Development of Portal Fatty Liver in Rats on Corn Diets; Response to Lipotropic Agents.* (20860)

MAURICE E. SHILS AND W. B. STEWART.

From the School of Public Health, and Department of Pathology, College of Physicians and Surgeons, Columbia University.

During the course of a study designed to assess the effects on rats of diets related to those consumed by children in whom Kwashiorkor develops, we have noted the consistent occurrence of fatty liver. The lipid is predominantly in the portal area of the lobule in contrast to the centrally located fat usually observed in rats on diets low in choline and methionine(1-3). Clinicians familiar with Kwashiorkor in various parts of the world agree that the fat accumulates first in the portal areas of the liver lobule and disappears last from them following proper dietary treatment(4-7). It would appear obvious that the development of the analog of this human disease in experimental animals requires the production of initial lipid accumulation in the portal areas of the liver.

Methods and procedures. Black rats derived originally from a mixed stock† were used. Two main groups with different nutritional histories were studied. One group termed "first generation" was the offspring of stock breeding females maintained on Rockland "D-free" pellets. The majority was fed the stock pellets for one week after weaning (21 days) and then placed on the experimental diets. In one experiment the rats were placed on the experimental diet at weaning. The second group, termed "second generation" was obtained from mothers maintained on the experimental diets (supplemented in the majority of instances by weekly subcutaneous injections of 3 μg of vit. B_{12}) for at least one month before mating. These second generation rats were, in turn, placed on the experimental diets at weaning. They were used in an effort to obtain more severe deficiencies with more obvious biochemical and pathological changes than might occur in the first generation rats. Diet 19 had the following composition: whole "water-ground" bolted white corn meal 76,‡ casein§ 3, Crisco 15, 5% cod liver oil in corn oil 2, salts (Hubbell, Mendel, and Wakeman) 1, vitaminized glucose 3 which contributed (in mg): thiamine-HCl 0.5, riboflavin 1, niacin 5, pyridoxine-HCl 1, calcium pantothenate 2.5, folic acid 0.02, and biotin 0.01. Diet 23 had a similar composition except that the 15 parts of Crisco were replaced isocalorically by 33.9 parts of glucose. Diet 39 had the following composition: casein§ 9, glucose 67, salts 4, 5% cod liver oil in corn oil 2, Crisco 15, vitaminized cerelose (identical with that for diet 19) 3. Animals were killed by exsanguination following intraperitoneal pentobarbital (Nembutal) anesthesia. Lipids were measured in the entire liver except for small portions of the left and median lobes which were taken for histological study. Chemical analysis involved gravimetric determination of the fatty acids and unsaponifiable matter. The sections of liver for histological study were placed in 10% formalin within a few minutes after death; paraffin sections were stained with hematoxylin-eosin, and frozen sections were stained with Sudan IV for fat and with hematoxylin as a counterstain.

Results. The weight increase on diet 19 averaged 1 to 3 g per week for the first 2 months. Female rats, during the first month on these diets, grew as well as, or better than the males; thereafter most of the males slowly outgrew the females. Frequent graying of

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† Obtained about 4 years ago from Dr. G. Emerson of the Merck Institute and since bred by one of us (MES).

‡ "Indian Head," Wilkins-Rogers Milling Co., Washington, D.C., purchased locally.

§ General Biochemicals, Inc., plain, untreated.

TABLE I. Microscopic Distribution of Lipid in Livers of Second Generation Rats on Corn Diet 19. (No. of rats and location of lipid in lobule.)

Diet	Sex	Total No.	Portal		Central		Diffuse†	Little or none
			Definite	Tendency*	Definite	Tendency*		
19	♂	23	13	1	1	0	8	0
	♀	17	8	2	1	1	4	1
19 + B_{12} (3 μg weekly)	♂	15	8	0	0	0	2	5
	♀	14	5	0	0	0	0	9
19 + 0.5% methionine	♂	9	7	0	0	0	0	2
Totals		78	41	3	2	1	14	17

* Tendency = fat occurred throughout lobule but was most prominent in region indicated.
† Diffuse = fat occurring throughout lobule without obvious localization.

hair was noted. Symmetrical alopecia of varying duration often occurred, and, when hair growth resumed, the new hair was gray or even white and of a fine soft texture; this new hair gradually became pigmented(8).

