

## THE EFFECT OF VARIOUS DIETS ON THE LIVER DAMAGE CAUSED BY EXCESS CYSTINE

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Previous reports (1-4) have described the rapid production of portal necrosis and hemorrhage in the livers of rats fed large amounts of *l*-cystine. In addition, more prolonged feeding of *l*-cystine causes the development of cirrhosis of the liver (4). The present paper is a study of the effects of variation in diet on the development of these liver lesions.

### *Methods*

Six different diets containing 10 gm. of *l*-cystine per 100 gm. of food (subsequently referred to as "10 per cent cystine") and 3 different diets containing "5 per cent cystine" were fed to male albino rats, 6 weeks of age. The protein of the "synthetic" diets was supplied by casein and yeast, the fat by lard and cod liver oil or by butter, and the carbohydrate by sucrose. The relative amounts of these ingredients were varied in the different diets. Table I shows the composition of the diets, the average daily food consumption per rat, and the number of rats fed each diet. An additional series of rats was fed 5 and 10 per cent cystine in the McCollum stock diet.

These diets permitted a comparison of the effects of different protein, fat, carbohydrate, and yeast concentrations on the hepatic lesions produced by 10 per cent cystine. It was also possible to compare the effects of different kinds of fat, namely butter *versus* lard and cod liver oil, as well as the effects of synthetic diets *versus* the natural food McCollum stock diet. In the 5 per cent cystine group, the McCollum stock diet was compared with a low protein, high lard, and cod liver oil diet. The effect of the addition of 1 per cent choline to this low protein, high fat diet was also examined.

The technique employed for classifying the liver lesions was similar to that described in a previous paper (4). Hemorrhage and necrosis were graded 0-4, each unit indicating involvement of  $\frac{1}{4}$  of the area of the section. Portal fibrosis and bile duct proliferation were also graded 0-4, each unit indicating involvement of  $\frac{1}{4}$  of the portal areas. In cases where there was any question as to the presence or absence of portal fibrosis, the Mallory connective tissue stain was employed. Hypertrophy and vacuolization of the liver cells were graded on the same basis as hemorrhage and necrosis. Estimation of the number of mitotic figures was made by counting the

number seen in 3 to 10 low power fields (magnification  $\times 100$ ) and obtaining the average per low power field.

The fat content of the livers was determined by the method previously described (4).

TABLE I  
*Per Cent Composition of Diets*

Diet	10 per cent cystine						5 per cent cystine		
	10HL	10HB	10LF	10HP	10HY	10 S	5HL	5HLC	5 S
Type	Low protein High lard	Low protein High butter	Low fat Low protein	Low fat High protein	Low fat High yeast	McCollum stock	Low protein High lard	Low protein High lard + Choline	McCollum stock
<i>l</i> -Cystine.....	10	10	10	10	10	10	5	5	5
Salt mixture.....	4	4	4	4	4	*	4	4	*
Brewer's yeast.....	5	5	5	5	20		5	5	
Casein.....	5	5	5	40	5		5	5	
Lard.....	20	—	3	3	3		20	20	
Cod liver oil.....	5	—	2	2	2		5	5	
Butter.....	—	25	—	—	—		—	—	
Sugar.....	51	51	71	36	56		56	55	
Choline HCl.....	—	—	—	—	—		—	1	
No. of rats.....	29	14	10	10	10	12	15	15	15
Intake, gm./rat.....	3.6‡	3.8	3.0	3.0	3.3	3.8	6.1§	6.8	6.1

The number of rats fed each diet and the average daily intake of food are indicated below the dietary system. The *l*-cystine and the choline were obtained from the Eastman Kodak Co., Rochester, New York.

\* The McCollum stock diet (5) has the following composition in parts per cent: bran 67.5, whole milk powder 15, casein 10, butter 5, NaCl 1.5, and Na<sub>2</sub>CO<sub>3</sub> 1.0.

‡ Average of 10 rats only.

§ Average of 7 rats only.

## RESULTS

In Table II are summarized the per cent mortality, the incidence per cent of the liver lesions, and the average fat content of the livers of rats fed the several diets described. These features have been divided into 2 periods, namely, those occurring before and those after 2 weeks of the dietary regimen. The grouping of the data followed the general scheme outlined in Table I except that the groups fed 10 per cent cystine in the 3 low fat diets (low protein, high protein, and high yeast) were combined. These 3 groups showed no significant differences among themselves in their response to the 10 per cent cystine, with one exception which will be mentioned later. The mortality of the 10 per cent cystine groups during the first 2 weeks was calculated on the basis of the number of animals that died and that survived throughout the

period. The animals sacrificed during these 2 weeks were not considered in the calculation.

