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Relation of Fat and Protein Intake to Fatty Changes, Fibrosis and Necrosis of the Liver.*

CHARLES A. HALL† AND VICTOR A. DRILL.‡

From the Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.

It is still undetermined whether the lesions seen in the livers of animals on a low protein diet or a high fat diet are all part of the same process, or are the results of two or more distinct processes. Several investigators have produced hepatic lesions of fatty change, necrosis, and fibrosis by dietary means.[1-6] Others observed the fatty change and fibrosis but no necrosis.[7-9] Daft and coworkers[4] suggested that these hepatic changes which had been considered as all having the same pathogenesis should be divided into two processes, one resulting in a cirrhosis and the other a necrosis. Himsworth and Glynn[10] reported that diets high in fat or low in lipotropic substances resulted in a fatty change followed by a diffuse fibrosis of the liver, while diets low in protein but containing lipotropic substances resulted in a massive hepatic necrosis with post necrosis scarring in animals that survived. The necrosis could be prevented by adding methionine to the diet.[11] It was also reported that necrosis could be produced by feeding diets in which the protein was supplied by an amino acid mixture omitting methionine and cystine.[12] Again methionine and also cystine prevented the necrosis. This has led to the development of the theory that dietary liver disease in rats can be divided into one type consisting of fatty change and fibrosis which is produced by a diet high in fat or carbohydrate and deficient in lipotropic substances, and a second type of damage consisting of necrosis and scarring which results from a deficiency of sulfur containing amino acids. The present study was designed to further observe the effect of a high-fat diet, and a low-protein diet, on the type of liver injury produced.

TABLE I.
Experimental Diets.

	Control diet, %	Diet I, %	Diet II, %	Diet III, %
Casein	16	16	6	4
Lard	6	51	6	6
Cornstarch	60	30	70	72
Sucrose	15	—	15	15
Salt mixture	3	3	3	3
	100	100	100	100

Methods. The animals used were male, Sprague-Dawley rats. The composition of the diets fed is listed in Table I. Each rat also

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† Post-war Fellow in Pharmacology, 1947-48, Rockefeller Foundation.

‡ Present address: Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Mich.

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TABLE II.
Microscopic Pathology in Livers of Animals on Diet I at Both Biopsies and at Autopsy.

Control group				Diet I		
Rat No.	Fat	Fibrosis		Rat No.	Fat	Fibrosis
1	0	0	Biopsy at 85-90 days	6	4+	0
2	0	0		7	4+	1+
3	0	0		8	4+	0
4	0	0		9	4+	0
5	0	0		10	3+	0
			Biopsy at 155-165 days	6	4+	1+
				7	4+	2+
				8	4+	1+
				9	4+	1+
				10	3+	0
1*	0	0	Autopsy at 190-200 days and	6	4+	2+
2*	0	0	animals dying earlier	7	4+	2+
3*	0	0		8	4+	2+
4	2+	0		9	4+	2+
5*	0	0		10	4+	0

* Animals dying before 190 days.

Grading of Fat

1+ Few droplets in every lobule.
2+ <half of every lobule infiltrated.
3+ >half of every lobule infiltrated.
4+ Almost all of every lobule infiltrated.

Grading of Fibrosis

1+ Strands of fibrous tissue scattered throughout section.
2+ Tissue divided into pseudolobules by fibrous bands.

received a daily oral supply of thiamin, 20 γ; riboflavin, 25 γ; pyridoxine, 20 γ; and calcium pantothenate, 100 γ. Vitamins A and D were supplied by adding 5 drops of Oleum Percomorphum§ per kilogram of diet to the melted lard as the diet was prepared.

Diet 1. The rats weighed from 140 to 170 g and one group of 13 animals were fed Diet 1, and a second group of 10 rats received the control diet (Table I). Water and food was allowed *ad libitum,* and daily food intake was measured in 5 rats of each group who were housed in individual cages. At 85-90 days liver biopsies were performed on all of the animals in the individual cages by laparotomy under ether anesthesia. The biopsy was taken from the edge of the left lobe of the liver. This procedure was repeated on the same animals in the same manner at 155-165 days. After 190-200 days on the diets the animals were sacrificed and specimens of tissue were taken from the center of the left and right lobes of the liver and from the kidney. The remaining liver of all animals was analyzed for total lipids by a modification of the method of Outhouse and Forbes.[13] All of the tissue sections were stained with hematoxylin and eosin and in addition sections stained with Sudan III were prepared from half of the animals in each group. In a few cases Masson, reticulin, acid fast stain and Best-carmine stain were also utilized.

