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HEPATIC LIPOSIS AND MYOCARDIAL DAMAGE IN MICE FED CHOLINE-DEFICIENT OR CHOLINE-SUPPLEMENTED DIETS**

The feeding of hypolipotrophic diets produces rapid and extensive hepatic liposis in several species including rats[4] and mice.[2,14] In addition to being deficient in choline the diets used in such experiments usually contain a large amount of fat and a marginal amount of protein.[10,14,18] Hypertension and renal glomerular lesions have been observed in rats fed such diets.[1] These observations have stimulated study of the actions of this type of nutritional deficiency in the production of lesions of arteries and myocardium.[5,7,22] There are contradictions related to the specific actions of the choline deficiency in several aspects of experimentally produced cardiovascular disease including injury to the heart and blood vessels,[17,20,21,30] and plasma levels of lipids and lipoproteins.[23,24]

In the present study major attention has been directed to hepatic liposis and to myocardial necrosis and fibrosis in young adult mice. The mice were older than the rats commonly used in such experiments. The selection of older animals was intentional so that a postweaning phase (four to six weeks) of adequate nutrition and normal growth could be established.

MATERIALS AND METHODS

Animals. Mice of the C (Bagg albino) stock with an initial body weight of 20-22 gms. were restricted to either the choline-deficient or choline-supplemented diet when 8 to 10 weeks of age.

Diets. From weaning (30 days of age) until eight to ten weeks of age these mice had been fed a standard laboratory ration that was adequate in choline, vitamins and minerals, being composed of approximately 25 per cent protein, 6 per cent fat, and 47 per cent carbohydrate. The composition of the choline-deficient diet was as follows:

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	Gms.
Vitamin-free casein	80.0
Sucrose	480.5
Lard	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

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 choline-supplemented diet contained 5 gms. of choline chloride in 1000 gms. of the diet described above.

Within the mouse cages the food was kept in metal containers constructed with partitions and wire-mesh covers to restrict contact with the diet to the mouth and snout. A wire-mesh floor was placed above the permanent floor of the cage to limit coprophagia.

Mice were weighed daily during the first month of feeding the experimental diets and weekly thereafter.

Histological technics. Abdominal and thoracic organs were fixed in 10 per cent formalin or in Lavdowsky's solution[29] or in both. Hearts were cut into two relatively equal frontal sections for fixation in 10 per cent formalin and Lavdowsky's. Thigh muscle from about one-half of the mice, and diaphragm from essentially all were fixed in Lavdowsky's solution. Routine paraffin sections (10 micra) of all of these organs and tissues were stained with hematoxylin and eosin (H. and E.).

Fat was demonstrated by staining frozen sections of formalin fixed material with oil red 0 in the same manner as with other Sudan dyes.[14, 27] It was essential to use gelatin embedding[13] to make adequate frozen sections of the hearts. As the result of technical errors only 25 per cent of these specimens were stained satisfactorily for fat.

The PAS (periodic acid-Schiff's reagent) technic was used to show glycogen and ceroid pigment. Ceroid pigment was also demonstrated by staining dehydrated and defatted (i.e., embedded in paraffin) specimens with dyes such as oil red 0 and Sudan black. The Masson (with aniline blue), the PAS and the Laidlaw reticulum methods were used to show fibrosis, and the von Kossa technic to demonstrate minerals. The staining and histochemical procedures mentioned above were used as described by Lillie.[12] The specific uses of material prepared by these histological technics are described later.

Selection of mice for study. At the intervals shown in Table 3, mice were killed by digital compression of the cervical spinal column. The usual procedure was to select those showing weakness, decreased activity, and respiratory difficulties plus extreme weight loss, provided the other signs of ill health just mentioned were evident. During the first 8 weeks of the experiment, such evidence of ill health was infrequent and

mice were killed on a schedule that produced a chronologic survey of changes in the livers of the members of the two dietary groups. Approximately 80 per cent of the mice killed after 8 weeks of feeding were females. Of those killed earlier, one-half were males. The males were extremely difficult to use in the experiments. They were active and expert in upsetting and burrowing into the food containers and also in becoming entrapped during such activities. In addition it was very time consuming to examine and weigh them since they objected very actively to handling.

Study of the material: Livers. One large lobe was fixed in Lavdowsky's solution (for subsequent paraffin-embedding) and another in 10 per cent formalin. Six to eight sections of the latter (across the entire lobe) were stained to show fat. Complete sections of the paraffin embedded specimens were processed as follows: 6 to 8, H. and E.; 4 to 6, PAS; and when so indicated by the PAS, and/or H. and E., 4 to 6 additional sections were stained by the Laidlaw reticulum method. Ceroid pigment is recognizable in H. and E. and in PAS preparations and also in frozen sections stained with Sudan dyes. When the pigment was so observed more sections (paraffin) were cut and then stained with Sudan dyes.

Hearts. One-half of each heart (complete frontal or saggital section as in Figure 13) was embedded in paraffin and the other was kept in formalin (10 per cent) for frozen sections and also as reserve material. At least 80 per cent of every heart and all of a majority were completely consumed in making the following preparations, all of which had some value in identifying areas of myocardial fibrosis and other lesions: H. and E., Masson, PAS, Laidlaw reticulum (in a variety of combinations with PAS and H. and E.), von Kossa, and frozen sections. Subsequent to frozen sectioning the remaining portion of the formalin-fixed half of the heart was dehydrated and embedded in paraffin and prepared for study by the staining procedures mentioned above. The numerous sections of the heart gave ample opportunity for studying structures located at or near the base, including aorta, vena cava, pulmonary vessels, thymus, lymph nodes, nerves, autonomic ganglia, unilocular and multilocular fat, esophagus, bronchi, and a large number and variety of small blood vessels within or near these several structures.