TABLE II  
Effect of Various Diets Containing 10 Per Cent or 5 Per Cent Cystine on Per Cent Mortality, Per Cent Incidence of Liver Lesions, and Liver Fat Content

Days on diet.....	No. of rats		Per cent mortality		Per cent incidence liver lesions						Per cent fat content			
	2-14 15+		2-14 15+		Hemor- rhage		Necro- sis		Cirrho- sis		2-14		15+	
											Aver- age	Range	Aver- age	Range
10 per cent cystine in McCollum stock.....	10	2	78	—	20	—	50	—	41	12	2.2 (4)*	1.6- 3.1	2.2	2.1- 2.2
10 per cent cystine in low fat.....	29	1	97	—	21	—	86	—	52	11	3.4 (20)	1.7-13.2	4.9	
10 per cent cystine in low protein, high lard.....	22	7	71	—	73	29	77	71	14	71	4.0 (11)	2.5- 7.9	15.3 (3)	13.1-19.1
10 per cent cystine in low protein, high butter.....	14	0	100	—	71	—	93	—	29	—	7.1 (12)	2.5-15.2		
5 per cent cystine in McCollum stock.....	5	10	0	0	0	0	60	20	80	50	1.4	1.1- 2.0	2.6	1.4- 5.0
5 per cent cystine in low protein, high lard.....	5	10	0	0	0	20	0	70	0	40	13.6	4.8-17.4	23.5	7.6-42.3
5 per cent cystine in low protein, high lard + choline.....	5	10	0	0	0	0	20	20	40	30	4.3	1.6-11.4	2.4	0.8- 4.6

\* The numbers in parentheses represent the number of livers in the group analyzed for fat. In all other instances the entire group was analyzed.

The average amount of a specific lesion in a particular group of rats is equal to its percentage incidence multiplied by its average severity (calculated from data derived as described under Methods). On this basis, the average amount of hemorrhage, necrosis, and cirrhosis was calculated for the groups of rats that died or were sacrificed during the first 2 weeks on the various diets

containing cystine. In Fig. 1, these values along with the average liver fat content are graphically presented.