Diet 2. Animals fed this diet weighed from 130 to 160 g. Ten rats received diet 2 and ten animals were fed the control diet (Table 1). Food was allowed *ad libitum* and daily measurement of food intake was made on five rats of each group. No biopsies were taken on these animals. Six rats in each group were sacrificed after 150 days and the remainder on the 200th day of the experiment. Tissue sections were made as outlined under Diet 1. Lipid analyses were made on half of the animals in each group.

Diet 3. The rats used weighed between 110 and 150 g. Ten animals were fed diet 3 and 10 received the control diet. All of the animals were housed in individual cages and each animal received *only* 8 g of food per day. The exact amount of food consumed by each animal was measured daily. Five

§ The authors wish to thank Dr. C. E. Bills of Mead, Johnson and Company for the Oleum Percomorphum.

[13] Outhouse, E. L., and Forbes J. C., *J. Lab. and Clin. Med.,* 1939, **25**, 1157.

TABLE III.
Microscopic Pathology of All Animals at Autopsy.

Group	Total animals	Duration of exp. (days)	No. of rats	No. died	No. with 1-2 + fat	No. with 3-4 + fat	No. with fibrosis	Total lipids mean + S.E.
Control diet	10	200		8	1	0	0	—
Diet I	13	200		7	0	13	11	20.50% ± 1.72
Control diet	10	150	6	1	2	0	0	8.98% ± 1.81
		200	4	1	0	1	0	9.40% ± 1.74
Diet II	10	150	6	1	0	6	3	28.19% ± 3.41
		200	4	1	0	4	3	23.14% ± 2.94
Control diet	10	60	5	0	1	0	0	—
		75	5	0	0	0	0	—
Diet III	10	60	5	0	0	4	0	—
		75	5	0	0	4	0	—

of the animals in each group were sacrificed at 60 days and the remainder on the 75th day of the study. A single piece of tissue was taken from the left lobe of the liver for microscopic examination and all sections were stained with hematoxylin-eosin.

Results. Diet 1. The animals fed Diet 1 all showed marked fatty changes in the liver on biopsy at 85-90 days (Table II). The fat was present as large droplets which almost filled the cells, pushing the nucleus and remaining cytoplasm into a narrow rim. Fibrosis had also appeared in one animal at this time.

At autopsy of the animals fed Diet 1, almost all of the livers were enlarged and all had a yellow-brown uniform color. In many cases parts of the liver, especially the lower edge of the left and median lobes, were scarred and nodular. Microscopically the end result was a trabeculation of the entire liver by strands of fibrous tissue, dividing the liver into small pseudolobules. These strands connected central veins, portal areas, and the capsule into an interlocking network. At this stage of the lesion there was less fat histologically than seen in the biopsies, and small fat droplets were more common whereas previously large fat droplets had predominated. Some of the pseudolobules were almost fat free. The progressive nature of fibrosis was apparent when the course of each animal was followed by biopsies to autopsy.

Enmeshed in the fibrous strands were scattered, isolated, large, fat-free hepatic cells, many of which were binucleated, together with masses of a light-yellow staining substance which was found to be acid fast and assumed to be ceroid. The ceroid was rarely seen other than in the fibrous areas. The findings on all animals sacrificed at 200 days or dying before that time is summarized in Table III. Only one animal fed the control diet showed any fatty change and this was of a minor degree.

The animals fed Diet I gained weight only during the initial part of the experiment and soon reached a plateau below that of the control group (Fig. 1). During the last 4 weeks of the study respiratory infections became evident in many of the animals. Of

the 13 animals fed Diet 1, 7 died before the termination of the study on the 200th day. All had severe hepatic changes and 4 had pneumonia. Eight of the animals receiving the control diet also died of pneumonia during this period, but without hepatic pathology. The respiratory infections in the control group

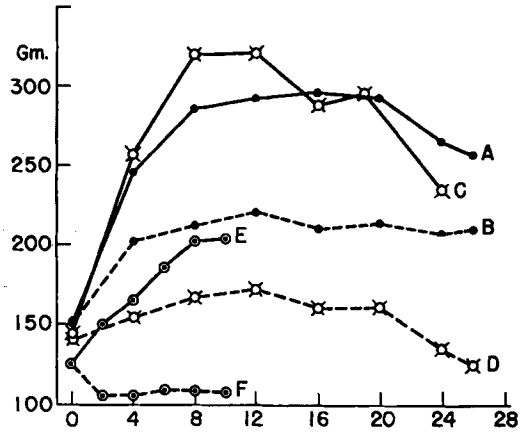

FIG. 1.

