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Prevention by Cystine or Methionine of Hemorrhage and Necrosis of the Liver in Rats.

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Necrosis, hemorrhage and cirrhosis of the liver in rats were described by György and Goldblatt.[1] They attributed these lesions to a deficiency of a part of the vitamin B_2 complex. By modifying the diet they were later[2] able to increase the incidence of the liver injury. In the report on this work, they stated that the pathogenesis of the necrosis and cirrhosis was related to the lipotropic effect of casein. Blumberg and McCollum[3] reported the production of liver cirrhosis in rats with or without accompanying necrosis and the prevention of the cirrhosis with choline.

Daft, Sebrell and Lillie[4] described the production of liver cirrhosis in rats and its prevention by choline, methionine or casein. Lowry,

1 György, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

2 György, P., and Goldblatt, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492.

3 Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598.

4 Daft, F. S., Sebrell, W. H., and Lillie, R. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 228.

Daft, Sebrell, Ashburn and Lillie[5] reported that treatment by choline or casein of rats with experimentally produced liver cirrhosis resulted in hyperplastic regeneration of liver cells and clinical improvement of the animals. In a paper describing the histology and histogenesis of this liver cirrhosis, Lillie, Ashburn, Sebrell, Daft and Lowry[6] stated that among the noteworthy features is the absence of hemorrhage and, in the earlier stages, of evident necrotic liver cells.

The composition of the diets used in these 3 laboratories is given in Table I. It will be noted that our cirrhosis-producing diet No. 545 contains a much lower percentage both of casein and of fat than the diets of György and Goldblatt[1, 2] and those of Blumberg and McCollum[3] and that our diet contains cornstarch while the others contain sucrose. Diet No. 545 also contains free cystine while the others do not. In view of the classic observations of Osborne and Mendel,[7] it is apparent that the 10% casein diets are deficient in the sulfur-containing amino acids. Recent work of Mulford and Griffith[8] indicates that even the 18% casein diet may be somewhat deficient in these amino acids. It is further apparent that all of these diets except the earlier one of György and Goldblatt[1] are low in protein and that all are low in choline. Diet No. 545 is probably not as low in choline as the others because of the appreciable choline content of cornstarch.[9]

In view of the differences in the diets and the concomitant dif-

TABLE I.
Composition of Diets.

	György and Goldblatt		Blumberg and McCollum[3]		Daft, Sebrell and Lillie[4]
	(1939)[1] Diet	(1941)[2] Diet	Diet (A)	Diet (B)	Diet No. 545
Casein	18	10	10	10	4.0
Cystine					0.5
Sucrose	68	64	29	14	
Cornstarch					86.5
Butter fat	8				
Lard		20	51	66	
Cod liver oil	2	2	2	2	2.0
Corn oil			2	2	
Wesson oil					3.0
Salt mixture	4	4	6	6	4.0

[5] Lowry, J. V., Daft, F. S., Sebrell, W. H., Ashburn, L. L., and Lillie, R. D., *Pub. Health Rep.*, 1941, **56**, 2216.

[6] Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V., *Pub. Health Rep.*, 1942, **57**, 502.

[7] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1915, **20**, 351.

[8] Mulford, D. J., and Griffith, W. H., *J. Nutrition*, 1942, **23**, 91.

[9] Fletcher, J. P., Best, C. H., and Solandt, O. M., *Biochem. J.*, 1935, **29**, 2278.

ferences in the results obtained in the three laboratories, it seemed advisable to study the effect of variations in the dietary levels of a number of ingredients. In the experiments, the results of which we are reporting at this time, we have incorporated varying amounts of methionine or cystine in the diet, replacing an equal amount of cornstarch, and have kept the other dietary constituents at constant levels. A supplement of thiamine, riboflavin, pyridoxine, pantothenic acid and nicotinic acid was given daily to each rat and with some of the diets we have further studied the effect of the inclusion of choline in the vitamin supplement. All of the rats received approximately 20% alcohol as a source of fluid. Similar results have been obtained in other experiments using water in place of alcohol.

A summary of the results of these studies is given in Table II. The figures are based on the results of histological examinations.

It is of interest that neither a high fat diet nor one containing free cystine is *essential* for the production of liver cirrhosis. Diet No. 542 contains 5% fat and 4% casein with no added amino acids and on this diet some rats developed cirrhosis of the liver.

Some rats on this same diet developed hemorrhage and necrosis of the liver. This necrosis appears to be identical with that seen by György and Golblatt.[1] It is primarily coagulative in type and centrolobular in location and is nearly always accompanied or

TABLE II.
Relation of Diet to Occurrence of Liver Hemorrhage and Necrosis and Cirrhosis.

