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Lipotropic Effect of Liver Extract on Dietary Hepatic Injury in Rats.*

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Many different supplements have been studied for their effect in preventing liver injury produced by dietary means. The possible effect of liver extract, however, has received little attention. Rhoads and Miller[1] observed that liver extract only partially restored to normal the abnormal liver function produced by a black-tongue diet in dogs. György and Goldblatt[2] studied the effectiveness of a liver concentrate in preventing dietary cirrhosis in rats and concluded that the "liver extract was completely ineffective in preventing hepatic injury". Gillman and Gillman[3] reported that liver extract had some lipotropic effect on the fatty livers of infants with pellagra. In view of the lack of experimental data on the effect of liver extract on experimental dietary liver disease, it was felt that further studies should be made.

Methods. Male rats of the Sprague Dawley strain, weighing between 150 and 170 g were used. All animals received food *ad libitum*. Five animals of each group were housed in individual cages, and their daily food intake was measured. The diet selected was one recently used by Himsworth and Glynn[4] which is high in fat but not low in protein and which produced fatty changes followed by fibrosis. The diet consisted of 16% casein, 51% lard, 30% cornstarch, and 3% salt mixture. Vitamins A and D were supplied by adding 5 drops of oleum percomorphum§ per kilo of diet to the melted lard as the diet was prepared. Each rat received a daily oral supply of the B vitamins as follows: thiamin, 20 μg; riboflavin, 25 μg; pyridoxine, 20 μg; calcium pantothenate, 100 μg. One group of 13 rats received the basic diet alone. The second group of 10 rats received a supplement of 1 cc of Lilly's Crude Liver Extract‡ (1 unit per cc) 3 times a week subcutaneously. A third group of 10 animals received 40 mg of choline chloride daily given subcutaneously in a 4% solution.

After 12 to 13 weeks of the study, a liver biopsy was performed on the 5 animals in each group in the individual cages. The biopsy was carried out by laparotomy under ether anesthesia, and tissue was taken from the edge of the left lobe. At 22 weeks a second biopsy was performed on the same animals in a similar manner. At 29 weeks all surviving animals were killed, and sections were taken for histological examination from the left lobe and the caudate lobe of the liver. The system of grading histological changes is listed with Table I. All liver sections were stained with hematoxylin and eosin. In addition, for half of the animals in each group, frozen sections were made and stained with Sudan III. The remaining liver was analyzed for total lipids by a modification of the

* This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service, and by a grant from Eli Lilly and Company.

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1 Rhoads, C. P., and Miller, D. K., *J. Exp. Med.*, 1938, **67**, 463.

2 György, P., and Goldblatt, H., *J. Exp. Med.*, 1942, **75**, 355.

3 Gillman, T., and Gillman, J., *Arch. Int. Med.*, 1945, **76**, 63.

4 Himsworth, H. P., and Glynn, L. E., *Clin. Science*, 1944, **5**, 93.

§ The authors wish to thank Dr. K. K. Chen of Eli Lilly and Company for the Liver Extract and Dr. C. E. Bills of Mead Johnson and Company for the Oleum Percomorphum. Each cubic centimeter of liver extract contained: Thiamin, 2.0-4.5 μg; Riboflavin, 45.0-60.0 μg; Pantothenic acid, 45.0-60.0 μg; Nicotinic acid, 100.0-140.0 μg; Folic acid, 2.0-3.8 μg; Methionine, 3.0 μg; and Choline, 1.0-1.6 μg. Total solids, 16-18%.

TABLE I.
Histological Changes in Liver at Biopsy and Necropsy.

