the role of choline in the transport, synthesis, and oxidation of fat.

Several studies have shown that in threonine induced fatty livers, the lipids which accumulate are predominantly, if not exclusively triglycerides (44, 47, 63, 73, 98, 101). Baker et al (8, 9) reported that at least 50% of the liver triglyceride turnover is accounted for by the release of triglyceride into the circulation. Other workers (64) have suggested that triglycerides rather than phospholipid or cholesterol esters are the major form in which fatty acids are transported from the liver to the extrahepatic tissues. On the other hand, phospholipids are now known to be essential components of plasma lipoproteins which transport triglycerides

from the liver to adipose and other tissues (55). Phospholipids have both polar and non-polar properties, and are believed to form bridges between the polar protein and the non-polar constituents, such as the triglycerides and cholesterol. Since phospholipid is an essential constituent of lipoproteins which transport triglyceride, a moiety of the very low density lipoproteins (d 1.019), from the liver to the extrahepatic tissues, a breakdown in the phospholipid moiety would result in a block in lipoprotein synthesis. Choline is an essential building stone for the fabrication of phospholipids, therefore, many workers have theorized that in choline deficiency, there is a defect in the normal utilization of dietary fat by the liver, because of insufficient synthesis of the phospholipid required for the conversion of chylomicron triglyceride to the low density lipoprotein triglyceride (44, 73, 98, 64), hence triglycerides accumulate in the liver. These findings are substantiated by reports of Olson et al (75) Lombardi (63) and Tinoco et al (90) who found lower levels of blood lipid concentrations in choline deficiency, suggesting lipid transport is decreased. Zilversmit et al (102) on the other hand, found that in the rabbit and dog the rate of incorporation of P^{32} into liver phospholipids was greater during periods of choline deficiency than during daily supplementation of the diet with 1.00% choline.

In addition to the enhanced transport of triglyceride into the blood from the fatty liver with choline supplementation, enhanced oxidation of fatty acids by the liver with supplemented choline has

been reported by Artom (5) from results of his in vitro studies with livers of protein deficient rats. However, the lipotropic action of choline cannot satisfactorily be explained merely as the result of an enhanced oxidation of fatty acids in the liver since these are only in vitro studies. He found no evidence of an increased rate of fatty acid oxidation in the rats fed choline-supplemented diets. Furthermore, he obtained similar results when low protein diets were supplemented with cystine or tocopherol, or by raising the level of dietary protein without substantially increasing the supply of methionine and cystine.

Another variable associated with choline deficiency that has been widely investigated is that which involves the study of fatty acid synthesis in the livers of normal vs choline deficient animals. Yoshida et al (101) in their study on the effect of threonine and choline deficiencies on the metabolism of C^{14} labeled acetate and palmitate in the rat, reported increased activity in the liver neutral lipid, and concluded that choline deficiency stimulated the synthesis of neutral fat in the liver. Using D_2O, it has been shown that the livers of choline deficient rats have more newly synthesized fatty acids than the normal rat livers (88). These results reflect the fact that the major site of fatty acid synthesis is the liver and suggest that in choline deficiency those fatty acids synthesized did not escape to the depots as rapidly as those in normal animals. On the other hand, other workers were unable to detect differences in the amount of newly synthesized liver

fatty acids from C^{14} acetate in choline deficient compared with normal rats (46).

Since fatty acids are structural components of phospholipid and neutral lipid molecules that may undergo alterations during choline deficiency, the fatty acid composition of various liver lipids of normal and choline deficient animals has been investigated. Tinoco et al (90, 91) found significant changes in the fatty acid patterns in both the triglyceride and phospholipid fractions in the livers of choline deficient animals, with a concomitant decrease in circulating lipoprotein and in all lipid classes in the serum. Contrary to these findings are those reported by Glende et al (44) who found that the fatty acid moiety of the liver lipids does not appear to be reflective of the altered lipid metabolism of the liver during choline deficiency. Morris[1] found lower liver fat levels for animals fed corn oil, than for those fed olive oil, when the level of choline in low protein, high fat, threonine deficient diets, was increased from 0.15% to 0.50%.

Since the fatty acid component of a dietary fat appears to influence liver fat levels associated with threonine and choline deficiencies, (Morris[1], Expt. I.) this experiment was designed in which different dietary fats containing varied fatty acid compositions, some very high in saturated, monounsaturated, or diunsaturated fatty acids have been selected and compounded in low protein, high fat (30%), threonine deficient diets with two levels

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[1] Unpublished data.

of choline (0.15% and 1.00%). These diets were fed to weanling rats in an attempt to gain further insight into the lipotropic action of choline. By comparing the fatty acid profile of the different dietary fats, and the fatty acid patterns of the liver lipids of animals on the respective deficient and supplemented choline diets, some clue concerning the role of choline in the accumulation of lipid in threonine deficiency may be obtained.

METHODS

Fourteen groups of eight animals each, were fed the basal diet (see page 21) with five different dietary fats replacing corn oil. One half the number of rats received diets in which the choline level was increased from 0.15% to 1.00% of the diet.

To eliminate the problem of obtaining adequate cage space, food cups, and water bottles, this experiment was conducted in two series, in both of which the corn oil diet served as the control. The animals were fed the following diets:

Series I.

Group 1 -------Corn Oil----------0.15% Choline

Group 2 -------Corn Oil----------1.00% Choline

Group 3 -------Coconut Oil--------0.15% Choline

Group 4 -------Coconut Oil--------1.00% Choline

Group 5 -------Olive Oil----------0.15% Choline

Group 6 -------Olive Oil----------1.00% Choline

Series II.

Group 7 -------Corn Oil----------0.15% Choline

Group 8 -------Corn Oil----------1.00% Choline

Group 9 -------Safflower Oil------0.15% Choline

Group 10-------Safflower Oil------1.00% Choline

Group 11-------Cocoa Butter-------0.15% Choline

Group 12-------Cocoa Butter-------1.00% Choline

Group 13-------Commercial lard-----0.15% Choline

Group 14-------Commercial lard-----1.00% Choline

The average food consumption and weight gain per week were recorded, and feed efficiency ratio calculated. Feces were collected and total fecal fat determinations made for all groups studied. Total liver moisture, fat and nitrogen content were determined on the livers, minus the left lobes. These procedures are described in the section labelled General Methodology.

The fatty acid composition of the diet fats and liver lipids were determined. Total lipids were extracted from the left lobe of the livers by the method of Folch et al (42). Aliquots of the lipid extracts were evaporated to dryness and the lipid content determined gravimetrically. Methyl esters of diet and liver lipids were prepared, separated, and quantitated by gas-liquid chromatography as described in Experiment 1. The oven temperature was programmed from 160° - 220° at 5°/min. for all fatty acid separations except coconut oil. For these mixtures the temperature was programmed from 90°- 220°(5°/min.).

RESULTS

Food intake, weight gain and feed efficiency ratios for rats fed a 9% casein diet and containing different sources of dietary fats with two levels of choline (0.15% and 1.00%) are summarized in Table I (Series I) and Table II (Series II). In Series I, food intake for choline deficient animals and choline supplemented animals within each set was not significantly different, except for the group fed coconut oil. In this set, animals fed diets containing 0.15% choline ate significantly more than the group which consumed diets containing 1.00% choline ($P<0.05$), and also more than the corresponding group fed corn oil ($P<0.05$). The increased food intake was reflected in the growth pattern of animals fed coconut oil containing 0.15% choline, which was higher than the corresponding group fed corn oil. However, this increased growth rate was not significantly different from those fed coconut oil with supplemented choline. The only significant difference in growth patterns between the respective sets was for the set fed olive oil. Animals that consumed olive oil containing diets with 0.15% choline weighed significantly more ($P<0.05$) than the group that consumed diets containing 1.00% choline. There was no significant difference in food consumption, and correspondingly no significant difference in growth patterns and feed efficiency ratios among the different sets or within the respective sets for

animals in Series II, except for those fed commercial lard containing 1.00% choline. These animals gained slightly more than the corresponding group fed corn oil ($P<0.05$). The different response of control animals of Series I, and those of Series II, in food consumption and growth patterns may be due to the difference in litter mates and the time of the year that each experiment was conducted.