Other organs and tissues. Adrenals and kidneys as a single "block" were cut into two equal portions in the usual fashion, one being fixed in Lavdowsky, the other in 10 per cent formalin. Two to four sections were stained with H. and E. and a similar number with PAS. Two to four frozen sections were stained with oil red O. Skeletal muscle (diaphragm, thigh and esophagus) and gonads and spleen were studied in two to four sections stained with H. and E. In most instances two to four sections of adrenals (embedded in paraffin and included in same section as kidney) and gonads were stained with Sudan black to demonstrate ceroid pigment.

The simple statistical analysis (means and standard deviations) of the data on body weights (Tables 1 and 3) was based on the methods and tables presented by Croxton.[8]

OBSERVATIONS

At autopsy the significant findings in mice killed after more than eight weeks of restriction to the diet were depletion of the usual fat depots, pneumonia, and fatty livers (in the choline-deficient group). Other tissues and organs, including the alimentary tract, appeared normal.

TABLE 1. WEIGHT CHANGES IN MICE FED HIGH FAT(LARD)-LOW PROTEIN DIETS

	Choline-deficient diet:				*Choline-supplemented:*			
Months fed	*Total no. of mice*	*Mean weight change*	*Per cent of mice: Gained weight*	*Lost weight*	*Total no. of mice*	*Mean weight change*	*Per cent of mice: Gained weight*	*Lost weight*
		%				%		
1	84	—1.2 ±6.5	41 (6.1) ±2.9	47 (7.7) ±4.6	47	+0.86 ±8.8	55 (6.8) ±8	38 (8.4) ±7.9
2	69	—4.2 ±9.8	29 (10) ±7.3	60 (12) ±7.8	37	—0.8 ±12.8	59 (8.4) ±4.5	41 (14.3) ±10.1
3	31	—1.0 ±11.1	45 (9.5) ±6	55 (10.2) ±7.5	34	—6.6 ±12.9	41 (5.7) ±6.2	50 (20) ±11
4	23	—3.9 ±14.8	52 (9.8) ±7.2	48 (18.7) ±10.3	28	—8.6 ±9.2	28 (3) ±3.3	64 (15) ±8.4
5	18	—4.3 ±11.9	44 (8.3) ±5.2	56 (14.4) ±10.8	24	—15.3 ±10.1	8 (2.5)	92 (16.9) ±9.6
6	15	—5.0 ±9.4	33.3 (7.7) ±2.4	66.6 (11.5) ±9.1	9	—12.4 ±7.2	0	100
7	12	—10.4 ±7.9	8.5 (3.2)	83 (13) ±8.3	8	—17.0 ±8	0	100
8	9	—11.7 ±13.1	0	100	2	—20.2	0	100
9	8	—20.0 ±8.4	0	100	No survivors			
10	6	—24.2 ±4.1	0	100	No survivors			

(a) Weights are those of all mice living at the stated intervals.

(b) Numbers in parentheses are means of weight changes (%) in animals (% of total in group) listed directly above.

(c) Standard deviations are those of changes in body weight (i.e., the mean listed directly above) and were not determined for groups smaller than 5 mice.

Livers. The results are summarized in Tables 2 and 4. In chronologic order the livers of mice fed the choline-deficient diet showed: (i) complete lobular liposis within 72 hours; (ii) formation of fatty cysts within 5 weeks; (iii) ceroid pigment within 9 weeks; and (iv) within 22 weeks a nodular hyperplasia of the parenchyma and an apparent increase of reticular fibers between these nodules. Some of these changes are shown in Figures 1, 2, and 5, and all have been described in detail previously.[14] The term fatty cyst as used here refers to structures previously described.[2, 3, 14]

TABLE 2. CHANGES IN LIVERS AND HEARTS OF MICE FED HIGH FAT-LOW PROTEIN DIETS

Livers:	*Choline deficient (% of mice)*	*Choline supplemented (% of mice)*
	12-40 wks.	*12-33 wks.*
Liposis Absent	0	45%
Complete Lobular Liposis	100%	0
Fatty cysts	100%	0
Ceroid pigment	100%	0
	27-40 wks.	*27-33 wks.*
Reticulinosis and Parenchymal Hyperplasia	100%	0
Myocardial lesions:		
1- 8 weeks:	0	0
9-15 weeks:	0	50%**
16-33 weeks:	25%*	72%**
34-40 weeks:	31%*	No survivors

* Small areas of fibrosis.
** Areas of extensive fibrosis.

No fat (sudanophilia in frozen sections) was observed in the livers of approximately one-half of the mice fed the choline-supplemented diet for the entire period of 1 day to 33 weeks (Tables 2 and 4). In an approximately equal number of mice the hepatic liposis was evident but was limited to very small sudanophilic droplets which did not fill or distort parenchymal cytoplasm or involve all zones of lobules (Figs. 3, 4, 6). Livers of mice fed this diet for as long as 33 weeks and who had lost as much as 25

per cent of body weight appeared normal when stained with hematoxylin and eosin or by the PAS (plus hematoxylin) method for demonstrating glycogen (Figs. 4, 6).