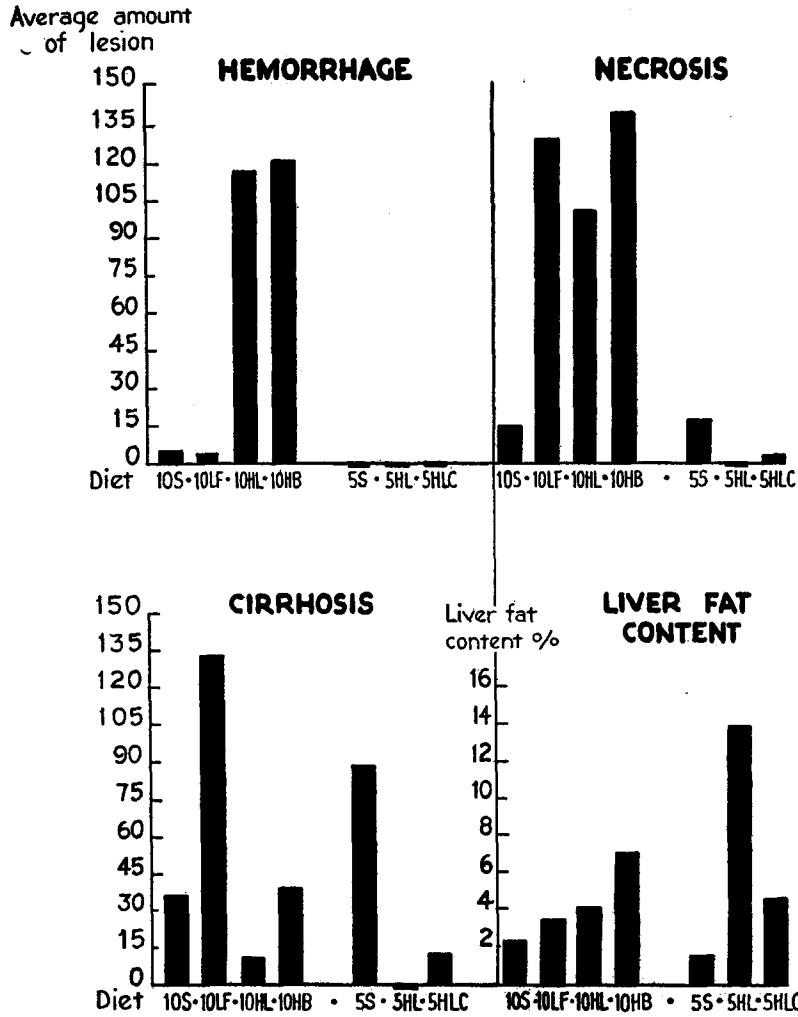


FIG. 1. Effect of different diets on liver lesions due to excess dietary cystine. First 2 weeks only. Ordinates represent average percentage incidence and average severity of liver lesion. Abscissa lists different diets, 10 = 10 per cent cystine, 5 = 5 per cent cystine, S = stock, LF = low fat, HL = high lard, HB = high butter, HLC = high lard and 1 per cent choline.

*Effect of Diets on the Course and Liver Lesions Due to 10 Per Cent Cystine*

These data show that the diet modified the mortality rate and the character of the liver lesion resulting from 10 per cent cystine. If the McCollum stock

diet, the natural food diet, is considered as the basis for comparison, the following results are apparent:

1. A low fat synthetic diet, regardless of the casein or yeast content was deleterious. This was indicated by a greater early mortality (only one of 30 rats lived more than 8 days) and by more necrosis and cirrhosis (Fig. 1). However, the incidence and severity of hemorrhage were not different.

2. A diet *high* in fat, consisting of lard 20 per cent, and cod liver oil 5 per cent had some protective action. This was indicated by a slightly lower mortality and a slower development of cirrhosis (bold faced figures, Table II). On the other hand, the incidence and extent of hemorrhage were far greater than that in the previous groups (McCollum stock and low fat diets). Necrosis was the same as in the low fat diets but greater than was observed in the McCollum diet.

3. The protective action of the high fat diet (lard 20 per cent and cod liver oil 5 per cent) was not due to fat alone, because 25 per cent butter substituted for the lard and cod liver oil, was followed by the most severe and harmful reaction to cystine. The mortality was greatest, the animals died in a shorter time (none survived more than 6 days), and hemorrhage and necrosis were most severe. In spite of the shorter survival period on this diet, these animals had much more cirrhosis in this 6 day interval than those on the 20 per cent lard and 5 per cent cod liver oil had in a 14 day interval.

#### *Effect of Diets on the Liver Lesions Due to 5 Per Cent Cystine*

Cystine was fed as 5 per cent of 3 different diets: the McCollum stock diet, the low protein, high lard, and cod liver oil diet, and the latter diet plus 1 per cent choline. There were 15 rats in each group. As none of these animals died, rats from each group were sacrificed after periods of from 3 to 42 days of cystine feeding. Five from each group were sacrificed during the first 2 weeks and 10 subsequently.

In general, the incidence and severity of the liver lesions were less than among rats fed 10 per cent cystine (Table II, Fig. 1) although the character of the lesion was the same. Portal hemorrhage occurred in only 2 of 45 rats fed 5 per cent cystine. Necrosis in the portal areas, when present, involved only a few cells.

Portal cirrhosis was present in each group and the incidence was modified by the diet (Table II and Fig. 2). Only 5 animals in each group were sacrificed during the first 2 weeks. There were insufficient data, therefore, to ascertain that the rate of development of cirrhosis was influenced by the diet. However, the available data indicate that the rate of development and the incidence of cirrhosis were related, that is, a rapid appearance of cirrhosis was associated with a high incidence of the lesion. Comparison of the 15 animals in each of the groups with those fed the 5 per cent cystine McCollum stock diet revealed the following with respect to the incidence of cirrhosis:

1. On the low protein, high lard, and cod liver oil diet the incidence was 4 of 15 as compared with 9 of 15 on the McCollum stock diet, that is, about one-half. During the first 2 weeks the incidence was 0 of 5 as compared with 4 of 5. In other words, the low protein, high lard diet had a protective action against the development of cirrhosis produced by either 5 per cent or 10 per cent cystine.

