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THE NATURE OF DIETARY FAT AND THE PATTERN OF HEPATIC LIPOSIS IN CHOLINE-DEFICIENT MICE†

The amount of hepatic liposis that occurs in animals fed hypolipotropic diets is to some extent dependent upon the chain length and degree of saturation of the lipid components of the experimental diet.[10, 21]

This report is concerned with the relation of the chemical nature of the lipid components of a choline-deficient diet to the intralobular pattern of hepatic liposis. Lard, butter, or synthetic triglycerides served as the lipid components of the several diets. The major purpose of the experiments was accomplished in that two distinct patterns of hepatic liposis were produced, one a rapid and complete lobular liposis, the other much less extensive and predominantly restricted to peripheral (portal) zones of lobules.

MATERIALS AND METHODS

A diet that had previously produced hepatic liposis characteristic of choline deficiency in rats[15] and in mice[19] was used. The composition of this diet is as follows:

	gms.
Vitamin free casein	80.0
Sucrose	480.5
Fat (see table 1)	400.0
Salt Mixture (No. 2, U.S.P.XIII)	40.0
l-cystine	5.0
Cod liver oil	4.5
Vitamin powder	10.0

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The vitamin powder contains:

Thiamine hydrochloride	0.500
Riboflavin	0.250
Pyridoxine hydrochloride	0.200
Calcium pantothenate	1.000
Nicotinic acid	1.000
Powdered sugar	997.050

The duration of the experiments, the composition (lipids) of the 8 diets, and the number of mice fed each of the diets are shown in Table 1. Young adult mice of the C (Bagg albino) stock with an initial body weight of 20-22 gms. were used. From weaning until restriction to the high fat diets the mice were fed the standard laboratory ration of Purina Fox Chow.

In diet I (Table 1) lard or butter was used as the fat component. Of the 80 mice fed this diet only 15 received butter as the dietary fat. Five of these mice were killed after one day of feeding, 5 after 3-7 days, and 5 during the 10-30 days' interval. These mice showed a pattern of hepatic liposis identical to that observed in mice fed the diet containing lard. In the remaining diets (II-VIII) the dietary lipid was supplied as one, two, or three synthetic triglycerides (Table 1).* The synthetic triglycerides used in the diets were the saturated compounds trihexanoin (tricaproin), trimyristin, tripalmitin, and tristearin, and the unsaturated compound, triolein.[2, 12]

All mice included in the histological studies were killed by digital compression of the cervical spinal cord. The livers were fixed for at least 48 hours in 10 per cent aqueous formaldehyde. Frozen sections of livers, hearts, kidneys, and abdominal aortas were stained with Sudan black B or with oil red O as previousuly described.[19] Routine paraffin sections of these same organs and of lungs were stained with hematoxylin and eosin.

OBSERVATIONS

The patterns of hepatic liposis in mice fed the 8 diets are summarized in Table 1. An identical pattern of rapid intralobular liposis, beginning centrally, extending peripherally, and rapidly involving the entire lobule, was observed in mice fed diets in which the fat component consisted of lard, butter, C_{14} and C_{16} saturated triglycerides, or C_{18} unsaturated triglyceride (Figs. 1, 3, 5, 7, 8, 9). A less extensive and predominantly peripheral liposis (Figs. 2, 4, 6) was observed in mice fed diet VII (lipid as 100 per cent C_{18} saturated triglyceride) and diet VIII in which 50 per cent of the lipid was C_6 saturated triglyceride plus 50 per cent C_{14} and C_{16} saturated triglycerides. The major portion of the study was devoted to a comparison of hepatic liposis in mice fed natural fats (lard or butter as in diet I) with that in mice fed a diet (VIII) containing 50 per cent (of total fat component) of a

* The synthetic triglycerides were obtained from D. P. I. Division of Eastman Kodak Co., Rochester, N. Y., and from the Hormel Institute, Austin, Minnesota. The suggestions and cooperation of Dr. Walter O. Lundberg, Director of the Hormel Institute, are gratefully acknowledged.

saturated C_6 triglyceride. The groups of mice fed these two diets (I and VIII) were large and the lack of significant changes in the body weights of the members of these two groups during the course of the experiments accentuates their value as material in which two contrasting types of liposis can be studied (Table 2).