Hearts. There was myocardial injury in both of the dietary groups. A precocity, higher incidence and greater extent of myocardial lesions were

TABLE 3. MYOCARDIAL LESIONS

	Choline-deficient diet:				*Choline-supplemented diet:*			
Weeks fed diet	*No. of mice killed*	*Per cent with lesions*	*Weight loss: (per cent) with lesions*	*Weight loss: (per cent) without lesions*	*No. of mice killed*	*Per cent with lesions*	*Weight loss: (per cent) with lesions*	*Weight loss: (per cent) without lesions*
1- 8	84	0	—	4.2* ±9.2	30	0	—	0.8* ±11.1
9-15	7	0	—	33 ±5.7	10	50	30 ±9.8	34 ±10.1
16-21	23	30	35 ±4.1	34 ±7.9	15	66.6	17 ±10.1	26 ±3.9
22-27	21	24	16 ±8.1	19 ±9.1	9	56	17 ±9.3	26 ±3.9
28-33	8	12.5	18 **	16 ±8.1	8	100	24 ±9.7	—
34-39	7	28.6	22 **	20 ±7.7	No survivors			
40	6	33	26 **	23 ±4.8	No survivors			
	9-40 wks. 72 mice	23.6%			9-33 wks. 42 mice	66.6%		

* Av. wt. changes in all mice (killed and surviving) at 8 weeks.
** No standard deviation determined for groups smaller than 4 mice.

obvious in the choline-supplemented group (Tables 2 and 3). A significant incidence of myocardial lesions (50 per cent) occurred in choline-supplemented mice killed during the 9-15 week interval of feeding this diet. This incidence was greater than in mice fed the choline-deficient diet for 16-40 weeks (Tables 2 and 3).

The most frequent cardiac lesion was fibrosis of the ventricular myocardium. In mice fed the choline-deficient diet these lesions were in-

TABLE 4. CHANGES IN LIVERS OF CHOLINE-DEFICIENT AND OF CHOLINE-SUPPLEMENTED MICE

Weeks on diet	*No. of mice*		*Per cent of mice showing*											
			Liposis absent		*Incomplete lobular liposis*		*Complete lobular liposis*		*Fatty cysts*		*Ceroid pigment*		*Reticulinosis and parenchymal hyperplasia*	
	—C	+C	—C	+C	—C	+C	—C	+C	—C	+C	—C	+C	—C	+C
1-4	42	15	0	40	0	60	100	0	0	0	0	0	0	0
5-8	42	15	0	60	0	40	100	0	100	0	0	0	0	0
9-15	7	10	0	70	0	30	100	0	100	0	14	0	0	0
16-21	23	15	0	60	0	40	100	0	100	0	100	0	0	0
22-27	21	9	0	11	0	89	100*	0	100	0	100	0	33	0
28-33	8	8	0	37.5	0	62.5	100	0	100	0	100	0	100	0
34-39	7	0	0				100		100		100		100	
40	6	0					100		100		100		100	

—C = choline-deficient diet. +C = choline-supplemented diet.

* Lobular pattern of parenchyma was greatly distorted after 24 weeks.

frequent (Tables 2 and 3), limited in size, and consisted of a restricted fibrosis. Lesions such as in Figure 7 appear to be early fibrosis in which fibroblasts and macrophages were numerous. Lesions of approximately the same size, but consisting chiefly of collagenous fibers were more characteristic of the hearts of choline-deficient mice. In this group of mice no lesions larger than the one shown in Figure 7 were seen.

In the hearts of choline-supplemented mice (Tables 2 and 3) the areas of fibrosis (Figs. 9-12, 15, and 16) were larger and multiple (Fig. 13). The fibrosis seemed to be a reparative process that was not encroaching upon myocardial components. Minimal and apparently initial fibrosis consisted of a small amount of ground substance and a large number of fibroblasts. In a more advanced stage of fibrosis the amount of interstitial material had increased and reticular fibers were present. A relatively definitive and older fibrosis was represented by a predominance of collageous fibers within the area. The areas of fibrosis usually contained what appeared to be fragments or remnants of myocardial fibers whose sarcoplasm was hypochromic when stained by eosin or by the acid fuchsin of the Masson technic (Figs. 9, 10, 11).

Active necrosis was not indicated by striking changes in myocardial fibers. In a few instances atrophy or necrosis (Figs. 9, 10, 11) was suggested by shrinkage of fibers, decreased eosinophilia, and poor definition of fibrillar structure. In one heart most fibers within the lesion were heavily stained with hematoxylin (Fig. 11). Ceroid pigment and mineral could not be demonstrated in such fibers and are assumed not to be responsible for the basophilia. Fibers that appeared hypochromic to eosin or acid fuchsin (Masson) also lacked the fine argyrophilic granulation of sarcoplasm that characterized normal myocardium stained by the Laidlaw method for reticulum (Fig. 14). Evidence of active myocardial necrosis was absent from hearts of all mice fed the choline-deficient diet and as described above was not clearly indicated in the cardiac lesions in mice receiving the choline-supplemented diet. Cellular reactions within the lesions were limited to an abundance of fibroblasts and to a small number of macrophages and lymphocytes. The number of neutrophils was not significant. Pericarditis (epicarditis) in which neutrophils and lymphocytes were abundant was present in approximately 10 per cent of the hearts with myocardial lesions. The pericarditis was similar to that described earlier in mice injected with large amounts of cortisone.[28] Endocardium, valves, and blood vessels of all layers of the hearts were normal. In the small number of satisfactory frozen sections of hearts stained with a Sudan dye there was no sudanophilia in walls of vessels or in the layers of the heart except the epicardium.