Liver composition data from rats in Series I and Series II are presented in Tables III and IV. In Series I (Table III) by increasing the level of choline from 0.15% to 1.00%, liver fat levels were significantly reduced (P 0.01) for all animals fed the three different dietary fats (Figure 1). This effect was most pronounced in the group fed coconut oil (28.3% vs 13.9%). The decrease in liver fat levels was balanced by an increase in the moisture content of the livers of rats fed the choline supplemented rations, except in the corn oil group, in which moisture levels were similar. Animals fed coconut oil and olive oil as the fat sources in choline deficient diets, had significantly higher liver fat levels ($P<0.01$) than animals fed corn oil. There was no significant difference between choline deficient animals fed coconut oil and those fed olive oil as the fat sources (28.3% vs 27.6%). No significant difference was observed in the nitrogen content between choline deficient animals and choline supplemented animals for those animals fed the corn oil diet. However, for the set fed

coconut oil, and for that fed olive oil, choline supplementation significantly increased the nitrogen content of the livers ($P<0.01$ and $P<0.05$ respectively). For animals in Series II, liver fat levels were elevated for all groups fed choline deficient diets except for those fed cocoa butter (Table IV, Figure I). Liver fat levels for animals fed cocoa butter were within the 10-14% range which is considered normal[1] for rats two weeks post weanling and above, on a stock diet. The addition of choline lowered liver fat levels, although the difference was significant at only 5% level (13.4% vs 10.7%). Choline supplementation significantly lowered liver fat levels for animals fed corn oil, safflower oil, and commercial lard. (23.0% vs 15.5%; 21.8% vs 15.5%; 22.5% vs 13.7% respectively). This difference was significant at the 1.00% level of significance or less for the corn oil and safflower oil groups and at the 5% level for lard group. The lower level of significance for commercial lard is probably due to the large standard error within the group fed the deficient diet. For the four sets of animals in Series II, choline supplementation significantly increased liver moisture content ($P<0.01$), but did not significantly alter liver nitrogen content, except for animals fed commercial lard. For this group choline supplementation significantly increased liver nitrogen content ($P<0.05$).

The Digestibility Data for the various dietary fats used in the experiment are shown in Tables V and VI. Choline supplementation had little or no effect on the digestibility of the various dietary

[1]Elvehjem,C.A., A report on "Amino Acid Imbalance" presented at the Symposium on Amino Acid and Protein (American Institute of Nutrition) held at Atlantic City, April 16-20, 1956.

fats; there was no significant difference between choline deficient and choline supplemented groups. Animals fed coconut oil, cocoa butter and commercial lard, the three most saturated dietary fats, excreted the greatest amount of fat, which was significantly different from the amount excreted by the corn oil group ($P<0.01$). However, the digestibility coefficient of coconut oil was not significantly different from that of corn oil. The digestibility coefficients of cocoa butter and commercial lard, which were not significantly different from each other, were significantly lower than that of corn oil. This may be due to the presence of tristearin in the dietary fats, which is poorly absorbed. This point was discussed previously in Experiment I page 41.

The data in Tables VII and VIII, and Figures 2, 3, 4 and 5, show the fatty acid composition of diet and liver lipids of rats fed 9% casein diets containing six different dietary fats with two levels of choline. Only the major fatty acids of these lipids are shown, namely: palmitic, stearic, oleic, linoleic, arachidonic, also lauric and myristic acids for coconut oil. The fatty acid patterns of the dietary lipids appear to be a major factor in controlling the fatty acid profile of liver lipids. The very high levels of linoleic acid in safflower oil, and oleic acid in olive oil, are strongly represented in the respective liver lipids of those animals. Rats fed coconut oil, the lone dietary fat that contains large amounts of lauric and myristic acids, were the only animals whose livers had appreciable quantities of lauric

and myristic acids. In contrast to some investigators (44, 71) choline deficiency appears to affect to some extent the fatty acid profile of liver lipids. The fatty acids seem mostly to be affected are $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$. In all the groups studied, animals fed choline deficient diets had lower levels of $C_{18:0}$ and $C_{20:4}$ fatty acids, and higher levels of $C_{18:1}$ and $C_{18:2}$, than animals fed choline supplemented diets. In all instances choline supplementation reversed the pattern, supporting higher levels of $C_{18:0}$ and $C_{20:4}$ and lower levels of $C_{18:1}$ and $C_{18:2}$ (Tables VII and VIII; Figures 2, 3, 4 and 5). In most cases, the difference is significant at the 1.00% level of significance or less. In animals fed coconut oil, choline supplementation causes a very significant decrease in $C_{12:0}$ and $C_{14:0}$ fatty acids in liver lipid ($P<0.01$). In all cases the level of $C_{16:0}$ fatty acids of liver lipids appears to be independent of dietary levels and choline seems to have no effect on this fatty acid. It is clear that liver lipid accumulation is not due to simple accumulation of dietary lipids.

Some fatty acids can be synthesized from two-carbon fragments with their origin from acetate, and can also be supplied from exogenous sources while others have to be supplied almost exclusively by the diet such as linoleic acid (Figure 6). A study of the interactions of one group of fatty acids versus the other group should give some useful information regarding the intermediary metabolism of the different fatty acids. A comparison of the different fatty acids within each group of fatty acids, in choline deficient

versus choline supplemented animals might reflect the possible role of choline in lowering liver fat levels. Table IX shows the ratio of fatty acids that can be synthesized from two carbon fragments originating from acetate, and can also be supplied by the diet to the non-synthesized fatty acids, linoleic acid and its derivative arachidonic acid, in diet lipids and in rat liver lipids of choline deficient and choline supplemented animals. When this ratio is high in diet fats (coconut oil and cocoa butter, Table IX) the animal accomplishes a significant decrease in the ratio of these two classes in the lipid deposited in liver tissues. However, when the dietary ratio is small, such a marked shift is not observed in liver lipid. It appears therefore that the animal tries to adjust the fatty acid composition in the liver lipid to achieve an equilibrium of the groups (A⇌B; Figure 6). This effect is especially pronounced when one is out of balance such as coconut oil with a ratio of 49:1 (Table IX); this ratio is drastically changed to 2.5:1, while corn oil with a ratio very close to 1 (0.8, Table IX) is hardly affected.

The ratio of linoleic acid to arachidonic acid of liver lipids for choline deficient and choline supplemented animals is given in Table X. In all cases choline supplementation significantly decreased linoleic acid levels with a concomitant increase in arachidonic acid; this effect is more pronounced for dietary fats high in linoleic acid.