Rapid and complete liposis in hepatic lobules. Livers of all mice except those fed diets VII and VIII (Table 1) showed centrolobular liposis within

TABLE 1. PATTERN OF HEPATIC LIPOSIS IN MICE

Fat composition of diets (Each diet contained 40% fat)	*Intralobular pattern of liposis after feeding diet for:*		
	1 day	*3-7 days*	*10-30 days*
I. 100% lard or 100% butter 80 mice	20 mice: central abundant	20 mice: complete lobular	40 mice complete lobular
II. 100% C_{18} Xsat. (triolein) 16 mice	4 mice: central abundant	8 mice: complete lobular	4 mice: complete lobular
III. 100% C_{14} sat. (trimyristin) 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
IV. 50% C_{14} sat. *plus* 50% C_{16} sat. (tripalmitin) 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
V. 15% C_{14} sat. *plus* 15% C_{16} sat. *plus* 70% C_{18}Xsat. 12 mice	4 mice: central abundant	8 mice: complete lobular	not studied
VI. 32.5% C_{14} sat. *plus* 32.5% C_{16} sat. *plus* 35% C_{18} Xsat. 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
VII. 100% C_{18} sat. (tristearin) 19 mice	3 mice: irregular and sparse	8 mice: sparse and predominantly peripheral	8 mice: predominantly peripheral
VIII. 50% C_6 sat. (trihexanoin) *plus* 25% C_{14} sat. *plus* 25% C_{16} sat. 50 mice	6 mice: sparse and predominantly peripheral	14 mice: predominantly peripheral	30 mice: predominantly peripheral

Abbreviations: sat.—saturated; Xsat.—unsaturated.

24 hours after restriction to the high fat diets. The lipid appeared first as small sudanophilic droplets in the cytoplasm of the parenchyma of central zones. During the subsequent two days the liposis spread in a peripheral direction and usually involved all lobular zones at the end of 72 hours

TABLE 2.

Fat composition of diets	*Wt. changes (av.) of mice fed diets for:*		
Diets producing complete lobular liposis within 1-3 days:	*7 days*	*14 days*	*30 days*
I. 100% lard	93 mice —0.3%	100 mice —0.7%	91 mice —0.3%
II. 100% C_{18} Xsat.	8 mice —15%	6 mice —18%	5 mice —24%
III. 100% C_{14} sat.	8 mice —13%	6 mice —16%	5 mice —18%
IV. 50% C_{14} sat. + 50% C_{16} sat.	7 mice —12%	6 mice —13%	4 mice —9 %
V. 15% C_{14} sat. + 15% C_{16} sat. + 70% C_{18} Xsat.	6 mice —2.7%	Not Studied	Not Studied
VI. 32.5% C_{14} sat. + 32.5% C_{16} sat. + 35% C_{18} Xsat.	7 mice —8%	6 mice —16%	3 mice —14%
Diets producing restricted and predominantly peripheral liposis of hepatic lobules during 1-30 days:			
VII. 100% C_{18} sat.	17 mice —8%	8 mice —8%	8 mice —26%
VIII. 50% C_8 sat. + 25% C_{14} sat. + 25% C_{16} sat.	46 mice +2.4%	38 mice +3.5%	30 mice +2.5%

Abbreviations: sat.—saturated; Xsat.—unsaturated.

NOTE: Included in this table are all mice for which body weights were recorded on the 1st day of feeding and on the 7th, 14th, and 30th day subsequently.

(Figs. 1, 8). During the subsequent 27 days of feeding these diets there was a progressive increase of intracytoplasmic lipid (Figs. 3, 5, 7) which was accomplished by formation of large globules or droplets, usually one per cell, as the result of fusion of smaller globules. This process enlarged the cells, and nuclei were often acentric. However, at 30 days cellular enlargement resulting from cytoplasmic accumulation of fat was not sufficient to produce extensive distortion of the basic parenchymal pattern of the liver. The least amount of fat was in the parenchyma of peripheral zones. There

was no specific central-peripheral intralobular gradient in the quantity of lipid in zonal cells, since mid-zonal cells often contained more stainable lipid than central cells. After three days lipid was abundant in both central and mid-zonal cells and was easily demonstrable in cells of the peripheral zones.