Vacuolation that might indicate liposis was lacking in the myocardium of the routinely defatted sections of heart.

Other organs. In sections stained with hematoxylin and eosin there were no significant changes in kidneys (also stained for fat), abdominal aorta, adrenals, or gonads. In adrenals and gonads the amount of ceroid pigment was usual for the age of the mice and apparently had not been influenced by the choline content of the diet. The blood vessels of these several organs were normal. Pneumonia was common in all mice fed the diets for more than two months. Mucosal inflammation was frequent in trachea and extrapulmonary bronchi. The great vessels at the base of the heart were normal. Multilocular fat (periaortic and perirenal) was unchanged. In mice fed the diets for three months or more unilocular fat was depleted in that cytoplasm was shrunken; nerves and autonomic ganglia appeared normal in H. and E. sections. The esophagus, including its smooth and striated musculature, showed no abnormalities. Lymph nodes, spleen and thymus showed no obvious depletion of mature forms or other alterations. Skeletal muscle (thigh, esophagus and diaphragm) was free of any changes including the deposition of ceroid pigment and calcium.

Relation of general health and of weight loss to changes in livers and hearts. After 8 weeks weight loss was greater in mice fed the choline-supplemented diet (Table 1). At all intervals the range in weight loss was broad in both dietary groups (Tables 1 and 3). After the first eight weeks, all mice killed for study represented the less healthy members of the dietary groups and the majority had lost considerable amounts of weight (Tables 1 and 3). The changes in the livers showed no relation to amount of loss in body weight. Very fatty livers were present in all of the choline-deficient mice and the other hepatic manifestations of the deficiency ensued in the expected relation to its duration (Tables 2 and 4; Figs. 1, 2, 5). Hepatic changes were not significant in the choline-supplemented mice (Tables 2 and 4; Figs. 3, 4, 5). The extent of liposis in the livers of members of this group seemed unrelated to changes in body weight.

Here it is not possible to present the complete history of weight changes of individual mice. There was no instance of rapid weight loss followed by significant gain of weight. Most commonly the rapid loss of weight took place during the two weeks immediately preceding the time at which the animal was killed.

A great majority of the heart lesions were in mice with extreme decreases in body weight. However, the weight losses of animals killed within a specific chronologic interval were not significantly or consistently greater in

those with cardiac lesions (Table 3). In mice killed during the 9-27 week-interval the incidence of myocardial lesions was more than 50 per cent greater in the choline-supplemented group. Extensive myocardial fibrosis was observed in five mice lacking excessive weight loss as follows: at 20 weeks, 4.5 and 5.3 per cent weight loss; 24 weeks, 1.8 and 4.2 per cent; and at 31 weeks, 9.8 per cent. In choline-deficient mice cardiac lesions were limited to mice with weight loss of more than 10 per cent at the time of death.

The constant finding of pneumonia in mice killed after eight weeks on the diets was mentioned earlier. Several experimental procedures seem to precipitate this disease in mice.[22, 24] It seems highly probable that pneumonia contributed significantly to the poor health including the weight loss observed in these animals.

DISCUSSION

The livers of mice fed the choline-supplemented diet appeared normal except for a very restricted and irregular liposis. The degree of protection that choline supplementation afforded to the liver was impressive. This same group of mice showed a precocity and an increased incidence of myocardial injury.

The frequent occurrence of myocardial lesions and mural liposis in aortas and coronary arteries of young rats fed high fat, low protein, choline-deficient diets has been responsible for the belief that an adequate intake of choline is essential for maintenance of the cardiovascular system in this species.[20, 21] Significant and perhaps contributory renal lesions occur very early in young rats fed similar diets.[1, 21, 22] The following were regular features of these earlier studies:[5, 6, 21] (i) The rats were young, often immediately post-weaning. (ii) Such diets produced a rapid and extensive hepatic liposis which was prevented by choline supplementation. Also, a considerable variety and number of dietary lipids were used.[20, 21] More recent observations in the rat,[10, 12, 17, 29] monkey,[13] and chicken[15] indicate that these earlier studies overemphasized the specific actions of the choline deficiency in the production of cardiovascular injury. In the present study the animals were of a different species and were older than the rats commonly used in studies of the actions of choline-deficient diets on the cardiovascular system, liver and kidneys.[1, 21, 22] Also in some of the studies on rats a majority of the animals were males[21, 22] rather than females as used here. There is evidence that testosterone aggravates actions of a high fat—low protein, choline-deficient diet on the cardiovascular system and the kidneys.[19]