2. Addition of 1 per cent choline to the low protein, high lard, and cod liver oil diet did not significantly alter the protective effect of this diet, that is, 5

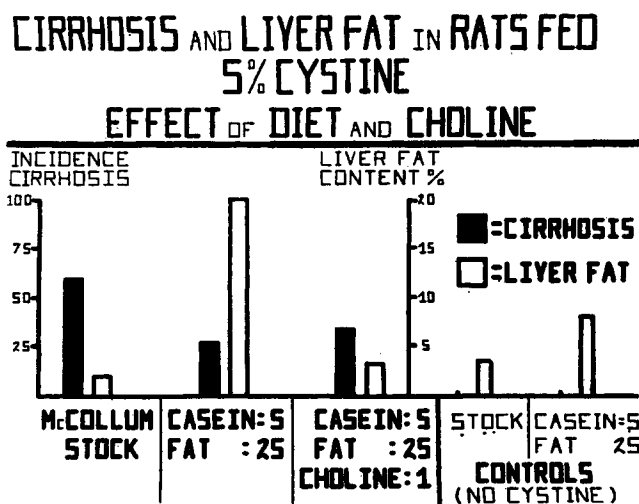


FIG. 2. There is no correlation between severity of fatty infiltration and cirrhosis in rats fed 5 per cent cystine. Choline, which prevented the fatty infiltration due to the diet, did not inhibit the development of cirrhosis. The incidence of cirrhosis was greatest on the McCollum diet, while the fatty infiltration was least.

of 15 fed choline had cirrhosis as compared with 4 of 15 on the diet without choline.

*Effect of Diet on the Fat Content of the Livers of Rats Fed Excess Cystine*

The average fat content of livers of 12 rats on the McCollum diet without cystine was 3.5 per cent (range 3.0–4.5). None of these control livers showed evidence of necrosis or cirrhosis. The average liver fat content of rats fed 5 per cent or 10 per cent cystine in the McCollum stock diet was 2.2 per cent. Although the livers of a few rats fed 10 per cent cystine in the low fat diets had slight increases in fat content, the average value was within the normal limits described above (Table II).

In contrast to the above, the average liver fat content of 8 rats on a low protein, high lard, and cod liver oil diet without cystine for 3 to 40 days was 8.1 per

cent (range 4.2–13.3). No liver lesions other than fatty infiltration occurred in this group. Addition of 10 per cent cystine to this low protein, high fat diet resulted in a further increase in the liver fat content in rats that survived more than 2 weeks. The 5 per cent cystine diet, however, resulted in an earlier and greater fatty infiltration of the liver than 10 per cent cystine (Table II). It is likely that this is related to the total food intake rather than to cystine alone, for, as Table I indicates, the animals that received 5 per cent cystine ate twice as much food as those receiving 10 per cent cystine. Therefore, both groups ingested the same amount of cystine, but the 5 per cent group ate twice as much fat.

The fatty infiltration observed with 5 per cent cystine in the high fat diet was inhibited by the addition of 1 per cent choline.

#### *The Relationship of Fatty Infiltration and Cirrhosis of the Liver Due to Excess Dietary Cystine*

The results described above offer an opportunity to determine any correlation between liver fat content and the development of cirrhosis in rats fed excess cystine. In these experiments, the diet modified both of these characteristics and if there were any causal relationship between them, the fatty infiltration and the cirrhosis should have shown some correlation.

Fig. 2 shows the incidence of portal cirrhosis of the liver and the average liver fat content of groups of 15 rats fed 5 per cent cystine in the McCollum stock diet, a low protein-high fat diet and a low protein-high fat diet plus 1 per cent choline. Cirrhosis was found in 60 per cent of the McCollum stock diet group and the average liver fat content was only 2.2 per cent. Although 1 per cent choline added to the low protein, high lard, and cod liver oil diets greatly lowered the liver fat content, *i.e.* from 20.7 per cent without choline to 3.0 per cent, it did not influence the incidence of cirrhosis which was approximately 30 per cent, with or without choline.

Fig. 3 is a scatter chart of the fat content and the severity of cirrhosis in the livers of rats fed excess cystine in the various diets.

No correlation between the liver fat content and the incidence or severity of cystine cirrhosis was evident from the above data.

#### *Other Findings*

Hypertrophy and vacuolization of the liver cells were found in each group. In general, the incidence and severity of these features paralleled the degree of fatty infiltration. However, in a number of instances, the liver cells had hydropic vacuoles and the liver fat content was normal or low. In these cases, the vacuoles did not take up Sudan III.