Basic diet					Liver extract					Choline				
		Fat					Fat					Fat		
Rat No.	Wt change	L.L.	C.L.	Fibrosis	Rat No.	Wt change	L.L.	C.L.	Fibrosis	Rat No.	Wt change	L.L.	C.L.	Fibrosis
						Biopsy at 12-13 weeks.								
1	+66	4+		0	14	+ 66	0		0	24	+175	0		0
2	+56	4+		0	15	+ 99	0		0	25	+126	0		0
3	+55	3+		1+	16	+ 90	0		0	26	+110	0		0
4	+74	3+		0	17	+100	0		0	27	+ 71	0		0
5	+32	3+		0	18	+124	0		0	28	+112	0		0
						Biopsy at 22 weeks.								
1	+80	4+		1+	14	+ 81	0		0	24	+179	0		0
2	+58	4+		1+	15	+107	0		0	25	+159	0		0
3	+60	4+		2+	16	+106	0		0	26	+116	0		0
4	+67	3+		0	17	+111	0		0	27	+ 71	0		0
5	+23	3+		0	18	+127	0		0	28	+112	0		0
						Necropsy at 29 weeks and earlier deaths.								
1	+58	4+	4+	2+	14	+ 48	0	0	0	24	+ 42	0	0	0
2	+28	3+	3+	2+	15	+ 65	0	0	0	25	+ 50	0	0	0
3	—16	4+	4+	2+	16	+ 52	0	0	0	26	+ 52	0	0	0
4	+30	4+	4+	2+	17	+ 50	0	+	0	27	+ 65	0	0	0
5	+ 3	4+	4+	0	18	+ 68	0	+	0	28	+ 35	0	0	0
6	+56	3+	3+	1+	19	+ 57	0	0	0	29	+ 59	0	0	0
7*	—32	4+	4+	2+	20	+ 82	0	+	0	30	+ 88	0	0	0
8*	+22	4+	4+	2+	21	+ 86	0	0	0	31	+108	0	0	0
9*	+ 3	4+	4+	1+	22	+ 98	0	+	0	32*	+ 86	0	0	0
10*	+26	4+	4+	1+	23	+ 28	0	0	0	33*	+ 52	0	+	0
11*	+40	4+	4+	0										
12*	+14	4+	4+	2+										
13*	+10	4+	4+	2+										

*Comments.

No. 7 died at 148 days of pneumonia.
No. 8 " " 167 " from anesthesia.
No. 9 " " 67 "—no infection found.
No. 10 " " 70 "—" " "
No. 11 " " 72 "—" " "
No. 12 died at 165 days of pericarditis.
No. 13 " " 170 " " "
No. 32 " " 175 " " pneumonia.
No. 33 " " 186 " " "

Grading of Fatty Change.

0—None or only a rare large droplet.
+—A few large droplets in each lobule.
++—More than +, but less than half of lobule involved.
+++—Over half of lobule involved but some cells fat free.
++++—Almost no fat free cells.
L.L. = Left lobe of liver; C.L. = Caudate lobe of liver.

Grading of Fibrosis.

0—None.
+—Thin strands of fibrous tissue scattered throughout.
++—Strands of fibrous tissue dividing liver into small sections.

method of Outhouse and Forbes.[5]

Results. The untreated animals fed the high-fat diet showed a marked increase in liver fat histologically, whereas treatment with liver extract or choline prevented the fatty changes in the liver (Table I). As indicated in Table I several animals died prior to the 29th week, generally from liver injury associated with infection.

In the untreated animals the change observed at the first biopsy was a marked infiltration with large fat droplets which almost completely filled the hepatic cells, pushing the nucleus and remaining cytoplasm into a narrow rim. The end result seen in the autopsy sections was a trabeculation of the entire liver with strands of fibrous tissue dividing the liver into sections smaller than the normal lobule. Large, isolated, fat-free hepatic cells with heavily staining cytoplasm and one or two large, dark staining nuclei were occasionally seen enmeshed in the fibrous strands. The progressive nature of the fibrosis in the untreated animals may be observed in the data in Table I. The fibrosis was never present unless there was also a marked fatty change. In following those animals with marked fatty change and fibrosis through biopsies to autopsy it was observed that as the fibrosis increased there was a slight decrease in the amount of fat in the cells.

The animals treated with liver extract or choline did not show any fatty change in the left lobe of the liver at the first or second biopsy or at autopsy. However, in a few of the animals in these groups additional examination of the caudate lobe at necropsy showed a slight (1 +) fatty change (Table I). However, this is a normal variation between lobes of the liver as other animals fed a normal diet of 16% casein, 6% fat, 75% corn starch, and 3% salt mixture showed a similar variation between left and caudate lobes in some cases.

The results of the lipid determinations are shown in Table II. The untreated animals showed a definite increase in lipid concentration. Treatment with liver extract or choline prevented the increase in liver lipids, and the resulting concentration of lipids in these animals are in the same range as rats fed a normal low-fat diet (16% casein-6% fat). Lipid determinations were not performed on the animals that died early in the experiment or who showed postmortem changes.

TABLE II.
Total Liver Lipids in % of Wet Weight.