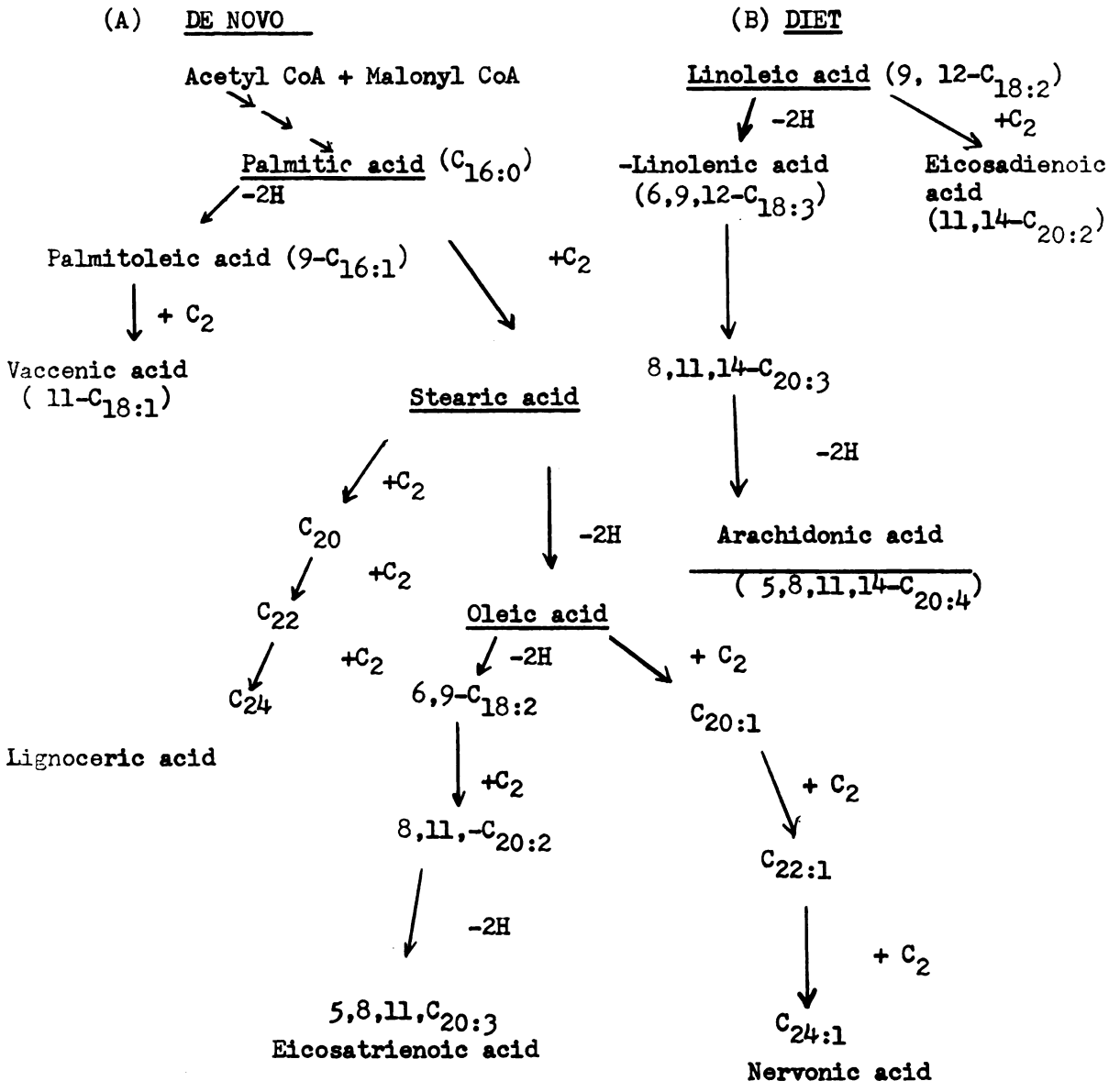

Figure 6. Biosynthesis of some fatty acids in mammals. (From: White, A., Handler, P., and Smith, E. L., 1964: Principles of Biochemistry; Third Edition, McGraw-Hill Book Company, U.S.A., page 451).

Shown in Tables XI, XII and XIII, are the ratios of palmitic acid ($C_{16:0}$) to stearic acid ($C_{18:0}$); stearic acid ($C_{18:0}$) to oleic acid ($C_{18:1}$); and palmitic acid ($C_{16:0}$) to oleic acid ($C_{18:1}$), of diet and liver lipids of choline deficient and choline supplemented animals. Since stearic acid appears to be preferentially conserved over palmitic and oleic acids, and since this fatty acid can originate from both palmitic and oleic acids, the first two ratios were calculated in an attempt to see the effect of choline on these fatty acids. The last ratio was calculated to see which, if any, route accumulates more stearic acid. From Table XI, it appears that choline supplementation either reduces the concentration of palmitic acid or increases the concentration of stearic acid or both. From Table XII, choline supplementation either increases stearic acid content or decreases oleic acid content or both. Table XIII, shows an increase in the ratio of palmitate to oleate with choline supplementation, which might be interpreted as a more pronounced decrease in oleic acid concentration over palmitic acid concentration; hence stearic acid appears to be preferentially conserved over oleic acid.

DISCUSSION

An increase in choline content from 0.15 to 1.00% of all the diets studied served to decrease liver fat levels in threonine deficient rats, regardless of the fat source of the diet. Thus the basal level of 0.15% choline represents a choline deficiency state under the present dietary condition and provides an opportunity of studying the role of choline in this syndrome.

The results of the present experiment cannot support the contention of some workers (26, 27, 12, 58) that the amount of saturated fatty acids in the dietary fat is directly related to the accumulation of liver lipid in choline deficient rats. Of the six dietary fats studied, coconut oil, which is very high in carbon twelve and carbon fourteen saturated fatty acids when fed to weanling rats produced liver fat levels equivalent to those obtained when olive oil is fed. Olive oil contains very high levels of a monounsaturated fatty acid. The liver fat levels of threonine deficient rats fed corn oil, safflower oil, and commercial lard were comparable although the fatty acid profiles of these fats are somewhat different. On the other hand, cocoa butter, which was selected as a source of dietary fat for its high levels of palmitic, stearic and oleic acids, when fed to weanling rats in threonine and choline deficient diets, produced the lowest liver fat levels (13.4% vs 23.0%). Hence the nature of the dietary fat appears to influence to some

extent liver fat accumulation in choline deficiency, but the theory that the amount of saturation appears to govern the deposition of fat in the liver in choline deficiency cannot be substantiated from these results. These results would indicate different metabolic pathways for fatty acids, some placing greater stress on the liver during choline deficiency while others appear to exert a sparing action on choline. Cocoa butter seems to spare both choline and threonine.

The low liver fat levels observed in animals fed a low protein diet deficient in threonine and choline and containing cocoa butter as the fat source, cannot be considered a reflection of its lower coefficient of digestibility to that of corn oil. The coefficient of digestibility of cocoa butter is similar to that of commercial lard, yet when animals are fed similar diets, with commercial lard as the fat source, liver fat accumulates to the same extent as that produced when animals are fed corn oil. Since animals fed cocoa butter did not consume significantly more than animals fed corn oil, an increased threonine intake cannot be attributed to the lower liver fat levels obtained when animals are fed cocoa butter. However, an outstanding peculiarity of cocoa butter, is its extraordinarily high content of stearic acid. Stearic acid makes up an average of 34% of the total fatty acids in cocoa butter; although this high level may seem to make this fat poorly absorbed, this is not the case, because of its high oleic acid content (35%). This will tend to produce disaturated-monounsaturated triglycerides which are well

absorbed. Stearic acid appears to be more protective against fatty livers associated with choline deficiency since liver fat levels were initially low and since the addition of choline only slightly reduced liver fat levels. This effect suggests the possibility of a different metabolic route for stearic acid as compared to linoleic or oleic acids. These acids are the primary fatty acids of the dietary fats that produced high liver fat levels in choline deficiency. This finding could be correlated with those of Stetten and Salcedo (86) who fed the ethyl esters of the saturated fatty acids (C_{14}-C_{18}) in the diets of choline deficient rats, and found that ethyl stearate produced the lowest liver fat levels. Bergstrom et al (14) fed carboxy-labeled stearate, palmitate and myristate to rats and compared the specific activities to the resulting glyceride and phospholipid fatty acids. Each acid was incorporated into triglyceride (corn oil) by transesterification before feeding. Four hours after feeding the C^{14} stearate-triglyceride, the intestinal phospholipids were labeled more heavily than the triglycerides. Palmitic and myristic acids on the other hand, were incorporated more readily into glycerides than phospholipids. In similar experiments MoreHouse et al (72) found that the phospholipid of both rat mucosa and liver incorporated stearic acid more rapidly than palmitic acid. Studies comparing the fatty acid composition of rat plasma glyceride and phospholipids led Le Breton and Pascaud (76) to conclude that stearic acid was transported selectively in phospholipids, linoleic acid was

principally carried in triglyceride form, while arachidonic and other polyunsaturated acids were incorporated into phospholipids or cholesterol esters. Other studies using cocoa butter as the dietary fat have been reported. Keys et al (62) studied the response of serum cholesterol to different dietary fats and found that cocoa butter did not increase serum cholesterol levels. Similar findings were reported by Connor et al (32) and Erickson et al (38) who used substantial amounts of cocoa butter in the diet yet found low serum cholesterol levels with supplemented cholesterol diets. Since increased serum cholesterol levels were found when other long chain saturated fatty acids were fed, and low levels when polyunsaturated fatty acids were fed, these workers suggested a cholesterol lowering effect of stearic acid, similar to that of polyunsaturated fatty acids. From the results of this experiment and from the reports of other studies, it is tempting to assume that phospholipids may play some part in the transport or metabolism of stearic acid. This fatty acid may give cocoa butter its peculiar characteristic, that of sparing choline in high fat, low protein, threonine deficient diets.

