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Chemistry of the Liver Cytoplasm of Normal, Fasted and Cirrhotic Mice.

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An impairment of the mechanism for the mobilization of lipide material from the liver has been reported to be present in both starvation[1] and cirrhosis.[2] MacLachlan[1] has observed that the liver of fasted albino male mice accumulates neutral fat in such amounts, that in the first 2 days, the quantity of total lipide increased 2 to 3 fold. As the starvation is continued, the amount of total lipide decreases even below that of the normal, and the phospholipides show a downward trend during this entire period of starvation. Wright[2] observed that the alcoholic cirrhotic liver is pale, enlarged, and most of the liver cells are distended with fat. This lipide may result from the inadequate diet observed in many cirrhotics. The liver cell has a cytoplasm which appears to be quite labile,[3] so that an investigation of the chemical changes occurring in this portion of the cell, during cirrhosis and fasting, might give some indication as to the integral processes involved.

Three experimental groups of animals were studied: 1. Normal, fully fed animals. 2. Animals which were fasted for 24 hours, and 3. Cirrhotic animals which were fasted for 24 hours.

Materials and methods. Male mice, 5 to 7 months of age, of the C_3H strain were utilized in this experiment. Hepatic cirrhosis was produced by feeding 0.1 ml of 40% carbon tetrachloride in olive oil to mice every 4 days for a period of 200 days.[4,5] Cirrhosis was confirmed by histologic examination.†

Livers, of mice anaesthetized with ether, were removed after clamping the portal vein and cutting the hepatic vein. The blood that was present in the liver was thus allowed to drain.

The cytoplasmic extract was prepared essentially according to the method of Claude.[6] Livers from 10 mice were pooled for each group, ground in a mortar, suspended in 4 volumes of alkaline saline (2 ml 0.1 N NaOH per liter of physiologic saline), then spun at 1400 × g in the Sorvall angle centrifuge. The decanted supernatant fluid was considered to contain a major aliquot of the total cytoplasm. Lipides that came to the surface in the various preparations were resuspended in the extract before the analyses were performed. It was assumed that all of the lipide that rose to the surface belonged to the cytoplasm, since Rosenfeld[3] observed that an increase of lipide during various pathologic conditions, *e.g.* phosphorus poisoning, was purely cytoplasmic in nature.

Separate aliquots were removed from the cytoplasmic extract for the determination of total nitrogen,[7] phosphorus,[8] "ribonucleic acid," and lipide. Total solids were obtained by heating an aliquot of the cytoplasmic extract in a tared weighing bottle over a boiling water bath until the fluid from the extract disappeared. The bottles were then placed in an oven at 105°C for one hour.

The "ribonucleic acid" was determined essentially by Brown's modification[9] of the

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† The authors are indebted to Dr. R. A. Huseby for the pathologic examination.

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TABLE I.
Chemistry of the Liver Cytoplasm of Normal, Fasted, and Fasted-Cirrhotic Mice.*

Experiment†	Mg per g wet weight liver					
	Total solids	N	P	"RNA"	N/P	N/"RNA"
Fully fed	206	22.2	2.54	11.2	8.75	1.98
	235	29.1	2.68	12.1	10.9	2.41
Fasted	294	24.1	2.47	11.3	9.78	2.13
	377	30.5	3.09	14.2	9.88	2.15
Fasted Cirrhotic	213	23.4	2.28	7.26	10.2	3.22
	241	27.0	2.46	8.18	10.9	3.31

* It was assumed that nearly all of the cells were ruptured[13] and the concentration of the chemical constituents, *e.g.*, N, P, "RNA", total lipide, and phospholipide in the cytoplasmic extract, was the same as their concentration in the original ground tissue. With this assumption it is possible to consider the chemistry on a wet weight basis and thus avoid the discrepancy that may arise in dry weight due to the large amounts of lipide material present.

† Each figure results from the analysis of one pool of 10 animals.

TABLE II.
Lipide Chemistry of the Liver Cytoplasm of Normal, Fasted, and Fasted-Cirrhotic Mice.

Experiment†	Mg per g wet weight liver			
	Total lipide	Phospholipide	N/P (atomic)	Iodine value
Fully fed	43.4	22.0	1.10	125
	46.5	22.2	1.24	129
Fasted	140	23.9	1.41	92.5
	177	25.2	1.48	95.5
Fasted Cirrhotic	67.5	16.1	1.50	90.2
	81.5	17.4	1.64	91.8

* Each figure results from the analysis of one pool of 10 animals.

orcinol-HCl method in which the pentose values are corrected for the presence of hexose. The "ribonucleic acid" was calculated by multiplying the total tetranucleotide ribose by the factor 2.14.