Gross and microscopic appearance of liver. Primary attention has been devoted to second generation rats on diet 19 in whom portal fat occurs quite consistently (Table I). Grossly, many of these livers had a "mottled" appearance with the lace-like yellow or light tan of the fatty liver surrounding tiny red or brown dots or streaks. This pattern was seen particularly consistently in the rats treated with 0.5% DL-methionine or vit. B_{12} and in which only a partial lipotropic response had been obtained (*vide infra*). Upon microscopic examination sudanophilic material was found predominantly in the portal areas of the lobules (Fig. 1). In general, the cells contained many small or medium sized fat droplets. In paraffin sections stained with hematoxylin and eosin the fat-containing cells had a "foamy" type of cytoplasm—a type of vacuolization different from that caused by glycogen. As the fat content became greater, large droplets of sudanophilic material were seen displacing the nucleus well to the side of the cell. When the lipid content of the liver rose above 10 to 15% as determined by chemical analysis, the fat was found to extend throughout the lobule. Administration of

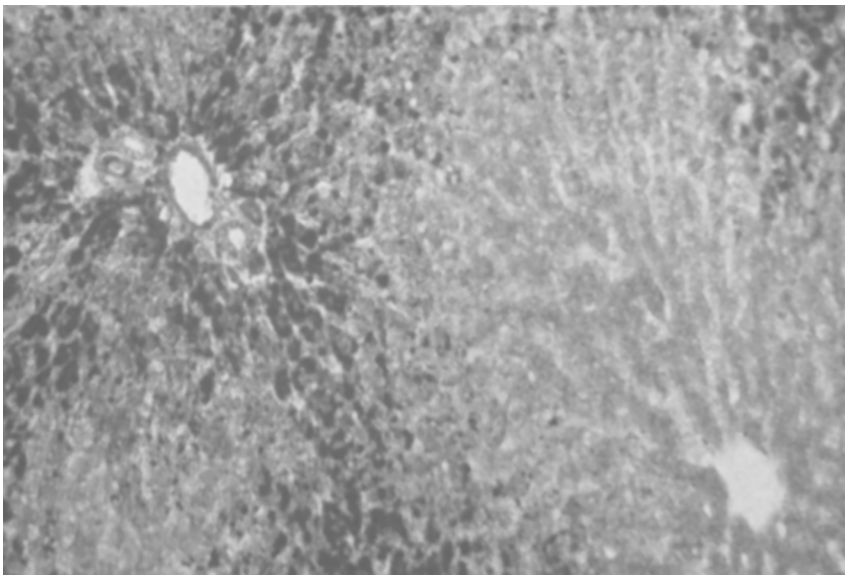

FIG. 1. Section of liver of a second generation male rat subsisting on corn diet 19 for 65 days. At upper left is a portal area, at lower right a central area. Sudanophilic material (black) is limited to portal portion of the lobule. Sudan IV and hematoxylin (× 300).

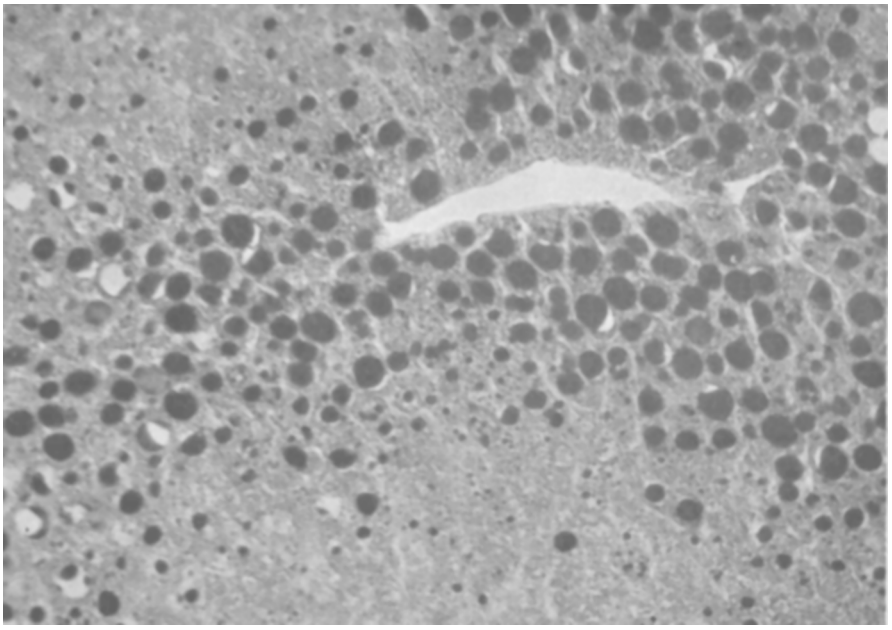

FIG. 2. Section of liver of a second generation male rat subsisting on diet 39 containing 9% casein. At upper right is a central vein. Large black sudanophilic globules cluster about this vein. Portal areas of this lobule were free of fat. Sudan IV, hematoxylin (× 300).

