

Fatty Cirrhosis in the Rat. VII. Influence of Different Levels of Dietary Fat on Early Deposition of Fat in Liver.* (30062)

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Previous studies from this laboratory described the fatty cirrhosis produced in rats fed for 15 weeks one of 3 hypolipotropic diets containing 5%, 20% or 38% lard(1). Each diet furnished the same amount of protein (casein and alpha soya protein). The diet containing 38% lard produced the greatest incidence and the most severe degree of cirrhosis. Liver disease was least severe in the diet containing 5% lard. In that paper, the statement was made "It is probable that the fat accumulation within the first two or three weeks was most severe in those animals that subsequently exhibited the most severe degree of cirrhosis at the end of the experiment." In the present study data are presented in support of the statement.

Methods and material. Male rats of the Holtzman strain individually caged in a constantly illuminated air-conditioned room were employed. The rats, age 35 days, weight 100 ± 10 g, were fed Purina fox chow for 5 days after their arrival at the laboratory and then started on one of the 3 varieties of a choline-deficient diet. These diets designated CA6-38, CA6-20 and CA6-5, respectively, are described in Table I. Sixteen experimental and 4 control rats were included in each of the 3 groups fed one of the above diets. The control rats were fed diets containing a supplement of 0.4% choline chloride. Since the diets were not isocaloric, the daily ration available to the 3 groups was 8, 9 or 10 g as indicated in Table I. The animals were not pair-fed; food consumption was not measured. As shown in Table II, the animals were killed after 2, 7, 14, 21 or 28 days. That portion of the study designated as Experiment A (Table II) was carried out in January and Experiment B, in June.

Specimens of liver tissue were fixed in neutral buffered formalin for demonstration of fat by the Oil Red O stain and in alcoholic

TABLE I. Composition of Diets.

Ingredients	CA6-38	CA6-20	CA6-5
Casein (vitamin free)	60.00 g	60.00 g	60.00 g
Alpha protein	60.00	60.00	60.00
Sugar	483.75	718.75	1005.75
Lard	379.50	225.00	62.50
L-cystine	6.25	6.25	6.25
Vitamin powder*	10.00	10.00	10.00
ADT† (in oil)	.50	.50	.50
Salts	40.00	40.00	40.00
Total	1000.00	1125.00	1250.00
Daily ration	8	9	10
Cal/rat/day	46.5	43.3	40.8

* Vitamin powder contains 0.3125 g thiamine, 0.5 g riboflavin, 0.3125 g pyridoxine, 1.25 g calcium pantothenate, 1.25 g nicotinic acid, 0.3125 g menadione (U.S.P.) and powdered sugar to make 1 kilo. The daily ration furnishes each rat with 25 γ of thiamine, pyridoxine and vita K, 40 γ of riboflavin and 100 γ of calcium pantothenate and nicotinic acid.

† Stock solution of ADT contains 8 g vit A (200,000 units per gram), 1 g vit D (50,000 units per gram), 24 g α -tocopherol and 23 g peanut oil. The daily ration furnishes the rat with 100 units of vit A, 25 units of vit D and 2 mg of α -tocopherol.

Bouin's fluid for hematoxylin-eosin staining. The liver was weighed before and after removal of sections for histologic study; the remaining portion was stored in a deep freeze chest for a later determination of total lipid and other fat studies. Total lipid of the liver was estimated by the method of Folch(2). The cholesterol, phospholipid and triglyceride fractions were determined on pooled samples of the extracted liver lipid. Liver pathology was graded on the basis of the gross appearance and the microscopic examination according to a system previously described(3).

Results and comments. The control rats were killed after 7 and 14 days. Their liver lipids were normal or very slightly elevated (Table II). The rats that received the 38% lard diet uniformly had a greater deposition of neutral fat (triglycerides) in their livers at each time interval. The speed with which the young growing rat develops fatty liver is evident by the elevated values for the liver lipid noticeable at 2 days and reaching a

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TABLE II. Early Fat Deposition in Liver of Rats Fed CA6 diet Containing 5, 20 or 38% Lard.

Exp	Dietary fat level	Days on diet	No. of rats	Body wt		Liver/b wt (%)	Liver lipid (%)	mg/g fresh wt of liver		
				Start	End			Cholesterol	Phospholipid	Triglycerides
B	5% Lard	2	2	106	110	5.3	9.4	3.5	13.5	77
	20% "	2	2	110	114	5.0	13.1	3.5	11.5	115
	38% "	2	2	114	110	4.8	15.7	3.8	13.3	140
B	5% "	7	2	119	110	4.5	12.1	5.0	13.3	103
	20% "	7	2	134	117	5.6	26.4	8.7	13.2	243
	38% "	7	2	130	117	5.5	29.7	10.4	13.4	273
B	5% "	14	2	119	125	8.0	26.2	7.5	8.6	246
	20% "	14	2	116	127	8.0	29.1	7.9	8.7	275
	38% "	14	2	122	141	7.8	36.3	8.3	8.3	346
B	5% "	21	2	127	145	8.5	31.2	9.4	10.3	292
	20% "	21	2	122	143	7.7	33.7	9.4	7.8	320
	38% "	21	2	133	160	8.0	41.6	9.9	8.3	398
A	5% "	21	4	99	155	6.5	27.2	10.3	9.5	252
	20% "	21	4	97	110	6.0	31.5	13.5	11.7	330
	38% "	21	4	100	122	7.8	41.5	11.6	9.5	393
A	5% "	28	4	95	120	8.7	24.5	8.1	6.8	230
	20% "	28	4	100	122	7.3	38.5	12.7	10.8	362
	38% "	28	4	98	130	8.2	42.5	13.5	10.6	399
Control animals (diet supplemented with .4% choline chloride)										
A	5% Lard	7	2	105	122	3.2	6.1	4.5	16.4	40
	20% "	7	2	120	123	3.8	6.8	6.3	14.8	37
	38% "	7	2	128	120	3.8	6.9	4.6	11.7	53
A	5% "	14	2	116	154	4.1	7.4	1.4	10.0	30
	20% "	14	2	118	158	4.5	7.5	1.3	8.1	37
	38% "	14	2	114	150	6.8	8.2	1.7	8.2	58