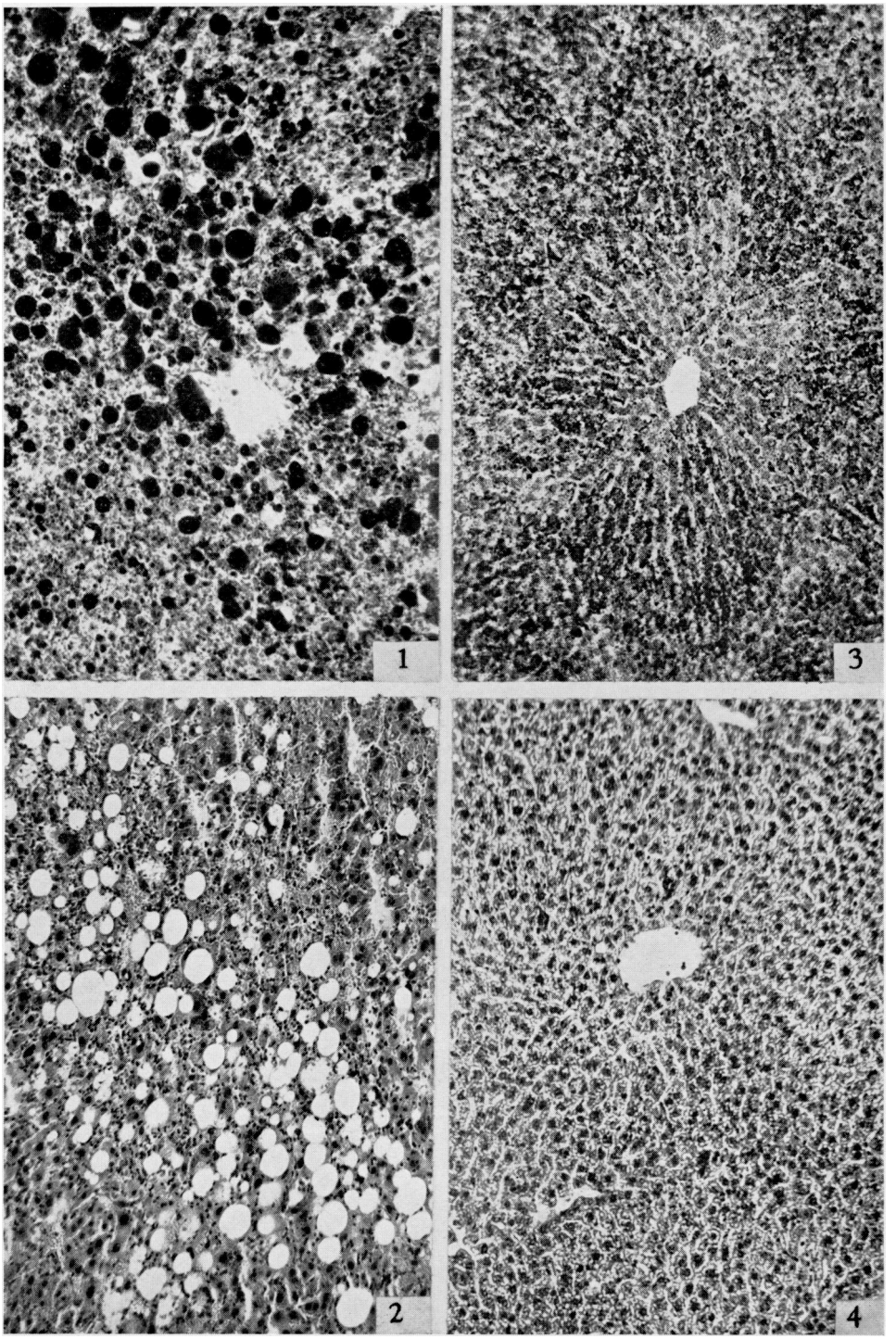


PLATE 1

Livers (x100) of mice fed choline-deficient (Figs. 1 and 2) or choline-supplemented diets (Figs. 3 and 4) for 5 months.

FIG. 1. Frozen section stained with oil red O and hematoxylin. All parenchymal cells contain fat. The large fat-filled structures (cysts) seem to be the result of rupture and fusion of adjacent cells.

FIG. 2. Same liver as in Figure 1. Paraffin section, H. and E. stain. Hyperplasia (apparently the first stage in formation of regenerative or hyperplastic nodules) is indicated by the large cells with hyperchromatic nuclei.

FIG. 3. The restricted and predominantly peripheral (portal) lobular liposis shown here represents the maximal amount seen in livers of choline-supplemented mice (frozen section, oil red O and hematoxylin).

FIG. 4. Same liver as in Figure 3, paraffin section, H. and E.