The supplementation of choline to these high fat diets resulted in decreased liver fat levels. However, the degree to which these levels were lowered differed with the dietary fat fed. The difference in liver fat levels between choline deficient and choline supplemented animals fed coconut or olive oil was greater than animals fed corn oil, safflower oil, cocoa butter or commercial lard. This may be interpreted as a greater stress imposed on the

livers of choline deficient animals by some fatty acid of coconut oil and olive oil. These fatty acids appear to increase the animal's requirement for choline. Since oleic acid, (Olive oil) lauric and myristic acids (Coconut oil) are the fatty acids of these two dietary fats, these fatty acids could have similar metabolic pathways.

The livers of choline deficient animals contained more oleic acid and linoleic acid than the livers of choline supplemented animals. On the other hand, significantly higher levels of stearic and arachidonic acids were found in livers of choline supplemented animals than those from choline deficient animals. In the normal rat, liver phospholipid levels are higher than triglyceride levels (54.3% vs 30.5%) (25), and phospholipids contain more stearic and arachidonic acids and less oleic acids than the liver triglycerides (72, 25). This observation seems to correlate positively with Holman's study (57) on the relationship between dietary fatty acids and liver fatty acids in the rat. He found a very high positive correlation between stearic acid and arachidonic acid. This could suggest a common metabolic path for these two fatty acids, presumably their incorporation into phospholipids. Data from the fatty acid composition of liver lipids suggest that in choline deficiency there might be an increase in the triglyceride content resulting in an increased ratio of triglyceride to phospholipid. When choline is supplemented to the diet, this ratio decreases suggesting a decrease in triglyceride levels or an increase in phospholipid content. These results seem to support the hypothesis of several workers (44, 63, 64, 66, 73, 88, 89, 98) that

during choline deficiency, fat transport from the liver is reduced. The concentration of phosphotidyl choline may be decreased in liver because of low choline levels in the diet. This would produce a slow turnover of the β-lipo-protein which transport the triglycerides out of the liver. If Kennedy's pathway (60) is reviewed,

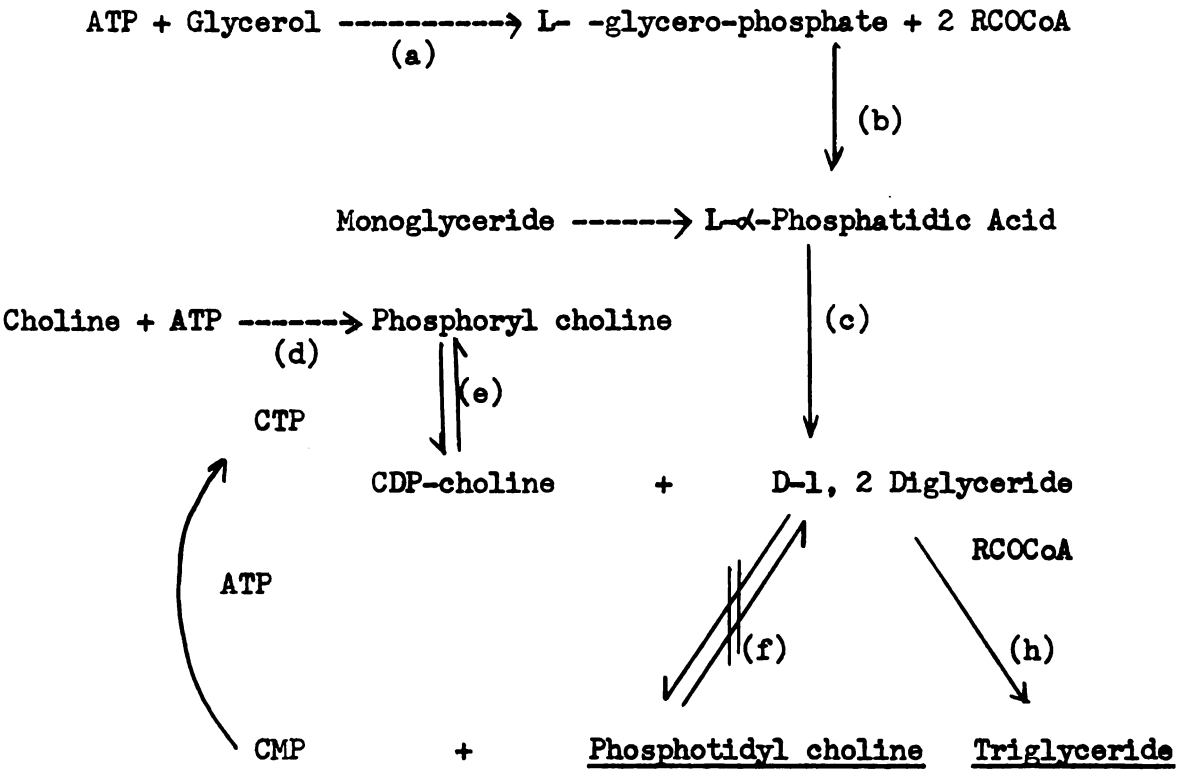

Figure 7: Pathway for enzymatic synthesis of phospholipids and triglycerides. (From Journ. Amer. Clin. Nutr. 6:217, 1958.)

it would appear that during choline deficiency pathway (f) would be highly reduced and therefore the D-1,2 Diglyceride would be channelled almost entirely to pathway (h); this would lead to an increase in triglyceride accumulation. Since phospholipid concentration in the

liver would be very low, and the β - lipo-protein complex would be synthesized at a much slower rate, triglyceride transport from the liver would be slow and therefore triglyceride accumulates. With this large ratio of triglyceride to phospholipids, an increase in the levels of oleic and linoleic acids respectively, would be observed. However when choline is supplemented to the diets phospholipid synthesis is increased, β -lipoprotein synthesis is increased, and triglycerides are removed at a faster rate. The ratio of triglyceride to phospholipid then decreases, getting closer to normal values. This would result in an increase in the stearate and arachidonate levels, the major components of the phospholipid moiety.

The consistent increase in the arachidonic acid concentration and concomitant decrease in linoleic acid levels in choline supplemented animals, might be indicative of an involvement of choline in the process of elongation of fatty acids within the mitochondria. This concept has been proposed earlier by Chalvardjian (29). Viviani et al (95) also found increased levels of linoleic acid and oleic acid in the livers of threonine and lysine deficient rats. The addition of threonine and lysine to these deficient diets significantly increased the levels of $C_{20:4}$, $C_{20:5}$, $C_{22:6}$ in the liver lipids. There seems to be an interference in polyunsaturated fatty acid metabolism in both choline and threonine deficiencies which could suggest that the threonine deficiency may interfere with phospholipid synthesis. However, this possibility is weakened by the fact that these two deficiencies (choline and threonine) are histologically different (see pages 4,5) which might argue in favor of two different biochemical mechanisms.

Table 1. Food intake, weight gain and feed efficiency ratio of rats fed a 9% casein diet containing different sources of dietary fats, with two levels of choline.

Group #	Diet[1]	# of animals /group	Food intake g/week	Wt. gain g/week	Feed eff.
Series 1.					
1	Corn oil with 0.15% chol.	8	73.0 ±3.2[2]	20.0 ±1.0[2]	0.27
2	Corn oil with 1.00% chol.	8	74.0 ±2.4	21.0 ±0.7	0.29
	Sign. of diff. (P)		ns	ns	
3	Coconut oil with 0.15% chol.	8	83.0 ±2.4[3]	23.0 ±0.5[3]	0.28
4	Coconut oil with 1.00% chol.	8	74.0 ±1.9	22.0 ±0.7	0.30
	Sign of diff. (P)		< 0.05	ns	
5	Olive oil with 0.15% chol.	7	77.0 ±1.6	22.0 ±0.7	0.28
6	Olive oil with 1.00% chol.	8	75.0 ±1.6	20.0 ±0.7	0.27
	Sign of diff. (P)		ns	< 0.05	

[1] Fat content of all diets = 30% w/w. Length of experimental period = 4 wks.