Incomplete and predominantly peripheral liposis in hepatic lobules. Complete and abundant lobular liposis did not occur in mice fed diet VII (fat as 100 per cent C_{18} saturated triglyceride) or diet VIII (50 per cent of fat as C_6 saturated triglyceride). Only in the latter group (diet VIII) was the number of mice sufficient to permit a chronologic view of the pattern of liposis. In these mice centrolobular liposis was restricted to very small globules of sudanophilic material and there was no progressive increase in the amount of lipid within the cytoplasm of the parenchyma of this zone. During 3-30 days of feeding there was an accumulation of fat in the parenchymal cytoplasm of peripheral zones of lobules (Figs. 2, 4, 6). The intracytoplasmic liposis in peripheral zones was extensive and in relation to the cytoplasmic enlargement and distortion of individual cells it approached an immediately "precystic" stage (Fig. 4) on the basis of earlier descriptions of hepatic liposis in rats[9] and mice[8, 19] fed hypolipotrophic diets.

In all of the mice used in this study the hepatic reactions to the high fat-choline-deficient diets had not reached the stages in which there is formation of ceroid pigment or changes in the stroma.[19]

Changes in other organs. Hearts, kidneys, and aortas showed no evidence of deposition of fat or other changes from normal. A majority of lungs showed small amounts of diffuse pneumonia. No renal lesions and a very low incidence of myocardial lesions were observed previously in mice fed high fat-choline-deficient diets for as long as 10 months.[19]

Changes in body weight (Table 2). The two large groups of mice fed diets (I and VIII) that produced contrasting types of liposis showed a satisfactory maintenance of body weight. The slight average loss of weight in mice fed lard and the small increase in those fed the C_6 compound do not seem significant (Table 2). The data derived from the relatively large group of mice fed lard as the dietary fat (diet I) and the small groups fed the other diets (II-VII in Tables 1 and 2) show that complete lobular liposis occurs in mice who maintain their initial body weight for 30 days and also in those that lose as much as 24 per cent (diet II) during the same period. Similarly, there was restricted liposis in mice maintaining initial weight (diet VIII) and also in those losing 26 per cent of weight during 30 days of feeding. The loss of weight in mice fed the two (saturated and unsaturated) C_{18} triglycerides (Table 2) gives reason to doubt effective absorption and/or utilization of these compounds in choline-deficient mice.[21]

DISCUSSION

Within hepatic lobules a liposis that was restricted in amount and peripheral (zonal) in location was limited to mice fed diets in which the lipid component was a C_{18} saturated triglyceride or predominantly a C_6 saturated triglyceride. The other synthetic compounds produced a liposis identical with that observed in mice fed lard or butter as the dietary fat. The major lipid components of lard are a 26 per cent level of C_{16} saturated compound and 62 per cent of C_{18} unsaturated; of butter, 11 per cent of $C_{4\text{-}12}$ saturated, 40 per cent of C_{14} and C_{16} saturated, and 36 per cent C_{18} unsaturated.[2, 12]

The study reported here permits no differentiation between deposition of fat supplied by the diet from that synthesized *in vivo*.[8, 17, 21] The present data are in agreement with the results of quantitative analyses[4, 5, 6, 21] demonstrating that maximal hepatic liposis occurs when the dietary lipid of a choline-deficient diet is chiefly composed of saturated C_{14}-C_{16} compounds fed as fatty acids, ethyl esters, or as natural fats. The same analyses demonstrated a decrease in hepatic lipid when C_4-C_{12} or C_{18} saturated compounds were fed. In contrast to the present findings an earlier study showed decreased (from maximal levels obtained with saturated C_{14}-C_{16} lipids) amounts of liver fat when unsaturated compounds including oleic acid (C_{18}) were fed.[4]