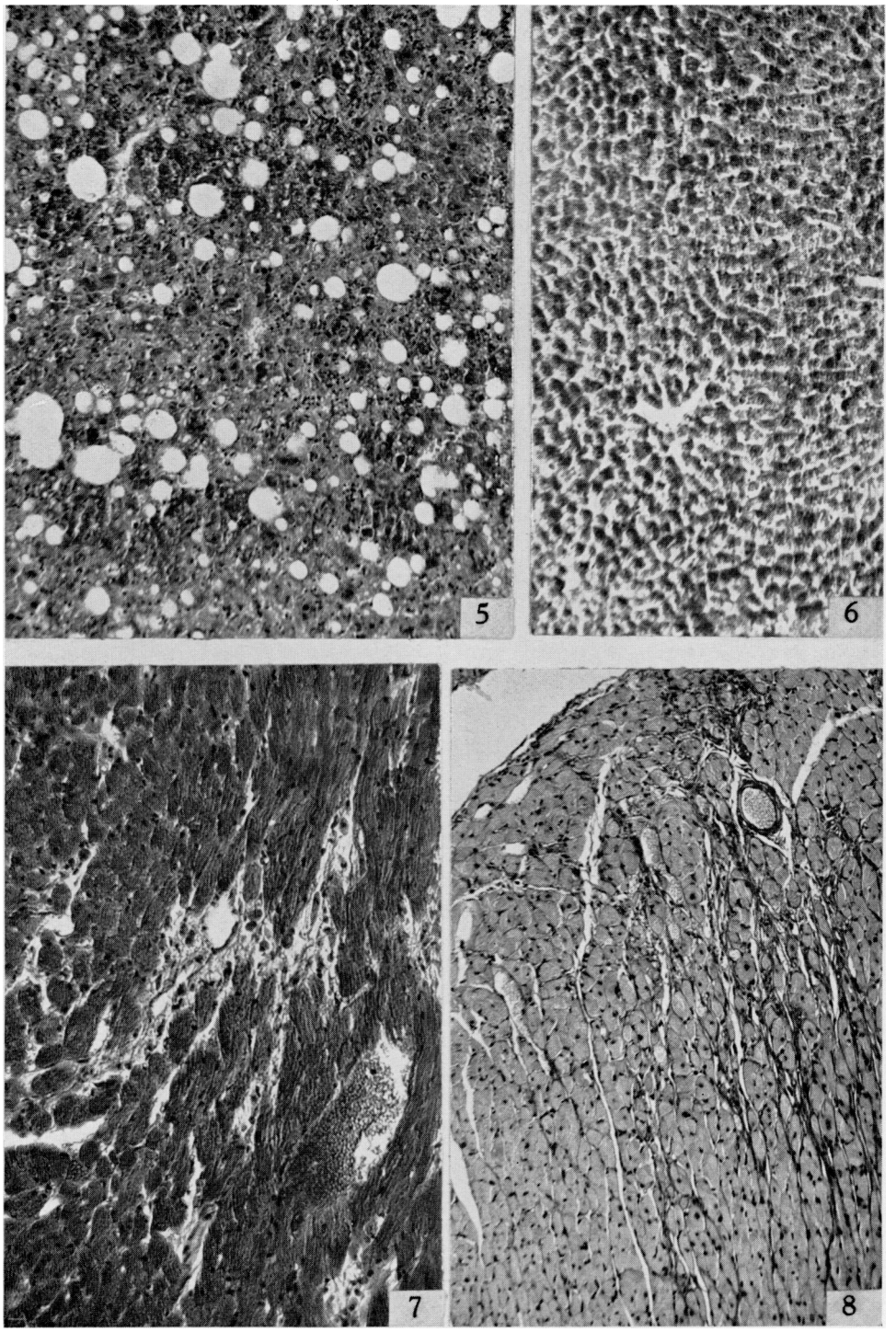


PLATE 2

FIG. 5. Liver of mouse fed choline-deficient diet for five months. PAS and hematoxylin. The darkly stained material is ceroid pigment. The cytoplasmic PAS-positivity indicating glycogen is very faint, x100.

FIG. 6. Liver of mouse fed choline-supplemented diet for five months. There is no staining reaction for ceroid pigment. Staining positivity indicating cytoplasmic glycogen is within normal range. PAS and hematoxylin, x100.

FIG. 7. Maximal (as to extent) type of myocardial lesion seen in choline-deficient mice, H. and E., x180.

FIG. 8. Restricted amount of myocardial fibrosis in choline-supplemented mouse, PAS, x115.