[2] Standard error of the mean.

[3] Significant difference from diet 1.

[4] Significant difference from diet 2.

Table II. Food intake, weight gain and feed efficiency ratio of rats fed a 9% casein diet containing different sources of dietary fats, with two levels of choline

Group #	Diet[1]	# of animals /group	Food intake g/week	Wt. gain g/week	Feed eff.
Series II					
7	Corn oil with 0.15% chol.	8	59.0 ±2.1[2]	17.0 ±0.9[2]	0.29
8	Corn oil with 1.00% chol.	8	59.0 ±0.7	16.0 ±0.3	0.28
	Sign of diff. (P)		ns	ns	
9	Safflower oil with 0.15% chol.	8	61.0 ±2.8	18.0 ±1.1	0.30
10	Safflower oil with 1.00% chol.	8	61.0 ±0.8	17.0 ±0.4	0.29
	Sign of diff. (P)		ns	ns	
11	Cocoa butter with 0.15 chol.	7	66.0 ±1.5	18.0 ±0.7	0.30
12	Cocoa butter with 1.00 chol.	8	65.0 ±1.8	19.0 ±0.9	0.29
	Sign. of diff. (P)		ns	ns	
13	Commercial lard with 0.15 chol.	8	64.0 ±2.0	20.0 ±1.1	0.31
14	Commercial lard with 1.00 chol.	8	64.0 ±2.1	19.0 ±1.1[4]	0.29
	Sign. of diff. (P)		ns	ns	

[1] Fat content of all diets = 30% w/w. Length of experimental period = 4 week.

[2] Standard error of the mean.

[3] Significant difference from diet 7.

[4] Significant difference from diet 8.

Table III. Liver composition of rats fed 9% casein diets containing different sources of dietary fats with two levels of choline.

Group # Series I.	Diet[1]	Moisture %	Fat % dry wt.	Nitrogen % wet wt.
1	Corn oil with 0.15% chol.	70.0 ±0.5[2]	20.6 ±0.9[2]	2.75 ±0.07[2]
2	Corn oil with 1.00% chol.	70.8 ±0.3	15.1 ±0.8	2.83 ±0.07
	Sign. of diff. (P)	ns	< 0.01	ns
3	Coconut oil with 0.15 chol.	67.6 ±0.3	28.3 ±1.6[3]	2.71 ±0.03
4	Coconut oil with 1.00 chol.	71.2 ±0.6	13.9 ±0.8	3.07 ±0.08[4]
	Sign. of diff. (P)	< 0.01	< 0.01	< 0.01
5	Olive oil with 0.15% chol.	67.6 ±0.05[3]	27.6 ±1.0[3]	2.49 ±0.06[3]
6	Olive oil with 1.00% chol.	70.4 ±0.7	16.6 ±0.7	2.75 ±0.08
	Sign. of diff. (P)	< 0.01	< 0.01	< 0.05

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Standard error of the mean.

[3] Significanct difference from diet 1.

[4] Significant difference from diet 2.

Table IV. Liver composition of rats fed 9% casein diets containing different sources of dietary fats with two levels of choline

Group # Series II.	Diet[1]	Moisture %	Fat % dry wt.	Nitrogen % wet wt.
7	Corn oil with 0.15% chol.	68.4 ±0.5[2]	23.0 ±1.5[2]	2.46 ±0.7[2]
8	Corn oil with 1.00% chol.	70.6 +0.3	15.4 +1.1	2.65 +0.06
	Sign. of diff. (P)	< 0.01	< 0.01	ns
9	Safflower oil with 0.15% chol.	67.9 ±0.4	21.8 ±1.3	2.44 ±0.05
10	Safflower oil with 1.00% chol.	70.6 ±0.3	15.5 ±0.7	2.51 ±0.13
	Sign. of diff. (P)	< 0.01	< 0.01	ns
11	Cocoa butter with 0.15% chol.	70.4 ±0.3[3]	13.4 ±0.7[3]	2.61 ±0.07
12	Cocoa butter with 1.00% chol.	71.5 ±0.1	10.7 ±0.6[4]	2.65 ±0.08
	Sign. of diff. (P)	< 0.01	< 0.05	ns
13	Commercial lard with 0.15% chol.	68.0 ±0.8	22.5 ±2.8	2.32 ±0.06
14	Commercial lard with 1.00% chol.	70.9 ±0.5	13.7 ±1.3	2.51 ±0.02
	Sign. of diff. (P)	< 0.01	< 0.05	< 0.05

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Standard error of the mean.

[3] Significant difference from diet 7.

[4] Significant difference from diet 8.

Table V. Digestibility data of various dietary fats in a 9% casein diet containing two levels of choline.

Group #	Diet[1]	# of animals /group	Fecal fat % dry wt.	Fecal fat Total amt. excreted[5]	Coeff. of Digest.
Series I.					
1	Corn oil with 0.15% chol.	5	9.25 ±0.48[2]	1.10 ±0.09[2]	90.8 ±0.7[2]
2	Corn oil with 1.00% chol.	5	9.37 ±0.29	1.16 ±0.06	90.8 ±0.7
	Sign. of diff. (P)		ns	ns	ns
3	Coconut oil with 0.15%chol.	5	9.53 ±0.49	1.45 ±0.12[3]	90.1 ±0.9
4	Coconut oil with 1.00% chol.	5	9.27 ±0.59	1.14 ±0.11	91.1 ±1.0
	Sign. of diff. (P)		ns	ns	ns
5	Olive oil with 0.15% chol.	5	8.58 ±0.57	1.09 ±0.13	91.7 ±0.1
6	Olive oil with 1.00% chol.	5	8.70 ±0.45	1.04 ±0.45	92.00±0.6
	Sign. of diff. (P)		ns	ns	ns

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Standard error of the mean.

[3] Significant difference from diet 1.

[4] Significant difference from diet 2.

[5] Total fat excreted in 4 day period, corrected for endogenous fat (determined in rats fed fat-free diet).

Table VI. Digestibility data of various dietary fats in a 9% casein diet containing two levels of choline.

Group #	Diet[1]	# of animals /group	Fecal fat		Coeff. of Digest.
			% dry wt.	Total amt. excreted[5]	
Series II					
7	Corn oil with 0.15% chol.	5	8.25 ±0.83[2]	0.72 ±0.14[2]	92.4 ±1.2[2]
8	Corn oil with 1.00% chol.	5	8.70 ±0.42	0.74 ±0.09	92.0 ±1.2
	Sign. of diff. (P)		ns	ns	ns
9	Safflower oil with 0.15% chol.	5	6.80 ±0.25[4]	0.50 ±0.00	94.7 ±0.2
10	Safflower oil with 1.00% chol.	5	7.15 ±0.11[4]	0.55 ±0.06	94.0 ±0.7
	Sign. of diff. (P)		ns	ns	ns
11	Cocoa butter with 0.15% chol.	5	12.71 ±0.75[3]	1.84 ±0.27[3]	85.0 ±1.7[3]
12	Cocoa butter with 1.00% chol.	5	12.51 ±0.67[4]	1.65 ±0.22[4]	86.5 ±1.5[4]
	Sign. of diff. (P)		ns	ns	ns
13	Commercial lard with 0.15% chol.	5	10.89 ±0.27[3]	1.39 ±0.08[3]	86.8 ±0.9[3]
14	Commercial lard with 1.00% chol.	5	10.77 ±0.20[4]	1.20 ±0.09[4]	89.1 ±1.3

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Standard error of the mean.

[3] Significant difference from diet 7.

[4] Significant difference from diet 8.

[5] Total fat excreted in 4 day period, corrected for endogenous fat (determined in rats fed fat-free diet).

Table VII. Fatty acid composition of diet and liver lipids of rats fed a 9% casein ration containing two levels of choline. (per 100 grams of total fatty acids.)

