Deficiency Diets in Young Growing Rats.* (24574)

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Choline biosynthesis is accomplished *in vivo* in the presence of serine, methionine, glycine, Vit B_{12} and folic acid. With ample serine and 1200 mg of methionine/100 g of ration, rats on choline deficient diet grow at normal rate and have normal liver lipids(1). However, Griffith states that hemorrhagic degeneration of kidney is wholly prevented by supplements of choline too small to affect deposition of liver lipids(2). An improved but reasonable working hypothesis involves the concept that the kidney requires a continuing source of choline for synthesis of lecithin(3). Hartroft *et al.* have shown pathological changes occur in major arteries of rats maintained on diets low in choline up to 126 days (4). These investigators indicate that choline is the essential dietary factor for cardiac muscle. Others believe methionine is the critical dietary factor for prevention of cardiovascular tissue damage in the rat(5). Force feeding of choline-containing diets devoid of methionine for 3 to 6 days induces fatty liver in adult female rats, but not in male rats(6). In the present study the effect of deficiencies of choline, methionine and cystine singly or paired was determined in young growing male rats. Organs were systematically examined for evidence of changes related to these deficiencies(7,8).

Materials and methods. One hundred and twenty young male rats of hardy, closely inbred modified Long-Evans strain, weighing 85 to 135 g, were employed. Animals were caged individually and kept at 70° to 80°F. The experimental dietary groups, each containing 15 animals, were established as follows: choline-deficient, methionine-deficient, methionine-cystine-deficient and cystine-deficient diets with 60 pair-fed controls (4 groups of 15 animals for each dietary regimen). Control diet consisted of 18% oxidized casein, 2% vitamin-free casein, 10% alpha soy protein, 35% corn oil, 4% salt mixture W (Nutritional Biochemicals Corp.), vitamin mixture(9) with addition/100 g of .85 g of choline and amino acid mixture containing 225 mg of tryptophan, 200 mg of tyrosine, 400 mg of cystine and 1200 mg of methionine. Sucrose was used to bring total diet to 100%. Experimental diets were obtained by eliminating from the control diet materials designated in the deficient diets. All animals were sacrificed at end of 6 weeks. Frozen sections (15 μ thick) of portions of thoracic aorta, kidney and liver of each animal were fixed in 10% formalin and examined for neutral fats employing oil red O stain and counterstained with Mayer's hemalum (Mallory, '52). In addition, portions of previously mentioned tissues and thymus, spleen, adrenals, pituitary and testes of each animal were fixed in Helly's fluid, sectioned in paraffin serially 7 μ thick, and stained with hematoxylin and eosin.

Results. Effect of dietary factors upon various organ weights are shown in Table I. Decreases in size of testes in methionine-deficient and methionine-cystine deficient groups are evident when compared to control pair-fed rats. Decrease of testes weight of cystine-deficient animals is statistically insignificant. Associated with these alterations are increases in thymic weights. The various diets resulted in no changes in splenic, pituitary and adrenal weights.

Grossly smaller testes were found in methionine-deficient and methionine-cystine-deficient animals. Microscopic examination of seminiferous tubules (Fig. 1) fails to show a compact and orderly arrangement. The seminiferous epithelium is similar in appearance to that of immature animals. Lack of sperm in the tubules was also noted.

Frozen sections of similar portions of thoracic aorta for all groups indicate that the amount of stainable fat does not differ significantly from that seen in pair-fed controls (Fig. 2).

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TABLE I. Effects of Dietary Factors upon Various Organ Weights* of Intact Rats. (Means and stand. errors.)