Diet	No. of livers examined	No. with no cirrhosis and no hemorrhage and necrosis	Total No. with cirrhosis	Total No. with hemorrhage and necrosis	No. with hemorrhage and necrosis and cirrhosis
Basal diet (No. 542)*	16	10	3	6	3
0.04% added cystine	17	4	9	10	6
0.08% " "	9	0	9	2	2
0.16% " "	12	1	11	0	0
0.5% " " (Diet No. 545)	94	9	85	0	0
0.05% added methionine	27	14	5	11	3
0.2% " "	4	4	0	0	0
0.7% " "	10†	10	0	0	0
Choline chloride (20 mg per rat per day) given in supplement.					
0.04% added cystine	10	4	0	6	0
0.5% " "	15	12	2‡	1§	0

*Differs from diet No. 545 in that the 0.5% cystine is replaced by cornstarch.

†Including 8 rats which received both 0.7% methionine and 0.5% cystine.

‡One rat dying after 201 days on experiment and another dying after 245 days on experiment had slight liver cirrhosis.

§This rat died after 296 days on experiment.

even replaced by hemorrhage of quite variable extent in the necrotic areas. Sometimes only small periportal islets of surviving liver cells remain. When this picture and the hepatic cirrhosis described by us[6] occur together they appear to be essentially unrelated to each other, the hemorrhage and necrosis being superimposed on almost any phase in the development of the cirrhosis. Rarely is there any evidence of marginal organization about the hemorrhagic necrosis, and usually the hemorrhage is so fresh that no hemosiderin is demonstrable.

The inclusion of choline chloride in the vitamin supplement at a level of 20 mg per rat per day had a preventive effect on the development of the liver cirrhosis. None of the rats receiving diet No. 545 survived longer than 113 days unless choline was given. Among the rats on this diet receiving the 20 mg of choline chloride per rat per day there have been survivals up to one year; 2 of the 15 livers examined have shown slight cirrhosis after 201 and 245 days, respectively. These results do not exclude the possibility that a higher level of choline administration would completely prevent this liver cirrhosis, but neither do they exclude the possibility that some other factor is involved. Methionine, a choline precursor, had a preventive effect if given in sufficient quantity. Cystine, on the other hand, even in small amounts definitely increased the incidence of hepatic cirrhosis. A supplement of cystine in a choline-deficient diet is known to increase the deposition of liver fat in rats[10] while choline[11] and methionine[12] are known to exert a lipotropic effect; that these effects may have had an influence on the results of our experiments is indicated by our observation[6] that a fatty liver is one stage in the development of this type of hepatic cirrhosis.

While exerting a preventive effect on the development of this cirrhosis, choline had no such influence on the hemorrhage and necrosis. Six of 10 rats receiving choline and a diet containing 0.04% of added cystine showed hemorrhage and necrosis of the liver. Either methionine or cystine in sufficient amounts, however, has consistently prevented these lesions. The only exception encountered to date is one rat receiving 0.5% cystine which died after 296 days on experiment. The significance of this single exception cannot be stated at the present time. The prevention of these lesions by the sulfur-containing amino acids indicates that there may be an etiologic relationship between the hemorrhages in the livers of these

10 Beeston, A. W., and Channon, H. J., *Biochem. J.*, 1936, **30**, 280.

11 Best, C. H., Hershey, J. M., and Huntsman, M. E., *J. Physiol.*, 1932, **75**, 56.

12 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

rats and the liver hemorrhage noted by Weichselbaum in rats with cystine deficiency[13] even though we have not observed the clinical symptoms which he described.

The observations recorded here do not indicate whether or not it is possible to vary the incidence of the hemorrhage and the incidence of the necrosis independently of each other.

The conclusions which seem to be justified by these experimental results are that *under these experimental conditions* (1) choline (and methionine, a choline precursor) have a preventive action on the development of this hepatic cirrhosis and (2) the sulfur-containing amino acids, cystine and methionine, have a preventive action on the development of the hepatic hemorrhage and necrosis. This suggests that the cirrhosis and the hemorrhage and necrosis are separate and distinct entities.

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Histochemical Studies of Phosphatase Distribution in Developing Teeth of Albino Rat.*

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The presence of phosphatase in teeth was first demonstrated by Robison and Soames[1] and confirmed by other studies made on extracts of the dental tissues.[2-7] It has been shown grossly that enzyme

[13] Weichselbaum, T. D., *Quart. J. Exp. Physiol.*, 1935, **25**, 363.

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[1] Robison, R., and Soames, K., *Biochem. J.*, 1924, **18**, 740.

[2] Mackenzie, A. S., *Brit. Dent. J.*, 1933, **54**, 153.

[3] Macfarlane, M. G., Patterson, L. M. B., and Robison, R., *Biochem. J.*, 1934, **28**, 720.

[4] Provissionato, A., *La Stomatologia*, 1935, **33**, 619.

[5] Berliner, F. S., *J. Dent. Res.*, 1936, **15**, 243.

[6] Shafer, Z. M., *Northwestern U. Bull. of Dent. Res., Grad. Study Quart.*, 1937, **37**, 26.

[7] Roche, J., and Bullinger, E., *Comptes Rendu Soc. de Biol.*, 1938, **129**, 976.