Gain in weight of rats on various diets. Curve A, control diet, and Curve B, Diet I. Curve C, control diet, and Curve D, Diet II. Curve E, control diet, and Curve F, Diet III.

is reflected in the loss of weight of this group during the last 4 weeks of observation (Fig. 1).

Diet 2. Animals fed the low protein diet (Diet 2) also showed marked fatty changes in the liver, similar to the findings in animals fed Diet 1 (Table III). Grossly the livers of the animals fed Diet 2 were enlarged and light yellow-brown in color. In some cases the edges of the left and median lobes were nodular and firm. Microscopically the type and distribution of both the fat and fibrosis in these animals were the same as those fed the 51% fat, 16% protein diet (Diet 1). No necrosis was observed either grossly or microscopically. In four of the animals the few fat-free cells that were present contained large amounts of glycogen, a finding that was not observed in animals fed the control diet. No important differences were observed histologically between the animals fed diet 2 for 150 or 200 days, except that the lesion at 200 days was slightly more advanced.

The animals fed the low protein diet (Diet 2) gained only a slight amount of weight (Fig. 1). Two of these rats died prior to sacrifice, at 125 and 164 days respectively, both with severe hepatic changes and one also had pneumonia. Two of the animals fed the control diet died from respiratory infections during this time.

Diet 3. Animals fed this diet, which contained only 4% casein, were limited to 8 g of food per day. At autopsy, either at 60 or 75 days, the rats showed hepatic changes similar to those observed with diets 1 and 2 (Table III). Grossly the livers appeared fatty, and nodularity or necrosis was not observed. Microscopically the fatty infiltration was characterized by large fat droplets, as previously described with Diet 1. There was no fibrosis or necrosis. Large deposits of glycogen, present in those cells not laden with fat, was observed in animals fed Diet 3, and not in animals fed the control diet.

The animals fed Diet 3, limited to 8 g a day, lost weight (Fig. 1). Rats receiving the control diet, also limited to 8 g of food per day, showed a moderate gain in weight.

Discussion. Although the 3 diets used were quite different the hepatic lesions produced by them appeared to be the same. Almost all of the livers were heavily infiltrated with fat. With Diet 1, a high fat diet, and Diet 2, a low fat-low protein diet, the total lipid determinations show that the actual amount of fat in the liver was the same for both diets (Table 3). Fibrosis was not seen unless fat was also present, and the fibrosis always followed the fatty change. The fibrosis was progressive, and not the type of scarring seen following an acute injury. Minor infiltrations with fat did not result in fibrosis, in agreement with other recent studies.[14] The fibrosis was not seen in the group on the 4% casein, restricted intake diet (Diet 3), as was to be expected, since all animals were killed by the 75th day, whereas animals on the other diets did not show fibrosis until after 75 days. It then appears that the fibrosis is a result of prolonged extensive hepatic fatty change which was produced by feeding a diet high in fat

[14] Glynn, L. E., Himsworth, H. P., and Lindan, O., *Brit. J. Exp. Path.*, 1948, **29**, 1.

TABLE IV.
Comparison of the Liver Injury and Protein Intake with Data of Himsworth and Glynn.

	No. of rats	Duration, days	No. with fibrosis	No. with necrosis	Food intake g/rat/day	Protein g/rat/day	Protein g/100 g/rat/day
			Diet II (6% protein, 6% fat).				
Present study	10	150-200	6	0	10.2	.614	.391
Himsworth	7	—	0	3	7.64	.458	.393
			Diet III (4% protein, 6% fat) and restricted food intake.				
Present study	10	60-75	0	0	7.6	.304	.283
Himsworth	7	—	0	4	7.18	.288	.258

or by feeding a diet low in protein.