Group #	Diet[1]	Lauric $C_{12:0}$	Myristic $C_{14:0}$	Palmitic $C_{16:0}$	Stearic $C_{18:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Arachidonic $C_{20:4}$
0[2]	Corn oil			13.9	2.2	27.8	56.2	[illegible]
1	" with 0.15% chol.			13.8±1.2[3]	8.4±1.4[3]	14.0±0.7[3]	33.2±2.1[3]	12.8± 1.7[3]
2	" with 1.00% chol.			15.8±0.7	15.9±2.1[5]	10.8±1.5	24.1±3.0[5]	20.3± 1.7[4]
0[2]	Coconut oil	45.9	17.8	8.9	2.5	7.1	2.4	
3	" with 0.15% chol.	16.5±3.4	13.5±2.6	24.7±1.8	6.9±0.8	18.3±1.4	5.5±0.4	6.1± 0.7
4	" with 1.00% chol.	4.3±0.6	5.7±0.4	21.5±1.2	15.1±1.4[4]	13.3±0.6	6.5±0.6	17.5± 1.3[4]
0[2]	Olive oil			11.4	2.7	78.1	7.9	
5	" with 0.15% chol.			16.7±0.9	7.0±0.5	60.2±1.7	7.7±0.1	6.8± 0.4
6	" with 1.00% chol.			16.6±0.6	11.7±0.8[4]	50.1±2.5	6.6±0.4	12.2± 1.1[4]

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Chemical analysis of diet fats.

[3] Standard error of the mean.

[4] Significantly different from control (fat with 0.15% chol.) ($P<0.01$).

[5] Significantly different from control (fat with 0.15% chol.) ($P<0.05$%).

Table VIII. Fatty acid composition of diet and liver lipids of rats fed diets containing various dietary fats with two levels of choline (per 100 gms. of total fatty acids).

Group #	Diet[1]	Palmitic $C_{16:0}$	Stearic $C_{18:0}$	Oleic $C_{18:1}$	Linoleic $C_{18:2}$	Arachidonic $C_{20:4}$
0[2]	Corn oil	13.9	2.2	27.8	56.2	
7	" with 0.15% chol.	14.5 ±0.5[3]	7.2 ±0.3[3]	16.5 ±0.6[3]	42.6 ±1.0[3]	11.1 ±0.4[3]
8	" with 1.00% chol.	15.0 ±0.4	9.8 ±0.8[5]	16.3 ±1.0	33.6 ±0.6	14.7 ±1.0
0[2]	Safflower oil	6.9	2.2	12.2	78.8	
9	" with 0.15% chol.	11.5 ±0.4	10.9 ±0.7[4]	8.3 ±0.2	53.9 ±0.8	10.4 ±0.4
10	" with 1.00% chol.	11.8 ±0.4	10.9 ±0.7[4]	8.0 ±0.3	43.4 ±1.7[4]	15.0 ±0.8[4]
0[2]	Cocoa butter	28.4	33.5	35.3	2.9	
11	" with 0.15% chol.	24.0 ±1.1	16.3 ±0.5	39.0 ±2.0	6.8 ±0.3	9.5 ±1.1
12	" with 1.00% chol.	21.1 ±1.2	17.9 ±0.2[5]	32.8 ±2.1	6.5 ±0.4	13.6 ±0.9
0[2]	Commerical lard	29.8	13.6	40.8	10.1	
13	" with 0.15% chol.	25.4 ±0.6	8.9 ±0.5	40.0 ±0.8	15.0 ±0.3	6.8 ±0.4
14	" with 1.00% chol.	24.1 ±0.4	13.4 ±0.4[4]	29.7 ±0.2[4]	11.8 ±0.3	12.2 ±0.3[4]

[1] Fat content of all diets = 30% w/w; Length of experimental period = 4 weeks.

[2] Chemical analysis of diet fats.

[3] Standard error of the mean.

[4] Significantly different from control (fat with 0.15% chol.) ($P<0.01$).

[5] Significantly different from control (fat with 0.15% chol.) ($P<0.05$).

Table IX. Ratio of the non-essential fatty acids to the fatty acids of the linoleic family of liver lipids of rats fed diets containing various dietary fats with two levels of choline.

Fat	Corn oil	Coconut oil	Olive oil	Corn oil	Safflower oil	Cocoa but.	C. lard
Diet	0.8	40.7	11.7	0.8	0.3	33.5	8.9
Liver(0.15% chol.)	0.8	7.1	5.8	0.7	0.4	4.9	3.4
Liver(1.00% chol.)	1.0	2.6	4.2	0.9	0.5	3.6	2.8

Table X. Ratio of linoleic acid to arachidonic acid of liver lipids of rats fed diets containing various dietary fats with two levels of choline.

Fat	Corn oil	Coconut oil	Olive oil	Corn oil	Safflower oil	Cocoa but.	C. lard
Liver(0.15% chol.)	2.6	0.9	1.1	3.8	5.2	0.7	2.2
Liver(1.00% chol.)	1.2	0.4	0.5	2.3	2.9	0.5	1.0

Table XI. Ratio of palmitic to stearic acid of liver lipids of rats fed diets containing various dietary fats with two levels of choline.

Fat	Corn oil	Coconut oil	Olive oil	Corn oil	Safflower oil	Cocoa but.	C. lard
Diet	6.3	3.6	4.2	6.3	3.1	0.8	2.2
Liver(0.15% chol.)	1.6	3.6	2.4	2.0	1.7	1 5	2.9
Liver(1.00% chol.)	1.0	1.4	1.4	1.5	1.1	1.2	1.8

Table XII. Ratio of stearic to oleic acid of liver lipids of rats fed diets containing various dietary fats with two levels of choline.

Fat	Corn oil	Coconut oil	Olive oil	Corn oil	Safflower oil	Cocoa but.	C. lard
Diet	0.08	0.4	0.4	0.1	0.2	0.9	0.3
Liver (0.15% chol.)	0.6	0.4	0.4	0.4	0.8	0.4	0.2
Liver (1.00% chol.)	1.5	1.1	1.1	0.6	1.4	0.5	0.5

Table XIII. Ratio of palmitic to oleic acid of liverlipids of rats fed diets containing various dietary fats with two levels of choline.

Fat	Corn oil	Coconut oil	Olive oil	Corn oil	Safflower oil	Cocoa but.	C. lard
Diet	0.5	1.3	0.2	0.5	0.6	0.8	0.7
Liver (0.15% chol.)	1.0	1.3	0.3	0.9	1.4	0.6	0.6
Liver (1.00% chol.)	1.5	1.6	0.3	0.9	1.5	0.6	0.8

