# EFFECTS OF EXCESS DIETARY CYSTEIC ACID, *dl*-METHIONINE, AND TAURINE ON THE RAT LIVER

By DAVID P. EARLE, Jr., M.D., KATHARINE SMULL, M.D., and JOSEPH VICTOR, M.D.

*(From the Research Service, First Division, Welfare Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York)*

Plate 25

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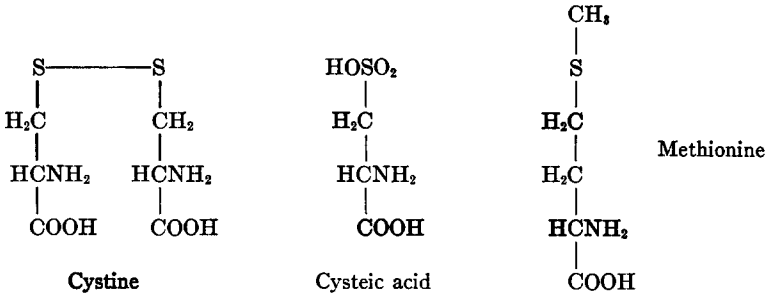
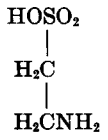
It has been shown that *l*-cystine, a sulfur-containing amino acid, fed in excess amounts to albino rats, causes portal hemorrhage and necrosis (1–4) and often cirrhosis of the liver (4, 5). *l*-Cystine feeding also causes the formation of large amounts of urinary sulfate (6). Cysteic acid, a closely related compound, when fed in amounts comparable to *l*-cystine, results in almost no increase in urinary sulfate (7). When *dl*-methionine, another sulfur-containing amino acid, is fed, large amounts of urinary sulfate are formed (8). The structural formulas of these compounds are:




Cystine Cysteic acid Methionine

If the liver lesions caused by excess *l*-cystine feeding result from the formation of large amounts of sulfate, then liver damage should occur when *dl*-methionine, but not when cysteic acid, is fed. However, it was recently demonstrated (9) that no correlation exists between the urinary sulfate excretion and the presence of liver damage in rats fed *l*-cystine, cysteic acid, and *dl*-methionine.

Both *l*-cystine and cysteic acid produce similar liver lesions. They have structural properties similar to that found in taurine which is:




Taurine

These 3 compounds, *l*-cystine, cysteic acid, and taurine possess an amino group separated from a sulfur molecule by a 2 carbon chain. If the liver lesions caused by cystine and cysteic acid were due to this structural similarity, taurine should also produce the liver lesions.

The present experiments attempt to define the structural relation of the cystine sulfur to the production of liver lesions. The general behavior and the lesions noted in rats fed large amounts of cysteic acid, *dl*-methionine, and taurine are described.

## *Methods*

Albino rats of both sexes and various ages were employed. Although previous cystine studies (4, 5) were carried out on 6 week old male rats, unpublished observations indicate that cystine liver damage may be produced in rats regardless of age or sex.

Cysteic acid was made from *l*-cystine by the bromine oxidation method and was fed as the sodium salt. The *dl*-methionine and taurine were obtained from the Eastman Kodak Company. The sulfur content of 15 per cent cysteic acid is approximately equal to that of 10 per cent cystine. Cysteic acid, in a concentration of 1.25 to 15 per cent in the McCollum stock diet, was fed to 15 rats. The food intake of each rat was restricted to 8 gm. a day. 6.4 to 12.4 per cent *dl*-methionine was added to the McCollum stock diet and fed to 26 rats. 12.4 per cent *dl*-methionine contains the same amount of sulfur as 10 per cent cystine. The daily food consumption of only 2 of the *dl*-methionine fed rats was measured and averaged 3.1 gm. a day, considerably below normal. The food consumption of the remaining rats also appeared to be low. Taurine was fed as 1 to 10 per cent of the McCollum stock diet to 130 rats. The daily food consumption was restricted to 10 gm.

Tissues from each rat were obtained at intervals after the onset of the feeding experiment and were fixed in Zenker's fluid and 10 per cent formalin.

Liver fat was measured in some of the methionine experiments by an ether extraction method (4).