Basic Diet		Liver Extract		Choline	
Rat No.	% Fat	Rat No.	% Fat	Rat No.	% Fat
1	27.3	14	8.5	24	8.9
2	25.8	15	10.4	25	6.2
3	18.2	16	7.5	26	7.6
4	27.1	17	6.4	27	7.4
5	20.1	18	7.1	28	8.3
6	14.3	20	11.9	30	8.3
8	20.4	21	9.2	31	10.2
12	15.1	22	11.2	32	5.4
13	15.9	23	7.4	33	3.9
Mean	20.50		8.84		7.36
S.E.	±1.72		±.65		±.64

Discussion. Previous investigators have produced similar hepatic lesions of fatty change and fibrosis in rats by dietary means.[4,6] Others have produced a dietary injury consisting of fatty change, fibrosis, and necrosis[2,4,7,8] Necrosis was not observed in any of the animals in the present study. All of the diets that have produced necrosis differ from that used here in several aspects. An important difference that is usually associated with the occurrence of necrosis is the use of diets which are low in protein, generally ranging from 6% to 10% protein. In the present study the diet contained 16% protein.

The supplement of choline prevented both the infiltration of the liver with fat and the fibrosis which followed the fatty change in untreated animals. This lipotropic effect of choline is well known, but a lipotropic effect of liver extract as obtained in the present study, has not been reported previously.

György and Goldblatt[2] studied the effect of daily doses of 0.2 g of a liver concentrate

5 Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1940, **25**, 1157.

6 Blumberg, H., and Grady, H. G., *Arch. Path.*, 1942, **34**, 1035.

7 Handler, P., and Dubin, I. N., *J. Nutrition*, 1946, **31**, 141.

8 Webster, G. T., *J. Clin. Invest.*, 1942, **21**, 385.

TABLE III.
Average Daily Intakes of Food, Protein, and Calories.

Treatment	Food g/rat	Protein g/rat	Protein G per 100 g rat	Calories per rat	Calories per 100 g rat
Basic diet	7.2	1.15	0.56	46.3	22.4
Liver extr.	8.0	1.28	0.54	51.6	21.6
Choline	9.1	1.45	0.55	58.5	22.1

in preventing the hepatic injury of fatty change, cirrhosis and necrosis produced in rats by a low-protein, high-fat diet. They concluded that the liver extract failed to prevent the induced liver injury. Either the differences in the types of injury produced, or in the liver concentrate used by György and Goldblatt may be responsible for the lack of effect of their concentrate as compared with the beneficial effect of liver extract in the present study. The animals in the present study received on the basis of total solids the equivalent of 0.51 g of powdered liver No. 343 per rat per week as compared to the 1.4 g of concentrate used by György and Goldblatt.

The effect of the liver extract does not appear to be due to an increase in protein intake resulting from stimulation of the appetite by the liver. The protein intake per rat was only slightly higher in the liver-treated group as compared to the untreated group, and the protein intake per 100 g of rat weight was the same in both groups (Table III). A group of animals not included in this report were given the basic diet with an additional 8% casein. This resulted in an average protein intake per rat of 2.12 g per day, and a protein intake of 0.79 g per 100 g of rat weight per day. Even with this protein intake, which is considerably above that of the liver extract and choline treated animals, all of these animals showed a 1+ or 2+ infiltration with fat.

The choline content of the liver extract is not responsible for its effectiveness. The liver extract used contained 1.0-1.6 mg of choline per cubic centimeter, and therefore each rat received only 0.56 mg of choline a day. Other data in the literature show that larger amounts of choline are necessary to prevent dietary lesions in the liver. The lowest effective dose used was that of Daft, Sebrell and Lillie[9] who found that a dietary supplement of 20 mg of choline protected against the lesion produced by a 4% protein-5% fat diet. Himsworth and Glynn[4] found that the fat content of the liver was normal at 34 days, if 4 mg of choline daily was used as a supplement to a diet containing 8% protein and 50% fat; but abnormal amounts of fat were present at 66 days in spite of continued choline administration. Furthermore, other supplements which will be reported in detail later, and which failed to prevent the fatty change, had a higher content of choline than the liver extract. It is possible that the liver extract contains an unknown lipotropic substance.

Summary. 1. Rats fed a 16% casein, 51% fat diet for 29 weeks all developed markedly fatty livers. Nearly all of the animals showed a diffuse, progressive hepatic fibrosis.

2. The fatty change and fibrosis were completely prevented by supplementing the high-fat diet with choline, or with crude liver extract.

3. The lipotropic effect of the liver extract is not due to an increase in protein intake resulting from a stimulation of appetite, and is not due to the small amount of choline present in the liver extract.

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