The purpose of this study was production of maximal hepatic liposis and no attention was directed to the amount of choline needed to prevent liposis when various synthetic triglycerides were substituted for natural fats such as lard or butter. Supplementation with choline chloride at a level of 0.5 per cent to 0.8 per cent prevents hepatic liposis in rats and mice fed lard containing diets, but not when some of the synthetic triglycerides used here are the dietary lipid.[19, 23]

An initial and immediate centrolobular liposis is a frequent response of hepatic parenchyma of several species including mice to a variety of insults including the actions of choline-deficient diets,[19] starvation,[24] polyhalogens[22, 24] and cortisone.[25]

The patterns of liposis and of cirrhosis in mice[8, 19] and rats[11, 14] fed hypolipotropic diet have received considerable attention. In general it has been concluded that liposis begins centrally[14, 19] and initial cirrhosis is not portal in location.[1, 11, 16] In animals fed such diets the stromal changes that have been described as cirrhosis or reticulinosis are not the direct result of liposis, but are due in considerable measure to the amount and pattern of nodular parenchymal hyperplasia.[11, 14] Rapid and complete lobular liposis, but without a specific central-peripheral pattern of spread within lobules, was ob-

Explanation of Figures

All figures show frozen sections of livers stained with oil red O or Sudan black to show fat.