## RESULTS

CYSTEIC ACID.—Five rats were fed cysteic acid as 1.25 per cent of the diet, 5 were fed cysteic acid as 6.25 per cent of the diet, 2 as 12.5 per cent of the diet, and 3 as 15 per cent of the diet. All animals were sacrificed after 8 to 14 days of this feeding.

*General Condition:* None of the rats fed cysteic acid died and all appeared to be in excellent condition at the time of sacrifice. They each received 8 gm. of food daily. The 10 rats fed 1.25 or 6.25 per cent cysteic acid maintained their weight. The 5 rats fed 12.5 to 15 per cent cysteic acid lost 7 to 43 gm. Five control rats fed 8 gm. *per diem* of the McCollum stock diet without cysteic acid lost 2 to 29 gm. in the same time.

*Pathological Findings:* When the concentration of cysteic acid in the diet was

6.25 per cent or less, it produced no anatomical changes. However, 12.5 to 15 per cent cysteic acid produced liver lesions in all 5 rats. The lesions resembled those described as due to *l*-cystine (4), particularly when 10 per cent *l*-cystine was fed in the McCollum stock diet (5). There was some portal necrosis of liver cells, with varying degrees of portal fibrosis. In 2 instances the cirrhosis was evident from the gross appearance. The livers of these 2 rats had finely granular capsules and the cut surfaces showed fine intercommunicating scars confined to the portal areas. A conspicuous microscopic feature was the extensive proliferation of bile ducts in some of the scarred areas (Fig. 1). The livers of the other 3 rats showed no gross changes. Histological examination, however, revealed numerous scarred portal areas with some proliferation of bile ducts. The portal scarring appeared to be due to both condensation fibrosis and connective tissue proliferation. The lack of changes visible in the gross in these 3 instances was due to the fact that the portal scarring showed little intercommunication with adjacent areas or extension into surrounding parenchyma. The liver cells were often hypertrophied but they showed little vacuolization and no fatty infiltration. A noteworthy trait was the absence of hemorrhage.

*dl*-METHIONINE.—A total of 26 rats were fed excess amounts of *dl*-methionine. One rat received *dl*-methionine as 6.4 per cent of the diet while all the other rats were fed 10 or 12.4 per cent *dl*-methionine.

*General Condition:* Unlike the rats fed large amounts of cysteic acid, the rats fed *dl*-methionine ate poorly and after several days appeared ill. They all lost weight rapidly, averaging 5.0 gm. a day. This was equivalent to a daily decline of 5.6 per cent of the original body weight. One rat lost 47 per cent of its original body weight in 13 days.

There was a considerable mortality among the rats fed excess *dl*-methionine. Disregarding the 14 rats sacrificed during the first 5 days, 8 of the remaining 12 rats were dead by the end of 1 week. One rat lived 17 days and was then sacrificed. Three of the 4 rats that survived more than 7 days were adults, all other rats in this experiment being 6 to 8 weeks of age.

*Pathological Findings:* The rats usually showed evidence of severe weight loss in the diminution or absence of subcutaneous and mesenteric fat and in the moderate to severe dehydration. The liver and spleen almost invariably were decreased in size and had sharp edges. The liver capsule was smooth, clear, and transparent and the organ had a dark brown color. In 2 instances the edges of the liver were greyish yellow in color and had a translucent appearance. This translucent portion was wedge-shaped on cross-section with the base of the wedge away from the liver margin. (Fig. 2. This photograph shows the liver of a rat fed 10 per cent *dl*-methionine for 4 days.) The liver parenchyma was very friable.