	Pituitary	Adrenal — Right	Adrenal — Left	Spleen	Thymus	Testes
Pair-fed controls† (60)‡	5.6 .1	10.9 .6	11.6 .4	305.5 27.4	128.2 4.8	851.4 70.3
Choline-deficient (15)	5.5 .2	10.6 .8	11.0 .9	270.8 17.6	131.1 6.0	787.7 103.7
Methionine-deficient (15)	5.8 .1	11.8 .6	12.6 .6	289.7 18.7	178.5 7.4§	387.6 22.3§
Methionine-cystine-deficient (15)	6.0 .2	9.6 .4	10.7 .7	229.3 22.9	175.6 3.6§	176.3 50.6§
Cystine-deficient (15)	5.3 .2	9.5 .4	10.2 .7	265.0 12.3	167.8 3.2§	591.4 97.2

* All weights expressed in mg/100 g body wt. † Pair-fed controls have been grouped due to similarity. ‡ No. of rats. § Indicates P 0.01 for values compared to controls.

Apparent widening of the tunica intima and media with some extension into the tunica adventitio. manifested by cellular vacuolization. is observed in paraffin serial sections of the thoracic aorta in methionine-deficient group (Fig. 3). Except for slight degree of cellular vacuolization, no tunica media hypertrophy occurs in methionine-cystine deficient animals. No increase in plaque formation is observed in any sections analyzed from dietary groups as compared to controls.

Myocardial fibers. cardiac vascularity. endocardium and valve leaflets appear microscopically normal in all groups.

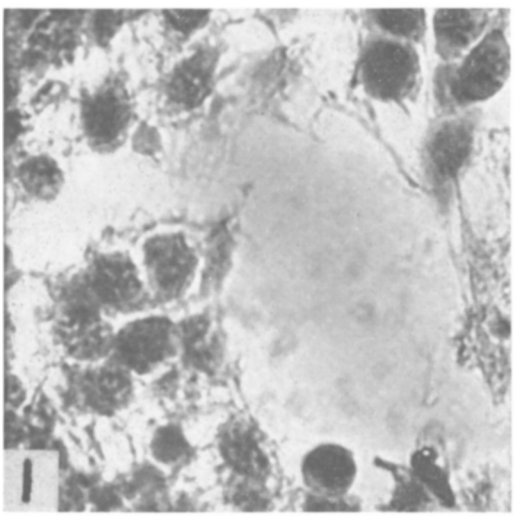

FIG. 1. Sections of rat testes fixed in Helley's fluid and stained with hematoxylin and eosin. High power view (430×) typical of methionine-deficient and methionine-cystine-deficient groups. Seminiferous tubules do not show a compact and orderly arrangement, similar to that of immature animals. Note absence of sperm in tubules.

Sections of liver from choline-deficient animals reveal no abnormalities. Frozen sections of liver stained for neutral fats reveal abnormal accumulations of stainable fat droplets within hepatic parenchymal cells of methionine-deficient animals and to a lesser degree in methionine - cystine - deficient animals. Similar periportal focal fatty metamorphosis (Fig. 3, 4) with moderate to severe nuclear activity and slight hepatocellular necrosis is evident in hematoxylin-eosin stained liver sections of methionine-deficient rats. All sections reveal preservation of lobular architecture. In paraffin liver sections of methionine-cystine-deficient animals, a moderate degree of periportal focal fatty metamorphosis (Fig. 5.6) with evidence of periportal fibroblastic reactions and microscopic areas of mild hepatocellular necrosis is noted. The over-all architectural pattern is well preserved.

Accompanying changes in liver sections are alterations in kidney sections. There is normal kidney structure in the choline-deficient animal. Fig. 7 and 8 reveal that in methionine-deficient rats glomeruli are compactly arranged and vary slightly in size and shape. Bowman's capsule is prominent with slight thickening (Fig. 8). Glomerular congestion and edema are conspicuous. There is variability in capsular spaces. Convoluted and collecting tubules exhibit a cloudy swelling. Although similar microscopic findings are observed for methionine-cystine-deficient animals. the changes are not as marked in this group (Fig. 9, 10). No significant differences from pair-fed controls in the amount of stainable fat are noted in any frozen sections