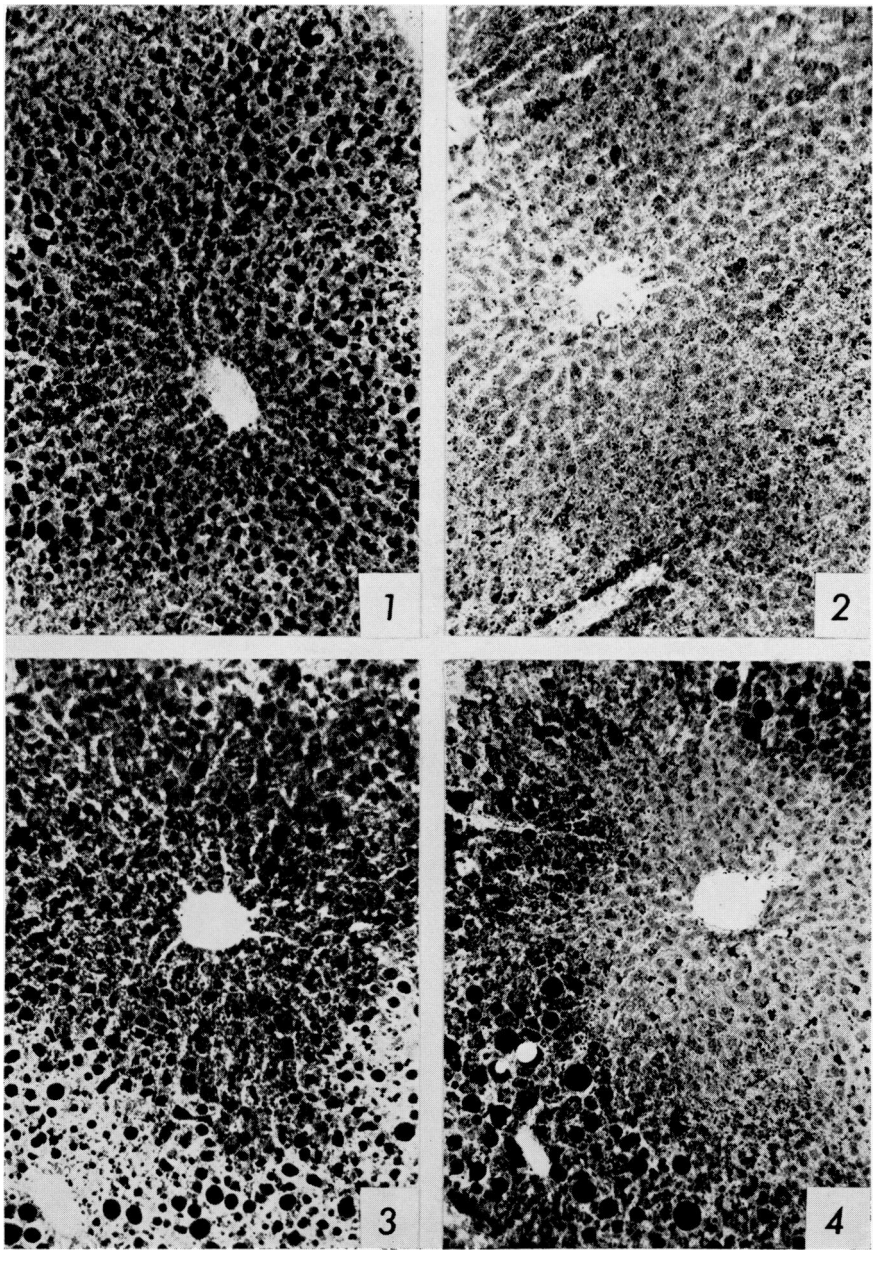

Plate 1

All sections are stained with oil red O, counterstained with hematoxylin; and show central vein.

Figs. 1 and 3. Abundant liposis in mice fed lard containing diet for 3 (Fig. 1) and for 30 days (Fig. 3). x100.

Figs. 2 and 4. Restricted liposis after feeding diet VIII (C_6 saturated triglyceride as 50% of total fat) for 3 days (Fig. 4). Observe the prominent and predominant peripheral (portal) liposis in Fig. 4. x100.