The essential change in the liver was the extreme atrophy of the liver cells

noted in 24 of the 26 rats fed excess *dl*-methionine. The diameter of the cells was much reduced and the cytoplasm was very dense and granular. The nuclei were also reduced in size, although this shrinkage was relatively less than that of the cells. The nuclei showed changes in the distribution of the chromatin which was condensed and often deposited at the periphery of the nucleus. The central nucleolus was more conspicuous than usual. The space between the nucleolus and the nuclear wall had very little stainable material. Fig. 3 is a photograph of atrophic liver cells of a *dl*-methionine fed rat. For comparison, Fig. 4 shows the same magnification of the normal liver cells of a rat fed the same McCollum stock diet without the *dl*-methionine supplement. The greyish yellow translucent parenchyma seen in the fresh liver in 2 instances showed much more severe atrophy than was seen elsewhere. No necrosis or other degenerative changes were evident, even in these areas. In one rat, a few isolated necrotic cells with hyalinized cytoplasm and dense pyknotic nuclei were scattered throughout the liver without any relationship to the lobular structure. No fibrosis, bile duct proliferation, or jaundice was found. The liver fat was much reduced in 7 methionine fed rats sacrificed after 4 to 6 days of feeding. It varied from 0.7 to 1.6 per cent of the whole fresh liver substance with an average of 1.2 per cent, in contrast to the livers of 12 control normal rats which had a range of 3.0 to 4.5 per cent (5).

An attempt to quantitate the degree of liver atrophy was made by counting with a microscope the number of portal spaces in 10 consecutive fields. At a magnification of 100 the average number of portal areas in 7 normal livers ranged from 1.2 to 3.6 per field with a mean of 2.2. In the 26 methionine fed rats the average number of portal areas ranged from 1.4 to 8.9, with a mean of 4.7. Of these, the 2 normal appearing livers of the methionine fed rats had average numbers of portal areas of 1.4 and 2.6 respectively. The remainder ranged from 3.1 to 8.9. One obvious source of error in the evaluation of the number of portal areas is the degree of distention of the liver sinusoids. In spite of this, however, the number of portal spaces in a unit area was much greater in the livers of the *dl*-methionine fed rats than in the rats fed the control McCollum stock diet.

The spleen showed microscopic evidence of atrophy, namely decrease in size of the Malpighian bodies, decrease in the number of cells in the pulp, and condensation of the pulp structures in 15 of the 22 cases examined. Hemorrhage was present in the pulp of one spleen and congestion of the sinusoids was prominent in another.

The kidneys were atrophied in only 3 of the 26 cases. These 3 instances occurred in rats showing the most extreme liver atrophy, the average number of portal areas in these livers being 6.3, 8.0, and 8.9 per low power field respectively. Dilatation of the convoluted and collecting tubules was the most usual alteration in the kidneys and occurred in half the *dl*-methionine fed rats.

The epithelial cells of these tubules were small, vacuolated, and had a basophilic cytoplasm. In one case, several mitotic nuclei were found among these vacuolated epithelial cells. Hyaline droplets were not seen in these cells. The glomeruli were not altered.

In 3 instances the stomach had submucosal hemorrhages in the antral portion beneath the squamous cell lining. The fundus showed no changes.

The lungs showed no specific lesions. Heart, testes, and pancreas were normal.

The results with feeding *dl*-methionine, as far as studied, were not influenced by the diet in which the *dl*-methionine was administered. It has been found that *l*-cystine fed in a low protein, low fat diet produced more severe liver lesions than when fed in the McCollum stock diet (5). For this reason, 5 rats were fed 10 per cent methionine in the same low protein, low fat diet. This diet consisted of 5 parts of casein, 3 of lard, 2 of cod liver oil, 5 of brewer's yeast, 4 of salt mixture, 10 of *dl*-methionine, and 71 of sucrose. This low protein, low fat diet did not influence the effect of the 10 per cent methionine.

TAURINE.—Rats weighing 80 to 100 gm. were fed excess amounts of taurine. This was fed as follows: 29 females—1 per cent taurine for 6 weeks, 68 females —2.5 per cent taurine for 8 weeks, 10 males—10 per cent taurine for 7 weeks, 20 females—stock diet without taurine for 7 weeks. The food intake was restricted to 10 gm. daily.

*General Condition:* The rats ate all their food and grew as well as their controls.

*Pathological Findings:* There were none in any of the organs examined which included liver, spleen, kidney, heart, aorta, lung, suprarenal, pituitary, thyroid, ovaries, testis, uterus, and brain.