All livers were studied for the presence of mitotic nuclei. In contrast to the other rats, many mitotic nuclei were found only among the rats fed cystine

as 10 per cent of the low fat, high *protein* diet. Among the rats fed 5 per cent cystine, no mitoses were seen in the livers of the stock diet group, but were observed in a few of those receiving low protein and high fat, with or without 1 per cent choline.

**LACK OF CORRELATION BETWEEN LIVER FAT CONTENT AND INCIDENCE OF CYSTINE CIRRHOSIS**

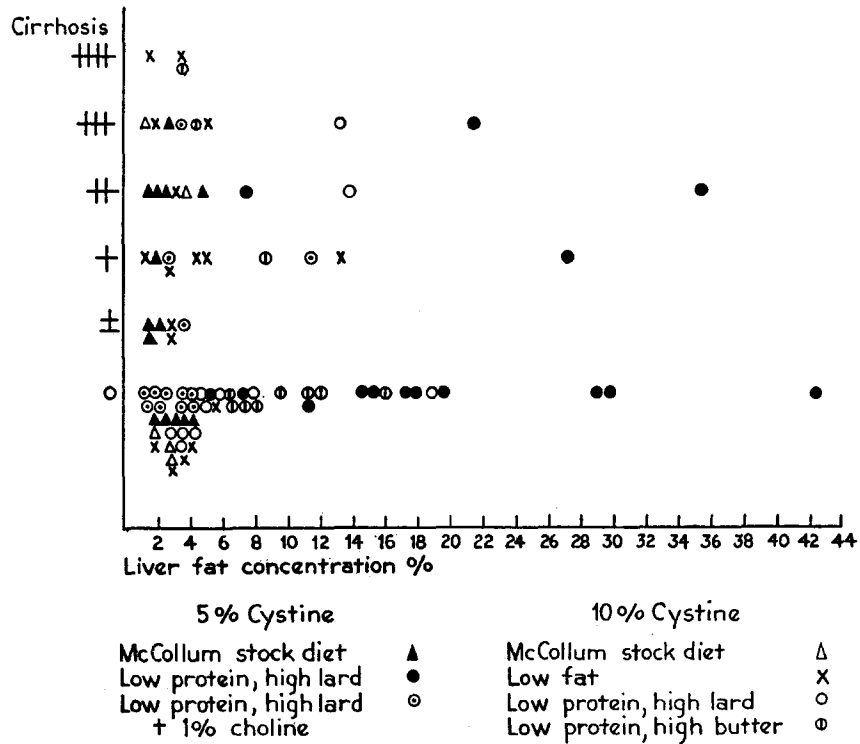


FIG. 3. Scatter chart showing degree of cirrhosis and fat content of the livers of rats fed excess cystine in various diets. For cirrhosis ± = fibrosis of less than ¼ of the portal areas, + = fibrosis in ¼ of the portal areas, ++ = fibrosis in ½ of the portal areas, and so on.

In a few instances, where severe liver damage had resulted from excess dietary cystine, enlargement of the spleen with congestion and fibrosis was noted.

Of 130 rats fed cystine as 5 or 10 per cent of various diets, hemorrhagic kidneys were found but once.



### *Summary of Results*

*Hemorrhage* in the liver was most severe with a high fat intake, whether the fat was lard and cod liver oil or butter. *Necrosis* was more severe with synthetic diets, regardless of the proportions of fat, protein, carbohydrate, or yeast, than with the natural food diet. *Cirrhosis* was less severe during the first 2 weeks on the high lard and cod liver oil diet than on the high butter, or low fat, or the McCollum diets. *Fatty infiltration* in the livers of rats fed excess cystine was also modified by diet. There were slight increases in the liver fat content of only a few rats on the McCollum stock and low fat diets. Marked increases were noted in the low protein, high fat groups. This increase in liver fat was prevented by 1 per cent choline. The number of *mitotic nuclei* in the liver cells was greatest among the rats fed the high protein, low fat diet. In other words, not only is the degree of cystine damage to the liver affected by diet, but the pathogenesis of the lesion is also dependent on the diet.

### DISCUSSION

The differences in liver lesions produced by cystine fed in various diets were related to differences in the diets and not to differences in consumption of cystine. The average daily food consumption of the rats fed 10 per cent cystine was essentially the same with all diets (Table I). The food consumption of rats fed 5 per cent cystine was approximately twice that of rats fed 10 per cent cystine, so that all groups consumed the same amount of cystine.