Lipide was extracted with Bloor's solution (alcohol:ether; 3:1). Bloor's solution was re-extracted with petroleum ether, care was taken to wash the lipide extract with water to remove any of the non-protein nitrogen contaminants.[10] A weight of lipide was obtained by evaporating the petroleum ether under reduced pressure from tared 10 ml volumetric flasks. The iodine value was determined by the method of Kretchmer *et al.*[11]

Experimental results. Table I shows the actual amount of nitrogen, phosphorus, and "ribonucleic acid" present in the liver cytoplasm obtained from one gram wet weight of liver. There are no differences between the various liver cytoplasms as concerns nitrogen, but the phosphorus appears to have decreased in the fasted-cirrhotic liver which is probably in part due to the decrease observed in the "ribonucleic acid" in these livers. This decrease in "ribonucleic acid" is obviously not due to the fast but to the cirrhosis since there is no decrease in "ribonucleic acid" in the fasted livers.

Total lipide (Table II) increases approximately 3 to 4 fold after a 24 hour fast, but this excess lipide seems to be entirely non-phospholipide in character since the phospholipide remains constant. The lipide phosphorus to nitrogen ratio indicates that the phospholipide present in the cytoplasm has been altered in that a lipide of a higher nitro-

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[11] Kretchmer, N., Holman, R. T., and Burr, G. O., *Arch. Biochem.*, 1946, **10**, 101.

[13] Barnum, C. P., and Huseby, R. A., *Arch. Biochem.*, 1948, **19**, 17.

gen content has entered the lipide extract. Cirrhosis affects the lipide content so that it increases above normal but not as high as the normal fasted liver cytoplasm. The phospholipide in the cirrhotic liver decreases, but here also there seems to be a lipide present with a high nitrogen content.

The iodine value of both the normal fasted and the cirrhotic-fasted liver cytoplasms decreased below that of normal. These results are in accord with those of Winter[12] in that he found that after feeding carbon tetrachloride to rats the iodine value of the liver fatty acids decreased from 111 to 100.

Summary and discussion. Chemical changes in the cytoplasm of the mouse liver cell after a short fast and at cirrhosis are reported. These changes occur most dramatically in the lipide fraction during a 24 hour fast resulting in an increase of 3 to 4 fold above normal. This lipide is non-phospholipide in character. Fasted cirrhotic liver differs from the fasted normal liver cytoplasm in that the lipide does not increase so markedly and the phospholipide decreases to about 60% of the normal fasted value. The iodine values of the normal-fasted and the cirrhotic-fasted liver cytoplasm fatty acids decrease below that of the normal values.

In addition to a lipide increase in the fasted-cirrhotic liver cytoplasm there is also a decrease of "ribonucleic acid" and phospholipide in one gram wet weight of the ground fasted cirrhotic liver.

It is possible that there is an interchange of phospholipide during a fast as evidenced by the lipide nitrogen to phosphorus ratio. This interchange would consist of substituting normal phospholipide with a lipide or phospholipide which contains a great deal of nitrogen.

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Electrical Alternation in Experimental Coronary Artery Occlusion.

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The purpose of this preliminary note is to report a high incidence of electrical alternation in experimental coronary artery occlusion.

Methods. In open-chested nembutalized dogs,[1] the left anterior descending coronary artery was occluded for periods of 30 seconds to 32 minutes. Electrocardiographic tracings were made with the exploring electrodes in the cavity of the left ventricle and on the epicardial surface or precordium. Thirty-three experiments were performed on 11 dogs.

Results. Electrical alternation occurred in 8 of 9 dogs (89%) which developed electrical signs of myocardial ischemia[2] following coronary artery occlusion. One dog died of ventricular fibrillation, and one dog failed to develop any electrical signs of ischemia following occlusion for 32 minutes. Electrical alternation occurred about 2 minutes after occlusion, was variable in duration (several seconds to 20 minutes), and disappeared either spontaneously or within 3 seconds to 5 minutes after release of the constriction. Alternation of the ST-T and QRS complexes, alone or together, was observed, the most common being of the ST segment (Fig. 1). There was alternation in the extent of ST elevation in the epicardial leads, and of ST segment elevation or depres-

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2 Bayley, R. H., and LaDue, J. S., *Am. Heart J.*, 1944, **28**, 54.