## DISCUSSION

The above data show that excess dietary cysteic acid produces portal necrosis and cirrhosis similar to that caused by comparable amounts of dietary *l*-cystine (4, 5). The ingestion of similar amounts of methionine or taurine did not produce such liver lesions. Urinary sulfate is formed in large amounts from *l*-cystine and *dl*-methionine feeding but not from cysteic acid (5). Since feeding *l*-cystine or cysteic acid produces liver necrosis and cirrhosis these lesions are not dependent on either the S-S grouping of cystine, the degree of oxidation of the sulfur in the cysteic acid, the formation or excretion of urinary sulfate which occurs with *l*-cystine and *dl*-methionine but not with cysteic acid, or the presence of the amino group and S molecules separated by a 2 carbon chain in the cystine, cysteic acid, or taurine.

The general reaction of rats fed 1.25 to 15 per cent cysteic acid differed from those fed similar quantities of cystine. Rats fed large amounts of cysteic acid ate 8 gm. of food daily while those receiving similar concentrations of *l*-cystine

ate only about 3 gm. daily (5). Those fed 12.5 to 15 per cent cysteic acid did not appear ill and none died within the 2 weeks during which they were studied. In contrast, the mortality during the first 2 weeks of 10 per cent *l*-cystine feeding varied from 71 to 100 per cent depending on the basal diet (5).

The atrophy of the liver caused by feeding excess amounts of *dl*-methionine is a most striking change. Although the food intake of the *dl*-methionine fed rats was not measured, the atrophy was certainly not due to starvation alone, since it was present in the livers of rats fed the *dl*-methionine for only 2 days. In the two instances in which the food intake was measured it was 3.1 per 100 gm. body weight daily. Six rats weighing about 100 gm. were fed 3.0 gm. of the stock diet for 2 to 6 days and showed no such atrophy. The severe general dehydration may have played some part in shrinking the liver cells, but if so, this effect appears to have been relatively specific for the liver since it occurred less frequently in the spleen and rarely in the kidney. *dl*-Methionine is acted on directly by liver cells (10). Unpublished observations show that the excised livers of *dl*-methionine fed rats have an increased metabolism as compared with livers fed the stock diet alone. The liver atrophy may be a reaction to this increased metabolism.

In spite of the usual severe atrophy of the liver and spleen, and occasional atrophy of the kidney, there is no apparent anatomical lesion that offers an explanation for the fatal outcome due to feeding excess *dl*-methionine.

## SUMMARY AND CONCLUSIONS

1. Cysteic acid fed to albino rats as 12.5 to 15 per cent of the McCollum stock diet caused portal necrosis and cirrhosis of the liver within 2 weeks. Concentrations of cysteic acid of 6.25 per cent or less in the diet produced no liver lesions within 2 weeks.

2. *dl*-Methionine fed as 6.4 to 12.4 per cent of the McCollum stock diet or of a low protein, low fat diet, resulted in severe atrophy of the liver cells but no cirrhosis of the liver.

3. Taurine fed as 1 to 10 per cent of the McCollum stock diet produces no liver lesions.

4. For reasons discussed in the paper, it is concluded that the liver necrosis and cirrhosis produced by cystine and cysteic acid are not dependent upon the S-S linkage of the cystine, the oxidation of the sulfur, the formation and excretion of large amounts of urinary sulfate, or the presence of an amino group separated from a sulfur molecule by a 2 carbon chain.