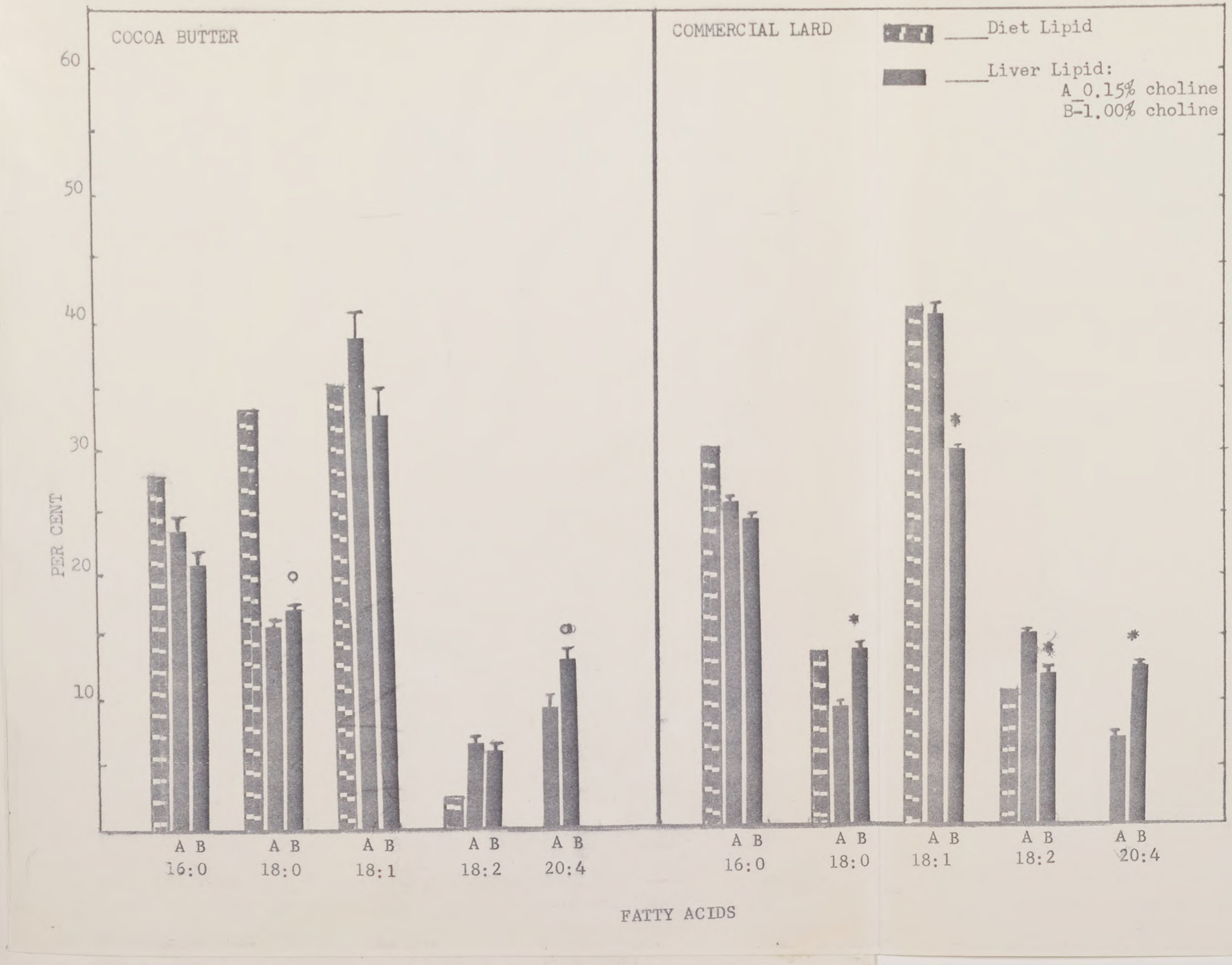

Figure 5. Fatty acid composition of diet and liver lipids of rats fed cocoa butter and commercial lard.
Significance of difference between experimental (B) and control (A) is indicated as: *--(P<0.01)
o--(P<0.05)

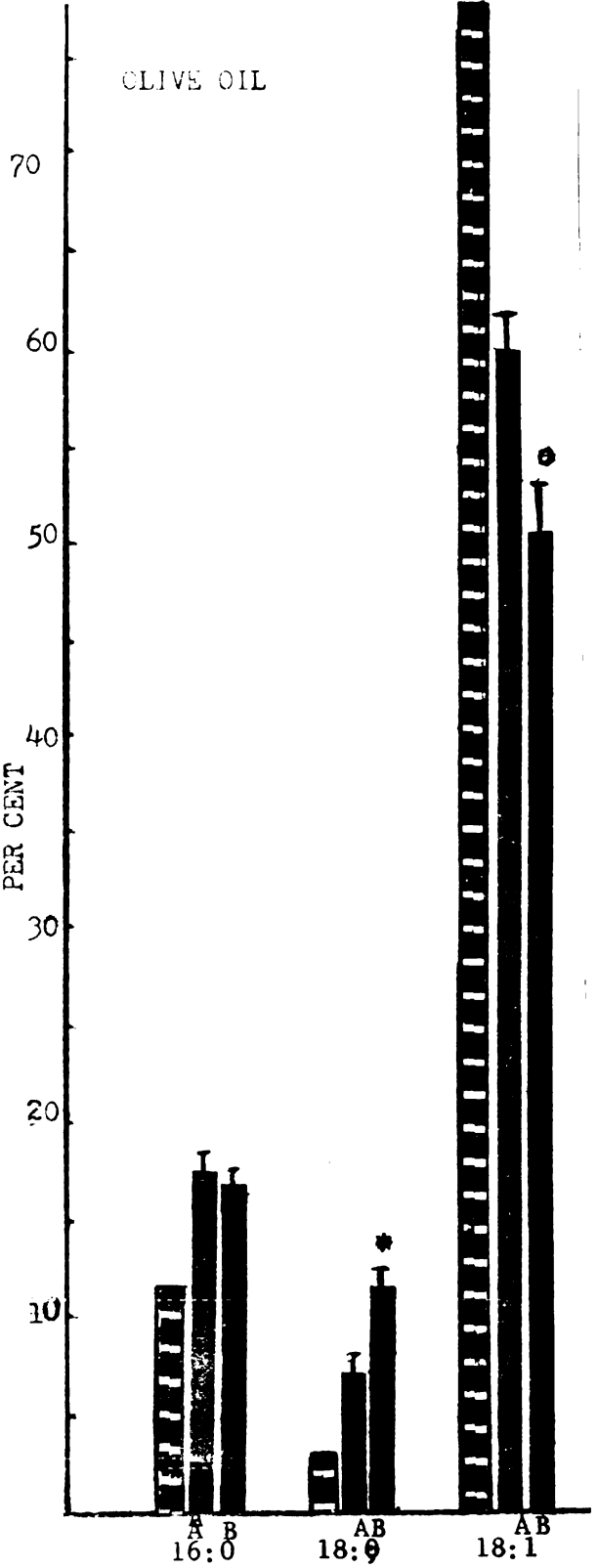

Figure 3. Fatty acid composition of diet and liver lipids of rats fed olive oil.
Significance of difference between experimental (B) and control (A) is indicated as: *--(P<0.01)
o--(P<0.05)