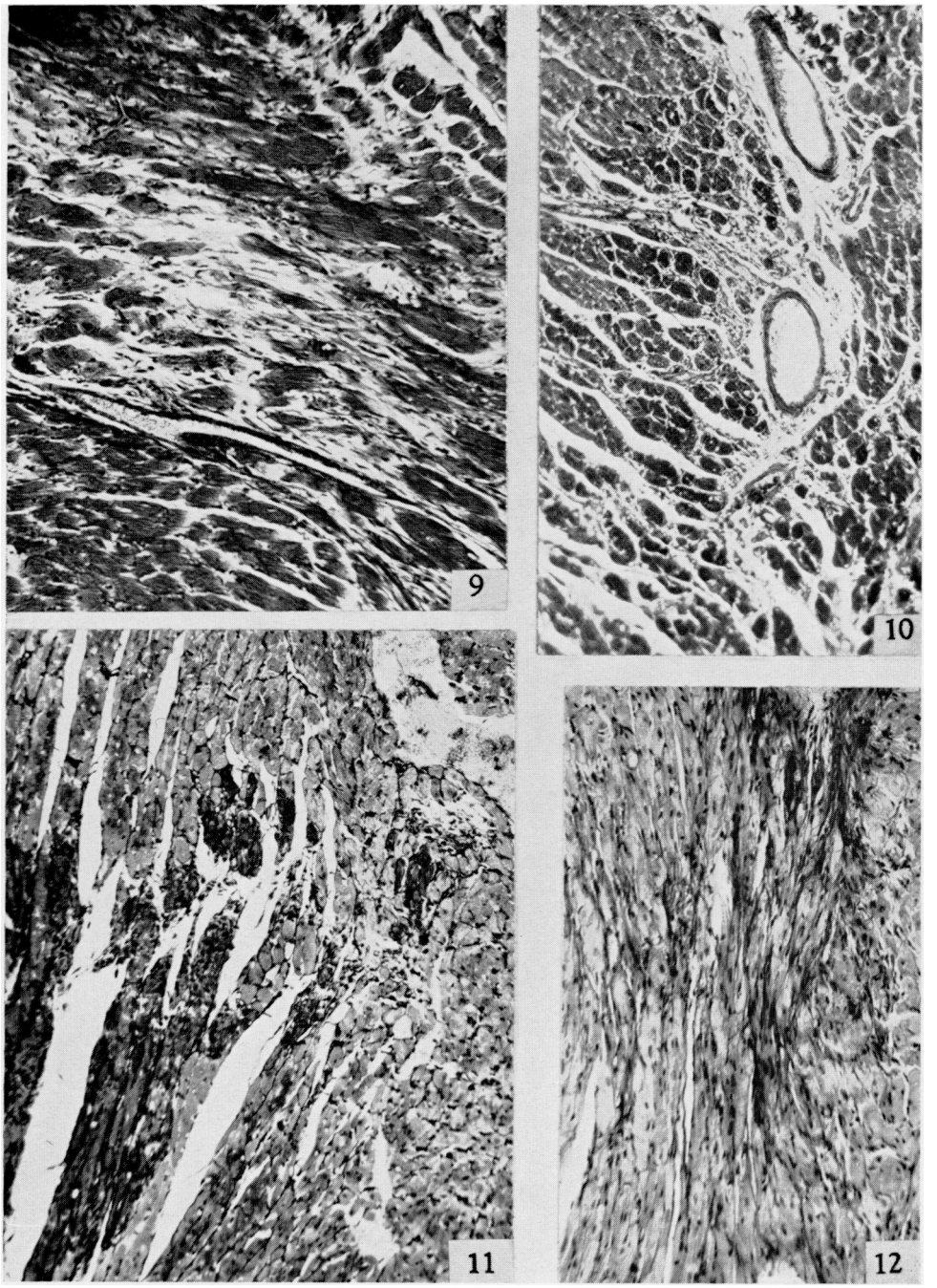


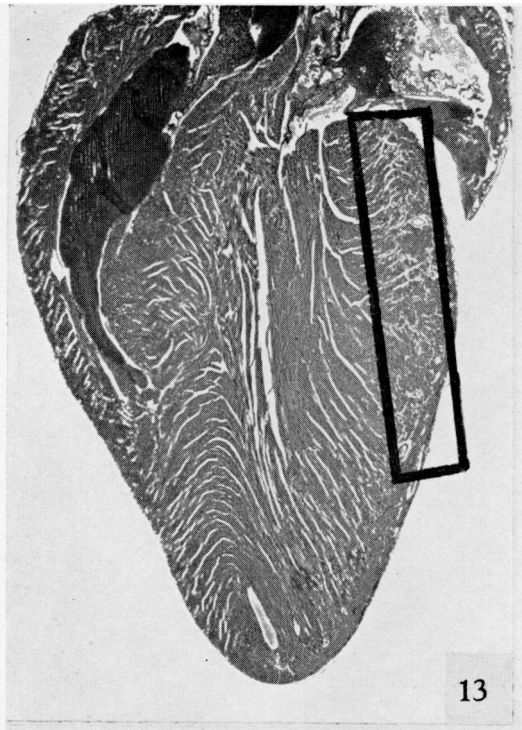
PLATE 3

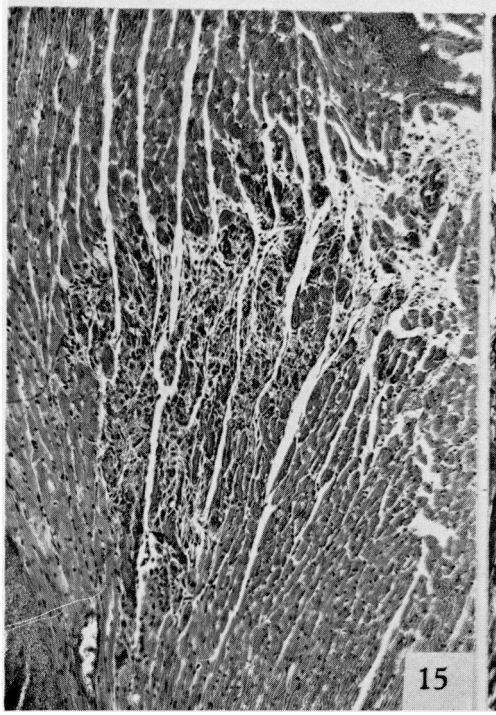
Hearts of mice fed choline-supplemented diets for four and five months.

FIGS. 9 and 10. Myocardial fibrosis representing the most frequent lesion seen in hearts of choline-supplemented mice, (Masson stain). Figure 9, x115; Figure 10, x90.

FIG. 11. Myocardial necrosis and small areas of early fibrosis (Laidlaw reticulum plus H. and E.). Calcium and ceroid pigment could not be demonstrated in the very basophilic myocardial fibers, x115.

FIG. 12. Myocardial fibrosis, PAS, hematoxylin, x115.





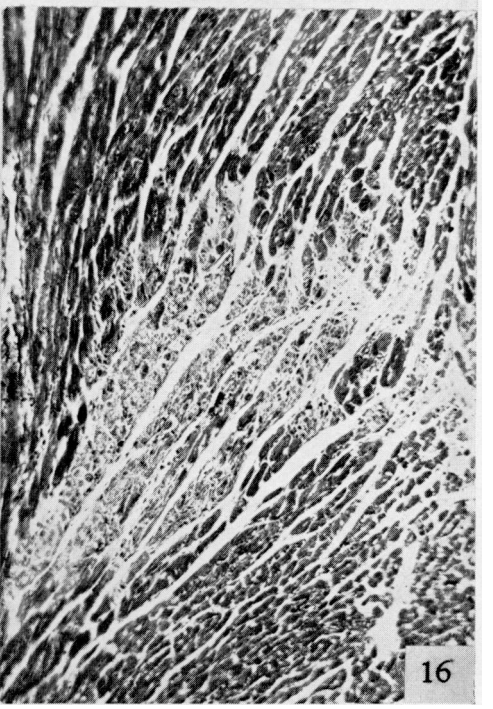


PLATE 4

Hearts of mice fed choline-suppiemented diets for four tc five months.