lipotropic substances which did not remove all of the fat resulted in a sharp portal localization of fat in these second generation rats.

It was possible that the portal accumulation of fat was a peculiarity of the strain of rat used in these experiments. To check this, rats of both sexes were placed on diet 39 in which the only source of protein was casein and in which choline was lacking. First and second generation[1] littermate rats were fed either diet 19 or a related diet containing 2% more salts and only 1.5% casein. These animals were carried for 11-60 days on the diets. DL-methionine (0.5%) or choline chloride (0.25%) were incorporated in the diets of some of these rats for 3 to 5 days before sacrifice in an attempt to localize the liver lipid more sharply. The livers of 31 rats on diet 39 have been examined. Twenty-four had lipid either localized in the central areas or with the largest lipid droplets occurring centrally (Fig. 2). The remaining 7 rats had fat distributed throughout the lobule with no discernible localization. The rats on the high corn diets had the fat in the periphery in every instance except where the lobule was so loaded with fat that no localization was discernible. Thus it appears that initial or preponderant accumulation of lipid in the portal areas is related to the presence of corn in the diet.

Lipid concentrations in the liver and the effects of lipotropic agents. Chemical analyses of the livers revealed amounts of lipid which corresponded well with the microscopic picture. The percentages found varied within the rather wide ranges which many investigators have noted in experimental fatty livers on a variety of diets.

Diets consumed in areas where Kwashiorkor exists tend to be low in fats. Consequently, it was thought desirable to study the effect on liver lipid accumulation of low versus moderate amounts of fat in corn diets. Such a study using first generation rats on the high corn diets (diet 19 with 17% by weight of fat versus diet 23 with 1.7% fat) revealed no appreciable difference, either in the fat content of the liver, or in rate of growth (Table II, exp. 1.) A further experiment extending

[1] Second generation rats placed on diet 39 were rats from mothers on the corn diet.

TABLE II. Comparison of Liver Lipids and Growth in Rats of the First Generation on Diet 19 and Effectiveness of Lipotropic Agents.*

Exp.†	Supplement	Days on Diet	Days on Supplement	% liver lipids Range	% liver lipids Avg‡	Avg wt gain (g)
1 ♂	—	92–115	—	9.6–22.9	13.8 (7)	149 (7)
	§	"	—	10.2–18.2	12.4 (5)	151 (5)
2 ♂	—	92–115	—	9.6–24.3	15.3 (12)	—
	Meth.‖	"	27– 37	3.5– 7.7	5.4 (6)	19: 39 (9)†† 19 + meth.: 39 (9)††
	B_{12}¶	"	92–115	2.7– 4.1	3.4 (8)	19: 148 (8)‡‡ 19 + B_{12}: 150 (8)‡‡
	Casein**	"	92–115	3.2– 3.6	3.5 (4)	19: 135 (6)‡‡ 19 + casein: 355 (6)‡‡
3 ♂ + ♀	—	65	—	—	—	117 (6)
	Meth.‖	65	65	—	—	104 (6)
4 ♂	—	255	—	20.9–24.1	22.5 (7)	—
	Meth.‖	"	31	11.3–13.9	13.1 (4)	—

* Within each experiment litter mates distributed among diet groups to compensate for litter differences in liver fat accumulation and wt gain.

† Exp. 1 and 2 started at 28 days of age; Exp. 3 at 21 days; Exp. 4 at 45 days.

‡ ( )—No. of rats.

§ 15 parts of Crisco were replaced isocalorically by 33.9 parts of glucose (diet 23).

‖ 0.5% DL-methionine in diet.

¶ 3 μg vit. B_{12} inj. intraper. once weekly.

** 15 g of GBI casein added to each 100 g of diet 19.

†† Wt gain for the last 27-37 days of exp. Rats with methionine compared with littermates on diet 19 only.