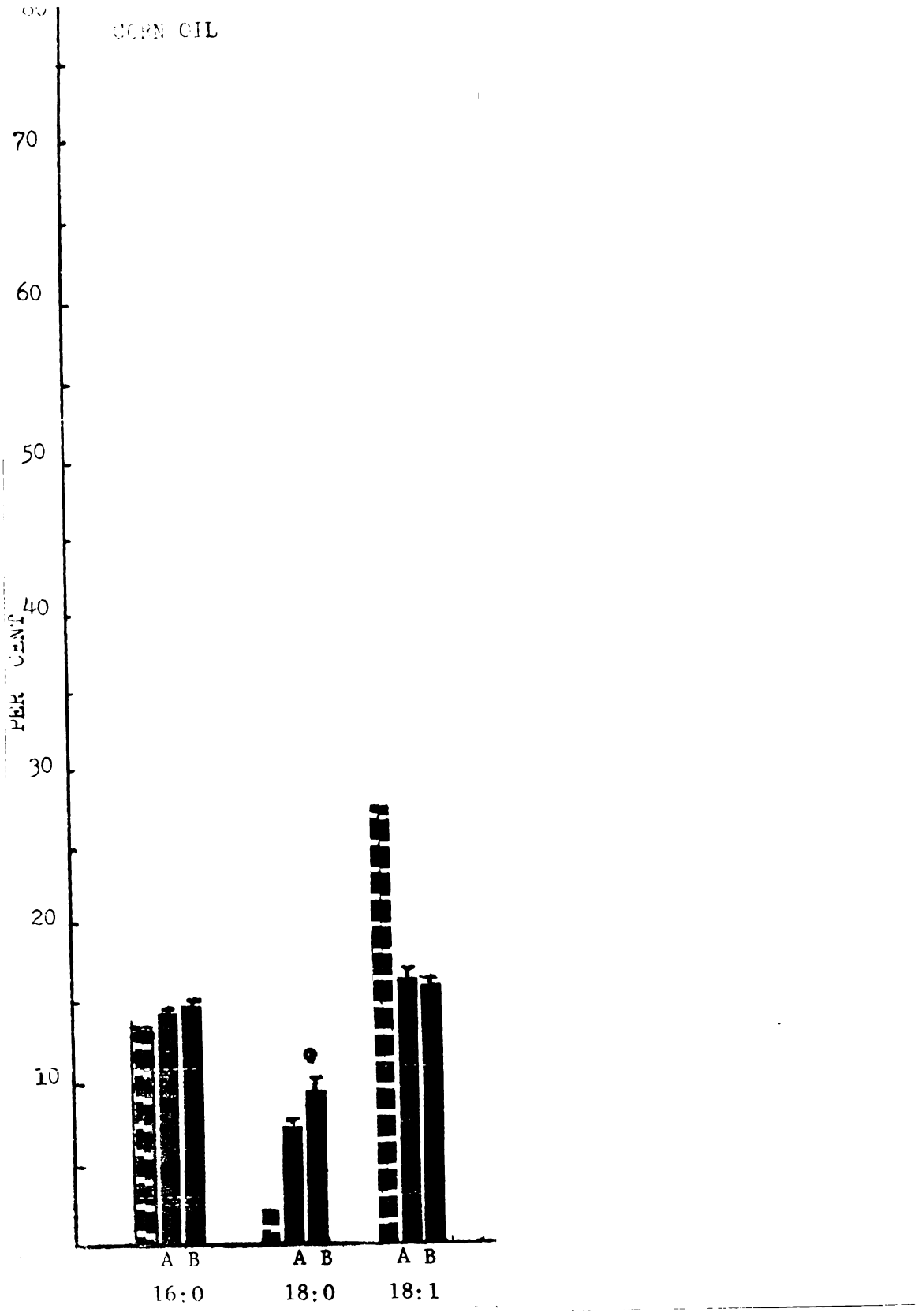

Figure 4. Fatty acid composition of diet and liver lipids of rats fed corn oil and safflower oil.
Significance of difference between experimental (B) and control (A) is indicated as *--(P<0.01)
o--(P<0.05)

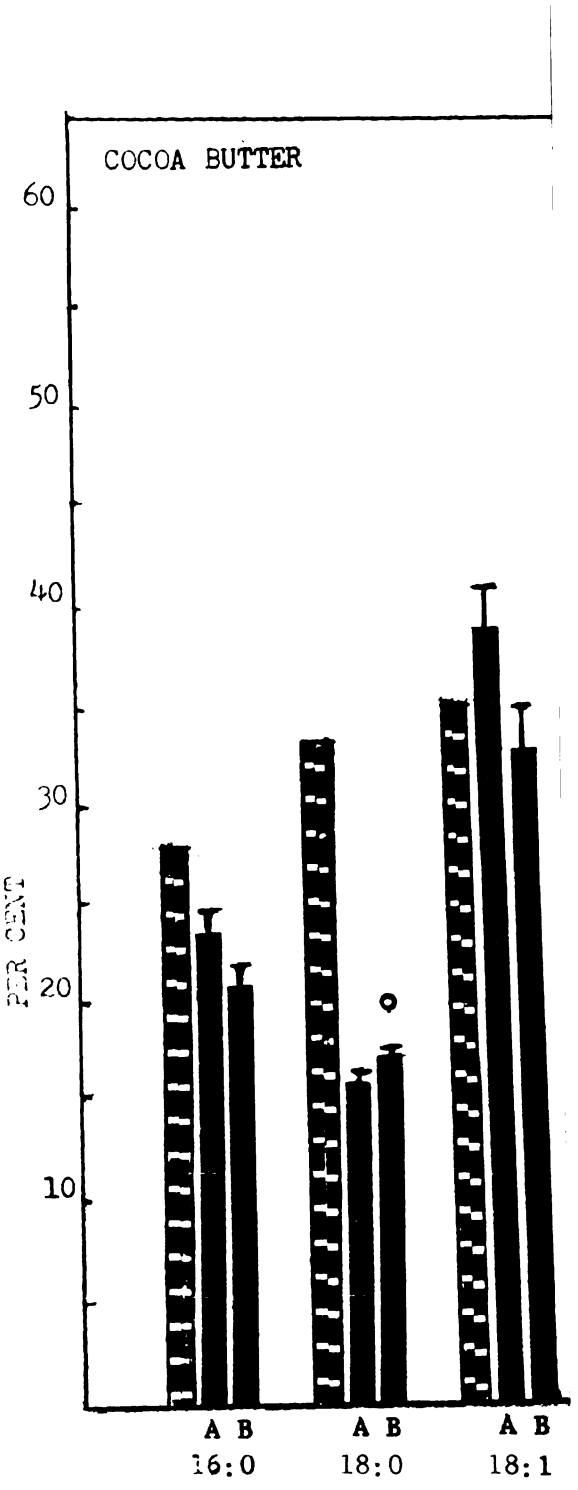

Figure 5. Fatty acid composition of diet and liver lipids of rats fed cocoa butter and commercial lard.
Significance of difference between experimental (B) and control (A) is indicated as: *--(P<0.01)
o--(P<0.05)

GENERAL SUMMARY AND CONCLUSION

Male weanling rats of the Sprague-Dawley strain were fed for four weeks, various isocaloric diets, deficient in threonine and choline. The control diet contained 9% casein, 30% corn oil and 0.15% choline. The following modifications were made to investigate their effect on liver lipids.

1. Varying the type of dietary fat to study:
   (a) Naturally hydrogenated versus chemically hydrogenated fats.
   (b) Dietary fats with different fatty acid compositions, some high in saturated, mono-unsaturated, or di-unsaturated fatty acids.
2. Increasing the level of choline from 0.15% to 1.00% in the diet, and its effect on the fatty acid patterns of liver lipid.

Food consumption and weight gain of control and experimental animals were determined and compared, liver moisture, fat and nitrogen content were studied. The digestibility coefficient of the different dietary fats were determined and the fatty acid composition of each of the dietary fats and of liver lipids extracted from control and experimental animals were determined and compared. From this study the following observations were made:

1. The chemical nature of the fatty acid component of the dietary fat influences the degree of liver fat accumulation in

threonine and choline deficiencies.

2. The degree of saturation of the dietary fat is not a major factor in the accumulation of fat in the livers of threonine and choline deficient rats. Neither hydrogenated corn oil (iodine value of 74) nor cocoa butter (65% saturated) caused fatty livers in threonine and choline deficient rats, whereas lard (iodine value of 55) did induce high liver fat concentrations (23%) in these animals.

3. Some fatty acids appear to spare the action of threonine and choline. Hydrogenated corn oil which contains 45% trans acids prevented liver fat accumulation in choline and threonine deficient rats as compared to corn oil, natural lard and commercial lard which are devoid of trans acids. Cocoa butter with its extra-ordinarily high concentration of stearic acid did not induce liver fat deposition in the threonine and choline deficient state as compared to the other dietary fats which are high in oleic and linoleic acids.

4. Choline supplementation causes a significant decrease in liver fat levels irrespective of the dietary fat fed. However, a more pronounced effect has been observed when the dietary fat has higher levels of the non-essential fatty acids, than the essential fatty acids.

5. Choline deficiency causes higher levels of linoleic and oleic acids in the livers of rats irrespective of the dietary fat fed.

6. The livers of choline supplemented animals contained higher levels of stearic acid and arachidonic acids than the livers of choline deficient animals.

7. The level of palmitic acid in the liver of both choline deficient and choline supplemented rats appears to be independent of its level in the dietary fats suggesting synthesis of this acid in the liver; therefore fat accumulation is not entirely of dietary origin.

8. Neither choline nor threonine deficiencies seem to have an effect on food consumption or weight gain.

9. The more saturated dietary fats- hydrogenated corn oil, cocoa butter, lard, and coconut oil are efficiently absorbed.

The possible lipotropic effect exerted by the trans isomers of hydrogenated corn oil, and stearic acid of cocoa butter, in the threonine and choline deficient state, may suggest a common metabolic path for these fatty acids. Since these acids have been found to occupy the α'-position of the phospholipid molecule, and these same acids have been found to be preferentially incorporated in the phospholipid molecule in the intestinal mucosa rather than the triglyceride molecule, one might assume that these acids do not travel via the liver but are transported directly to other tissues, where they may have a specific function.

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