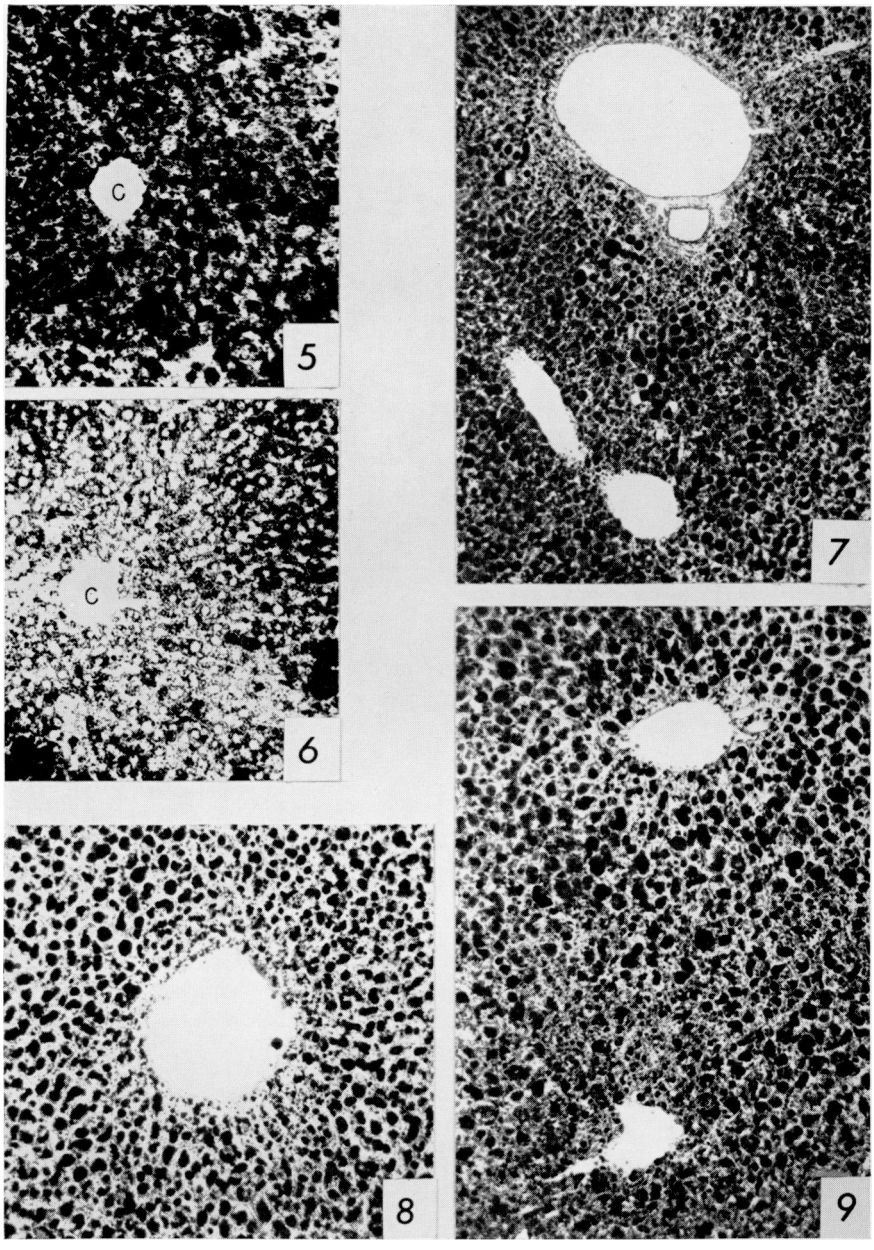

PLATE 2

FIGS. 5 and 6. Complete lobular liposis after feeding diet I (lard) for 30 days (Fig. 5) and a predominantly peripheral liposis in mouse fed diet VIII (50% C_6 saturated triglyceride) for 30 days. "C" indicates central vein. Sudan black. x80.

FIG. 7. Lard diet fed for 3 days. Portal vein, hepatic artery and bile duct are shown in upper one-third of photograph. The extent of liposis throughout the entire lobule is shown. Oil red O. x50.

FIG. 8. Diet V (15% C_{14} and 15% C_{16} saturated plus 70% C_{18} unsaturated triglycerides) fed for 3 days. All portions of lobules were filled with fat in this liver of which the central portion of a lobule is shown. Oil red O stain. x100.

FIG. 9. Diet II (100% of fat as C_{18} unsaturated triglyceride) fed for 7 days. The central vein is in lower portion of photograph, the portal vein in the upper. Lobular liposis is complete, but is more obviously unilocular in peripheral portion of this lobule. Oil red O stain. x100.

served in mice fed choline-deficient diets in which soya flour was the source of protein.[3]

A choline-deficient diet containing C_4 saturated compound (tributyrin) as the lipid component did not produce a typical centrolobular liposis in young rats, but a peripheral liposis was not described.[23] A predominantly peripheral lobular liposis was observed in rats fed a choline-deficient and moderately high (15 per cent) fat diet in which the protein sources were a 76 per cent level of corn meal plus a 3 per cent level of casein.[20] In rats[15] and in mice[19] fed the basic diet (containing lard as fat) used here the initial liposis was centrolobular. The present study was terminated 4 to 6 months before stromal changes occur in livers of mice fed choline-deficient diets.[19]

An initial and continuing peripheral liposis was observed here in mice fed two of the diets and a similar pattern has been described previously in rats[20] fed choline-deficient diets containing corn meal as the major source of protein. This type of liposis does not substantiate the explanation that the fairly broad susceptibility of centrolobular cells to a variety of injurious agents and conditions is due to a regional deficiency of essential blood-borne elements in central zones because of prior utilization of such materials in the more peripheral portions of lobules.[7, 13, 18, 24] It seems clearly demonstrated that both the quantity[4, 5, 6, 21] and the intralobular pattern of hepatic liposis are controlled to a considerable degree by the chemical composition of the lipid components of choline-deficient diets. A better understanding of the liposis awaits demonstration of the separate and combined contributions of the three atypical components to the process. These components are an inadequate level of choline, a high level of dietary fat,[21] and the low level of protein.[14, 15, 16]

SUMMARY

1. An initial centrolobular liposis that involved all hepatic lobular zones within 72 hours was observed in mice fed choline-deficient diets containing butter, lard, C_{14} or C_{16} saturated triglyceride, or a C_{18} unsaturated triglyceride as the lipid component.

2. When the dietary fat was a C_{18} saturated triglyceride or predominantly (50 per cent of total dietary fat) a C_6 saturated triglyceride, the liposis was restricted chiefly to peripheral (portal) zones of lobules and had not extended throughout all lobular zones after 30 days of feeding.

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