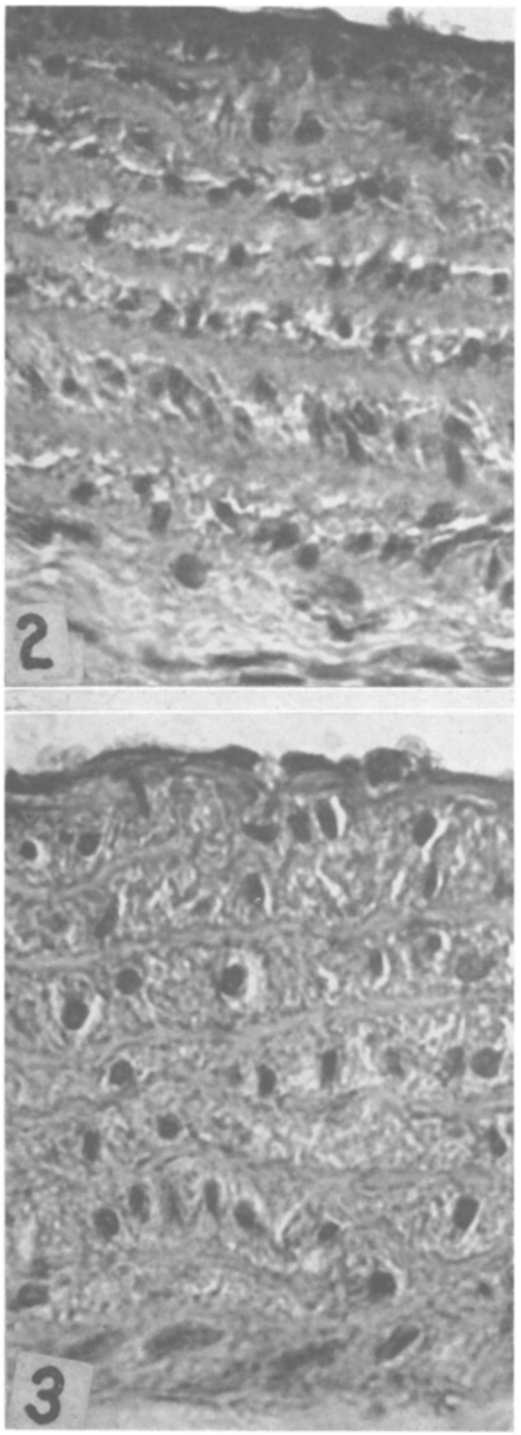

Fig. 2-3 represent sections of rat thoracic aorta fixed in Helley's fluid and stained with hematoxylin and eosin. 430×.

FIG. 2. Typical of pair-fed control, choline-deficient and cystine-deficient animals. Normal architecture.

FIG. 3. Methionine-deficient rat. Widening of tunica intima and media with some extension into tunica adventitio.

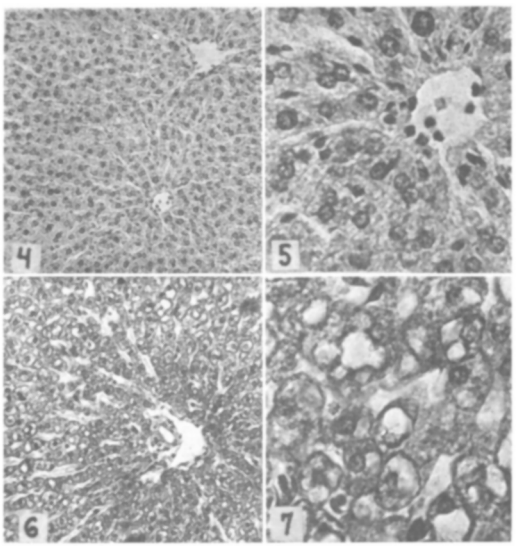

Fig. 4-7. Sections of rat liver fixed in Helley's fluid and stained with hematoxylin and eosin.

FIG. 4-5. Methionine-deficient rat. 50× and 215×. Observe periportal focal fatty metamorphosis.

FIG. 6-7. Methionine-cystine-deficient animal. 50× and 215×. Moderate degree of periportal focal fatty metamorphosis with evidence of periportal fibroblastic reactions is apparent.

of kidney from the deficiency groups.