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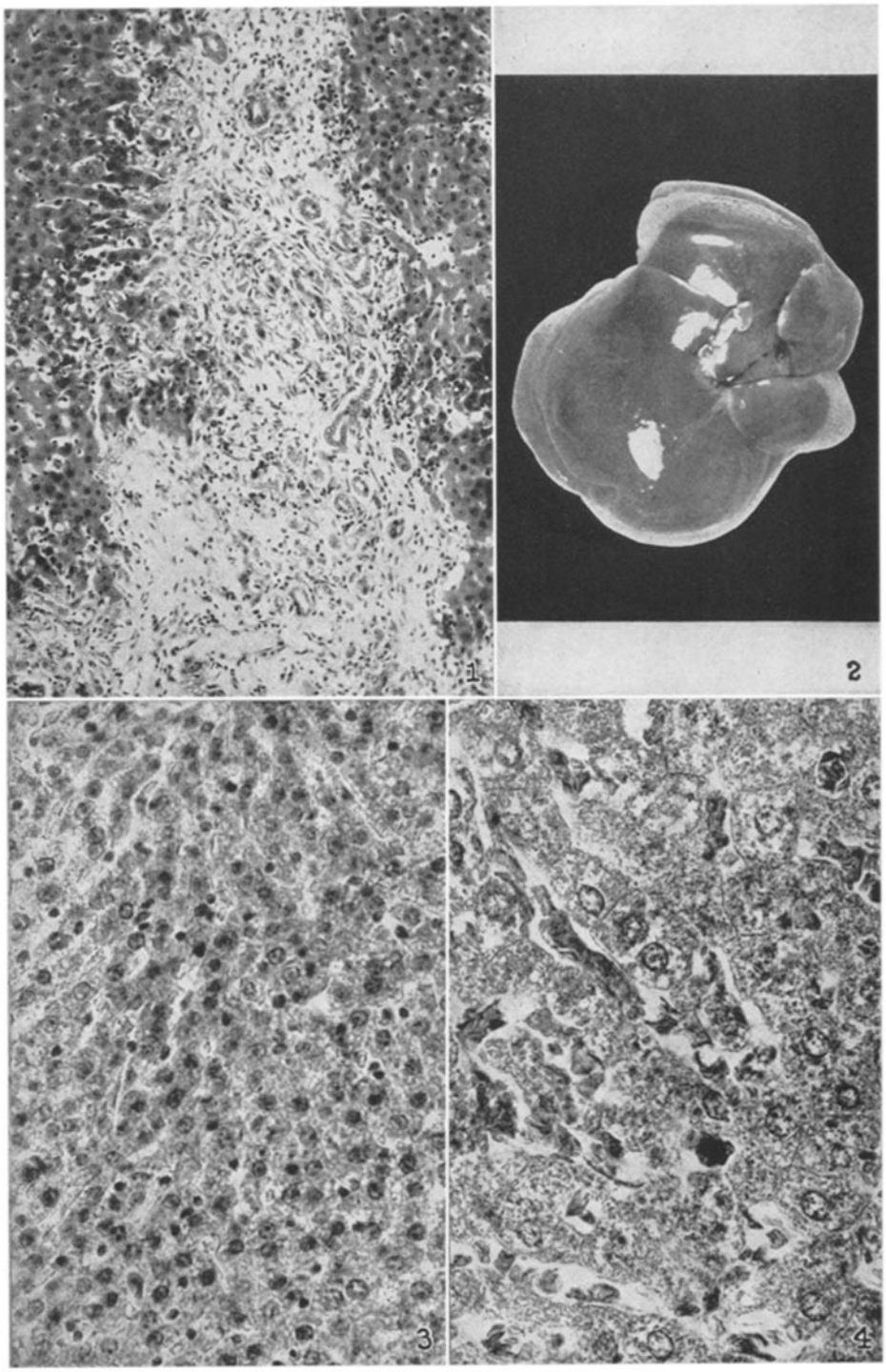
## EXPLANATION OF PLATE 25

FIG. 1. Cirrhosis of the liver following ingestion of 15 per cent cysteic acid for 8 days. Fibroblast and bile duct proliferation in the portal areas with relatively slight liver cell necrosis. Hematoxylin and eosin stain. Magnification × 100.

FIG. 2. Liver atrophy in rat fed 10 per cent *dl*-methionine for 4 days. The edges appearing as light grey in the photograph were yellow in the fresh organ, while the remainder was brown. The white areas on the surface of the liver are reflections from the light source. Natural size.

FIG. 3. Atrophy of liver cells in rat fed 10 per cent *dl*-methionine in McCollum stock diet for 4 days. Hematoxylin and eosin stain. Magnification × 460.

FIG. 4. Normal liver cells of rat fed McCollum stock diet. Hematoxylin and eosin stain. Magnification × 460.




(Earle *et al.*: Relation of cystine sulfur to liver lesions)