FIG. 13. The rectangle encloses a lesion (or lesions) occupying a large area. The lesions, mostly fibrosis, are similar to those shown in Figs. 15 and 16 (below), x14.

FIG. 14. Argyrophilic granulation of sarcoplasm is lacking, but reticular fibers are stained in the several myocardial lesions shown here. Laidlaw method for reticulum, no counterstain, x140.

FIGS. 15 (H. and E.) and 16 (Masson) show large areas of myocardial necrosis and early fibrosis, x90.

The high incidence of cardiac lesions observed here might suggest that correction of the choline deficiency (with reference to hepatic liposis) produces a nutritional state conducive to the previously demonstrated myocardial necrogenic actions of low protein diets in mice.[8, 26] However, the choline-supplemented mice failed to show two common features of protein deficiency in this species. These are hepatic necrosis, and deposition of ceroid pigment and calcium in skeletal and myocardial muscle[8, 26] Dietary components acting separately and collectively in the production of injury to the liver and to the myocardium of mice and/or rats include the quantity and type of fat,[6, 11, 16, 27] amount of protein,[8, 26] levels of choline[21, 22, 30] and vitamin E,[26] and content of cystine[26] in relation to that of protein.

When results in rats[10, 17, 20, 21] and mice[2, 8, 26] are compared, the cardiovascular system seems to show considerable diversity in its reactions to atypical diets. In relation to a high fat diet neither species is as susceptible as the rabbit to production of atheromatous lesions.[18] In most of the studies mentioned earlier dietary lipid has consisted of lard, butter, or oil furnishing a wide range as to the chemical nature (degree of saturation and chain length) of the fats. Several studies indicate that the chemical nature of the dietary fat is important in relation to the amount and pattern of hepatic liposis[16, 27] and to the extent of injury to the cardiovascular system.[5, 6, 21, 22] Ethyl-laurate displays a great capacity to produce myocardial damage in young rats fed choline-deficient diets.[11] Buckley and Hartroft observed fat in coronary arteries and myocardium of choline-deficient mice, but questioned that the incidence was significantly greater than in their choline-supplemented group of mice.[2]

The following observations which seem significant are from studies[20, 21] of very young rats fed 12-60 days on high fat diets with protein contents of 15-30 per cent: (i) food intake was low in the several dietary groups; (ii) the incidence of myocardial liposis and necrosis seemed dependent upon the presence of a choline deficiency and to some degree the type of dietary fat; (iii) the incidence of such lesions was extremely low in the choline-supplemented rats; (iv) myocardial lesions were produced by feeding fat-free, choline-deficient diets. The incidence of myocardial damage is high in young rats fed high fat (40 per cent lard or butter plus 5 per cent cholesterol) diets containing propylthiouracil and sodium cholate with and without choline supplementation.[17] Supplementation with choline seemed to enhance the development of lesions described as coronary thrombosis and myocardial infarction.[17] The results of the present study indicate that a dietary level of choline sufficient to prevent hepatic liposis increases the susceptibility of the myocardium to high fat, low protein diets.

Within a given period the great range in weight changes of mice, both with and without myocardial lesions, precluded valid analysis of the relation of weight loss to the incidence of cardiac damage (Tables 1 and 3). The data permit no evaluation of the relation of food intake to weight loss. Because of the marginal dietary content of protein a true deficiency would exist when intake was low. As mentioned earlier the amount of weight loss of mice killed at the specific intervals could not be correlated with the presence or absence of myocardial lesions (Table 3). Objective consideration of the situation demands a broader view. In the choline-supplemented mice the early occurrence and relative rapidity of weight loss are obvious when the chronology and extent of changes in weight are considered for all of the mice (Table 1). The condition or conditions responsible for the early and rapid loss of body weight may have contributed significantly to the high incidence of myocardial lesions in the choline-supplemented mice.

The apparently healthy state of livers of choline-supplemented mice deserves attention in relation to the possible contribution of decreased food intake to weight loss and cardiac damage. In choline-supplemented mice with extreme losses in body weight the decreases in glycogen and increases in fat within hepatic parenchymal cytoplasm were less than are produced by relatively short as well as longer periods of fasting.[9, 23, 28] These livers also lacked the extreme changes including necrosis, cirrhosis and in some instances deposition of calcium that have been described in mice fed diets containing marginal amounts of protein, but with low fat content.[8, 26] The true actions of inanition could be determined by precise and extended studies of food intake with reference to essential requirements provided by the dietary protein, fat and carbohydrate in relation to several levels of choline-supplementation. The separate and joint actions of at least four dietary components must be evaluated. These are the choline-deficiency, the high fat content, the adequacy of the choline supplementation in relation to organs other than the liver, and the deficient (or at least marginal) level of protein.

SUMMARY

1. In chronological order the livers of mice fed a high fat, low protein, choline-deficient diet showed complete lobular liposis, formation of fatty cysts and ceroid pigment, nodular parenchymal hyperplasia, and distortion of the stromal pattern.

2. Supplementation of the diet with choline chloride prevented all of these hepatic changes except a very limited amount of parenchymal liposis.

3. In mice fed the high fat, low protein, choline-deficient diet for 9-40 weeks there was an incidence of 23.6 per cent of myocardial lesions consisting of small areas of fibrosis. In mice fed the choline-supplemented diet for 9-33 weeks 66.6 per cent showed myocardial lesion. Fibrosis was the predominant feature of a majority of these lesions. No significant changes were observed in kidneys or blood vessels of either dietary group.

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