Routine histologic examination of other organs (thymus, spleen, pituitary and adrenals) reveals no apparent changes.

Discussion. Our results indicate that with a diet containing ample protein and sufficient vitamin and mineral supplement choline is not a critical dietary factor in growth and lipotropism of the male rat subjected to dietary regime of short duration (6 weeks). No evidence was found that exogenous choline-deficiency may be responsible for the pathologic changes noted.

Absence of dietary factors from exogenous sources is often corrected by biosynthesis when necessary basic materials are present for synthesis and conflicting priorities do not exist. Methyl donor function of choline can be provided by methionine or betaine. The lipotropic action of choline, which may be the result of its incorporation into the phospho-

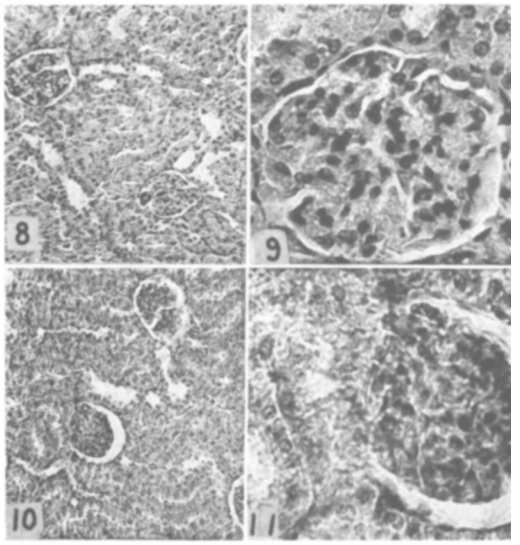

Fig. 8-11. Sections of rat kidney fixed in Helley's fluid and stained with hematoxylin and eosin.

FIG. 8-9. Methionine-deficient rat. 50× and 215×. Glomeruli compactly arranged and vary slightly in size and shape. Glomerular congestion conspicuous. Convoluted and collecting tubules exhibit cloudy swelling.

FIG. 10-11. Methionine-cystine-deficient animal. 50× and 215×. Findings similar to those of methionine-deficient group but not as marked.

lipid molecule, cannot be replaced. However, in the diet there is ample starting material for synthesis of choline and it may be assumed that the lipotropism of the choline-deficient diet under our conditions is the result of endogenous biosynthesis.

The requirements for sulfur amino acids can be satisfied by methionine alone but not by cystine(10). We eliminated methionine from the basal diet by the method of Welch (11) with the result that that methionine and methionine-cystine deficiencies caused development of cardiovascular changes in accord with previous investigations(12,13). Of interest is the finding that methionine and methionine-cystine deficiencies cause increase in thymic weights with accompanying decrease in testes weights. A possible explanation may be found in the work of Martin and Lehr(14) who demonstrated similar thymus-testes inverse weight relation in experimentally induced obstructive nephropathy. They believe that the thymus gland seems to suppress formation or release of excess of certain steroid hormones.

It is extremely interesting that pathologic changes were more extreme in methionine-deficient than in methionine-cystine-deficient animals. The methionine-deficient diets contained an adequate supply of cystine. Cystine added to sulfur-amino acid-deficient diets improves the nutritive value of the diet, increases the demand for methionine for growth and reduces the quantity available for lipotropic activity(15). This appears to be an adequate explanation for our findings.

Summary. 1. Young growing male rats fed 6 weeks a diet containing ample balanced protein and mineral supplement do not require exogenous choline. 2. Similar diets deficient in methionine alone or methionine and cystine cause pathologic changes in testes, kidney, liver and cardiovascular system. 3. Gross and histologic changes are demonstrated in methionine-deficient and methionine-cystine-deficient animals. 4. These changes are more marked in animals fed the diet deficient in methionine alone. Presence of cystine increases methionine requirement for growth and decreases methionine available for lipotropism. 5. An inverse thymus-testes weight relation is noted in animals fed a methionine-deficient diet.

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