It is possible, though not probable, that there were differences in the amounts of cystine absorbed from the gastrointestinal tract on the various diets. In this connection it should be noted that Wilson (6) found that rats could absorb cystine from the gastrointestinal tract at the rate of 30 mg. per hour, or 720 mg. in 24 hours. In the present experiments, the greatest amount of cystine consumed by an individual rat in 24 hours was 630 mg., while the average intake for the groups of rats on the different diets varied from 300 to 380 mg. in 24 hours.

The liver lesions produced by cystine are related to the proportion of cystine in the diet but not to the amount of cystine ingested. This is illustrated by the fact that there were differences in the liver lesions between rats fed 5 per cent and 10 per cent cystine (Table III), although both groups ingested the same amount of cystine, the food consumption of rats fed 5 per cent cystine being twice that of rats fed 10 per cent cystine (Table I).

It has been reported that high dietary fat enhances the deleterious effect of various hepatotoxins (7-12) or may produce cirrhosis (19, 20). The present studies indicate that certain fats such as lard and cod liver oil, when fed as 25 per cent of the diet, have some protective effect against liver damage caused

by excess cystine, while butter does not. In liver damage due to selenium, it has been found that a diet containing 39 per cent beef fat and 2 per cent cod liver oil protected against the development of cirrhosis of the liver (13).

Most of the reported studies state that protein (8-12) and yeast (14) are protective against certain liver poisons. In the present experiments, liver damage resulting from 10 per cent cystine in low fat diets was not influenced by 5 or 40 per cent casein or by 5 or 20 per cent brewer's yeast. The studies reported in this paper agree with the findings that yeast *per se* has no protective action on carbon tetrachloride liver damage when the food intake is controlled (15).

György and Goldblatt (16) have found necrosis and cirrhosis of the liver in rats fed, for 100 or more days, a diet devoid of vitamin B, but supplemented with thiamine, riboflavin, pyridoxine, and pantothenic acid. The diet was also low in protein (casein 10 per cent) and high in fat (lard 20 per cent, cod liver oil 2 per cent). The addition of small amounts of cystine increased the severity of liver damage while choline prevented it. Similar findings were reported by Blumberg and Grady (17). More recently, Webster (18) has reported necrosis and cirrhosis in rats fed low protein, high fat diets which also contained adequate amounts of brewer's yeast. These lesions were made more severe by the addition of small amounts of cystine and were prevented by choline. The present experiments show that the liver damage caused by 5 per cent cystine fed in a 5 per cent casein, 20 per cent lard, and 5 per cent cod liver oil diet was not prevented by 1 per cent choline, although the fatty infiltration of the liver was markedly inhibited (Fig. 2).

This difference in the response to choline is a distinction between the pathogenesis of the liver lesion produced by cystine and that caused by low protein high fat diets (16-20). Other differences are found in the liver lesions as well as in the rate of their development. On the cystine diet, the lesions originated in the portal areas while those described by György and Goldblatt (16) showed diffuse degeneration. The cirrhosis caused by cystine may appear within a week, while under the conditions summarized above (16-18) about 4 months on the diet were required for the appearance of cirrhosis. The cirrhosis caused by cystine was associated with considerable bile duct proliferation (4) and not necessarily with fatty infiltration. That found under conditions mentioned by others (16-20) had considerable fatty infiltration and little bile duct reaction, even when small quantities of cystine were added to the diet (16).

#### SUMMARY AND CONCLUSIONS

1. The effect of 9 different diets on the liver lesions resulting from excess dietary cystine has been studied in 130 rats.
2. The incidence and severity of each of the following liver lesions were

varied by changes in the composition of diets containing 5 or 10 per cent cystine:

- (a) *Hemorrhage* was least severe with low fat diets.
- (b) *Necrosis* was most severe with synthetic diets.
- (c) *Cirrhosis* was delayed by a diet high in lard, 20 per cent, and cod liver oil, 5 per cent, but not by a diet high in butter, 25 per cent.
- (d) *Fatty infiltration* was found consistently only with low protein, high fat diets.

In other words, the pathogenesis of the liver lesion due to excess dietary cystine can be modified by diet.

3. In the presence of cystine as 5 per cent of a low protein, high fat diet, 1 per cent choline inhibited fatty infiltration but did not protect the liver against damage by cystine.

4. In these experiments there was no apparent correlation between fatty infiltration of the liver and the incidence or degree of cirrhosis.

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