All 3 groups of animals on the control diet contained at least one animal with some increase in liver fat. In all cases the amount of fat was small and fibrosis was absent. The slight fatty change in the control animals was associated with the presence of respiratory disease.

It is difficult to compare the findings in the present study with previous ones because of the great variation in diets and techniques. However, since the methods used here were very similar to those of Himsworth and Glynn[10] a comparison can be made for each of the diets used. In both studies the 51% fat, 16% protein diet (Diet 1) produced the same fatty changes. In the present study the appearance of fibrosis was more rapid. In both cases low protein diets (Diet 2) also resulted in fatty change but Himsworth and Glynn did not find this to be followed by fibrosis as in the present study. Some of the animals fed 6% and 4% protein diets by Himsworth and Glynn developed acute hepatic necrosis, with post necrotic scarring in the surviving animals. This lesion was not observed in our animals fed the same diets (Table IV).

Himsworth and Glynn concluded from the data collected on several low protein diets that a protein intake between 200 and 500 mg per rat per day resulted in the massive necrosis. Since our 6% protein group (Diet 2) received 614 mg the failure to develop necrosis may be explained on the basis of the higher protein intake (Table IV). The protein intake, when calculated as protein per 100 g of rat weight per day, is the same in both studies. This explanation cannot hold for the failure of the 4% protein group (Diet 3) to develop necrosis, since the animals were limited to 8 g of food per day as was done by Himsworth and Glynn, and the protein intake falls well within the critical range (Table IV). Himsworth and Glynn also found that after 40 days on such a diet many of the animals became ill and died with hepatic necrosis surviving only an average of 64 days when a 4% protein diet was fed. With a similar protein intake (Diet 3) our animals did not die during the study and failed to show hepatic necrosis, either grossly or microscopically. The average calorie and protein intake for the animals on Diets 1 to 3 is summarized in Table V.

There were some minor differences in the diets used in the two studies although the percentage of constituents was the same.

TABLE V.
Average Daily Food, Calorie and Protein Intake.

Group	Food, g per rat	Calories per rat	Calories per 100 g rat	Protein, g per rat	Protein, g per 100 g rat
Control diet	13.1	54.7	20.3	2.12	.778
Diet I	7.2	46.3	22.4	1.15	.555
Control diet	14.9	62.3	23.5	2.37	.898
Diet II	10.2	42.8	27.2	0.61	.391
Control diet	7.9	33.0	19.2	1.26	.736
Diet III	7.6	31.8	29.6	0.30	.283

Himsworth and Glynn used lard in the high fat diets and arachis oil in the low fat diets, and used cod liver oil as a source of fat soluble vitamins. In the present study lard was used in all diets and the vitamins were supplied by oleum percomorphum.

Summary. 1. Groups of rats were fed diets of 16% protein and 51% fat; 6% protein and 6% fat; and 4% protein and 6% fat. These diets resulted in a fatty infiltration of the liver, and in the long term experiments this was accompanied by a diffuse, progressive, hepatic fibrosis.

2. The hepatic lesions produced by all 3 of these diets appeared to be the same type and probably had the same basic pathogenesis.

3. Hepatic necrosis was not produced by the diets used, and deaths from acute hepatic necrosis were not obtained with a low protein intake.

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Susceptibility of the Guinea Pig to Action of Alloxan as Compared with the Rat.*

FRIXOS C. CHARALAMPOUS AND D. MARK HEGSTED.
(Introduced by Fredrick J. Stare.)

From the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School, Boston.

The susceptibility to the diabetogenic as well as the general toxic action of alloxan has been studied in several species[1-9] including the human being.[10] Goldner[5] reported lesions in the islets of the guinea pig after alloxan administration but no diabetes was produced because the animals died within 24 hours. Other workers[11,12] observed changes in the blood sugar levels after alloxan injections in this species but West and Highet[13] were unable to produce alloxan diabetes in the guinea pig.

The work presented in this paper is in agreement with that of West and Highet and compares the susceptibility of the guinea pig and the rat to the action of alloxan.

Experimental. Twenty-five male guinea pigs weighing between 500 and 600 g each and kept in individual cages were divided into 5 groups. Group I consisted of 6 guinea pigs which were injected with alloxan monohydrate intravenously at various doses as shown in Table I; Group II of 7 animals partially depancreatised prior to alloxan administration. One month after the operation the animals were injected with 200 mg per kg body weight alloxan intravenously and

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