maximum at 21 to 28 days. The initial manifestation of a choline-deficient diet in the rat is the centrolobular accumulation of fat. In the present experiment this was evident at 2 days and prominent at 7 days. The accumulation of centrolobular fat involving approximately half of the conventional liver lobule has been designated as Stage I according to the grading system employed. This quickly gives way to a situation in which the entire liver lobule is fatty; most of the livers at 14, and especially 21 and 28 days, were classified as Stage II in which virtually every liver cell contains fat droplets, these being largest in the cells nearest the centrolobular vein.

Our previous study(1) indicated that rats receiving the 38% lard diet for 15 weeks exhibited the most advanced degree of pathology, *viz.*, hypertrophic or atrophic nodular cirrhosis with thick fibrous trabeculae. Rats fed the 20% lard diet for a similar period showed less severe damage, *i.e.*, early cirrhosis with thin trabeculae. Those receiving the 5% lard diet exhibited lesser changes; one-fourth failed to develop cirrhosis.

Hartroft(4) stated "The rate of accumulation rather than the mere presence of abnormal liver fat would seem to be the essential factor in initiating hepatic fibrosis." The results of the present study confirm this contention. The diet that produces the greatest accumulation of fat in the liver in the first four weeks results in the most severe degree of hepatic pathology after 3 or 4 months.

The 3 diets employed in this and the previous experiment are regarded as capable of producing 3 degrees of choline-deficiencies: severe, moderate and mild, corresponding to the 3 levels of fat (38%, 20% and 5% lard) in the diets. Each of the 3 diets is capable of producing fatty liver in rats but the severity of the pathological changes that follow vary according to the amount of fat deposited early in the course of the experiment.

The primary exclusive role of fatty infiltration in the pathogenesis of experimental dietary cirrhosis is questioned by some(5). On the basis of our observations we believe that excessive fat accumulation is the primary cause of the development of cirrhosis

in the choline-deficient rat.

Summary. Liver fat accumulation was studied in groups of young rats fed one of 3 low protein diets containing, respectively, 5%, 20% and 38% lard. Each diet furnished the same quantity of protein (casein and soy- α -protein). The rapidity and magnitude of fat accumulation was greatest in rats fed the 38% lard diet and least in those receiving the one containing 5% lard. In previous studies the same diets were fed for 15 weeks; the 38% lard diet produced the greatest incidence and the most severe degree of cirrhosis, whereas the group fed the 5% lard diet had the least severe degree of liver dam-

age. The severity of the fatty cirrhosis that ultimately develops in choline deficient rats is chiefly determined by rate and magnitude of fat accumulation in the liver in the first 3 weeks of the experiment.

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Further Purification of TSH-Releasing Factor (TRF) from Sheep Hypothalamic Tissues, with Observations on the Amino Acid Composition.* (30063)

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We have reported previously(1) on the existence in crude extracts of hypothalamic tissues, of a polypeptidic substance (TRF) which specifically stimulates *in vivo* the secretion of adeno-hypophysial thyrotropin (TSH). We have subsequently described the early stages of purification of TRF(2,3) as well as its *in vitro* activity(4) on a system of isolated pituitary fragments. Evidence has been presented(1,2,4) to propose that TRF is a substance different from LRF (LH-Releasing Factor), CRF (Corticotropin-Releasing Factor), α - and β -Melanocyte Stimulating Hormones, bradykinin, and Von Euler's Substance P, all polypeptides known to be present in hypothalamic tissues. This paper reports the further purification of hypothalamic TRF.

Experimental procedures and results. a. *Starting materials, acetic acid extraction, gel filtration on Sephadex G25, bioassay.* Table I summarizes description of starting materials and of early stages of the purification

procedure as described earlier(1,2). The 25 kg of hypothalamus without median eminence were lyophilized in industrial freeze-drying equipment prior to extraction with acetone (100 vol/1 g material). Two sizes of Sephadex G25 columns were used: 5.5 $\times$ 150 cm to which were applied 7.0 g of lyophilized acetic acid extract of the hypothalamic tissues; or 15 $\times$ 150 cm on which were applied 120-140 g of the same extract (Fig. 1). The bioassay for TRF used throughout this study is the *in vivo* method described earlier(5). Extraction and studies of biological activity through the Sephadex G25 filtration stage were conducted on aliquots of the 2 types of starting material used here, *i.e.*, median eminence-stalk fragments and hypothalamus fragments without median eminence (Table I). Because of the similarity of the results obtained (see *Discussion*), the bulk of the 2 types of material was pooled and processed further as in Table I.

b. *Counter current distribution.* The conditions as in(3) are summarized in Fig. 2,3; yields are reported in Table I.

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