‡‡ Wt gain over period of 90 days. Rats with supplement are compared to littermates on diet 19 only.

the time on these diets to 191 days confirmed this finding.

The effect of lipotropic substances in the prevention and cure of fatty liver was considered of interest in view of the occurrence of portal fat. The findings with first generation rats are given in Table II. It is seen from the data (exp. 2) that vit. B_{12} and casein were very effective in preventing fat from accumulating in the liver. DL-methionine (0.5%) added 27-37 days before the end of the experiment reduced the amount of fat; however, in 5 of the 6 rats the concentration was still above normal. When 0.5% methionine was added to the diet of rats during the last month of an 8½-month period on diet 19 (exp. 4), an appreciable reduction in lipid occurred, but considerable fat still remained. Preliminary evidence suggests that choline chloride (0.25%) was as active as vit. B_{12}.

It is apparent that diet 19 was deficient in choline and substances that yield or allow formation of labile methyl groups for choline synthesis. Methionine, and vit. B_{12} were not limiting factors for growth in this diet (Table II, exp. 2 and 3) whereas casein caused a marked increase of growth and prevented excessive accumulation of fat in the liver (Table II, exp. 2).

Methionine, choline and vit. B_{12} were also effective in reducing liver fat in second generation rats. However, here again, one-half percent methionine was rarely completely effective in preventing accumulation of fat (Table III). This level of methionine did not improve growth. Increasing the level to 1.0% did not appreciably improve the lipotropic action and depressed growth in some of the animals. In the small number of animals thus far tested, choline effectively prevented fat accumulation.

Vit. B_{12} injected into second generation rats was erratic in its lipotropic activity. In some rats it was completely effective in preventing fatty livers; in others considerable fat accumulated. In all instances, however, administration of this vitamin resulted in lower lipid levels than in the untreated rats. An example

TABLE III. Effect of Methionine and Choline on Liver Fat in Second Generation Rats.*

Supplement	Exp. 1 None	Exp. 1 .5% methionine	Exp. 1 .25% choline Cl	Exp. 2 .5% methionine	Exp. 2 1.0% methionine
No. animals	6†	11‡	5§	7♂	9♂
Littermate wt gain (g)	104	102	—	86	69
Avg % lipid (wet wt)	15.3	6.2	3.4	7.2	5.5
Range	8.4–24.4	4.0–9.1	3.2–3.8	3.0–11.2	3.7–8.4

* Diet 19 begun at 21 days and continued for 65 days. † 4♂, 2♀. ‡ 8♂, 3♀. § 3♂, 2♀.

of the results obtained is given in Table IV for littermates weaned by mothers subsisting on diet 19. Similar results have been obtained with young from females on diet 19 supplemented with vit. B_{12} with or without additional salt mixture. In this generation, as in the first, B_{12} did not improve growth despite its lipotropic action and despite the fact that these second generation rats came from mothers who had not received the vitamin for several months or longer. In 11 out of 12 female littermate pairs, the animal receiving B_{12} grew less than its sister not receiving this vitamin. In male littermates there did not appear to be any consistent trend.

Discussion. While methionine was not completely effective in preventing some accumulation of fat, it had significant lipotropic action in our diet 19. Similarly, Dean(9) noted that unspecified amounts of methionine prevented fatty liver in weanling rats fed *matoke*.¶ Our "mottled" livers resemble some of the fatty livers described by Gillman *et al.* (10) in rats on a diet of cooked corn meal and sour milk. Some of their rats also had portal fat. Corn cannot be the unique etiological factor in the development of the portal fatty liver of Kwashiorkor because the liver changes occur even under conditions where corn is not an important dietary component. However, corn appears to have a characteristic in common with some other plant materials that predisposes to the syndrome under certain nutritional conditions. Our experiments emphasize the fact that there is some factor (or factors) either present or absent in corn which results in portal rather than central fat. It is hoped that the ability to develop the portal type of fatty liver in the rat may help shed light on some of the nutritional factors operating in Kwashiorkor.

TABLE IV. Effect of Vit. B_{12} on Hepatic Lipid and on Body Weight Gain in Second Generation Rats from Mothers on Diet 19. (Young started at 21 days, killed after 65 days on diet.)

Litter No.	Sex	No supplement % lipid	No supplement Wt increase (g)	Vit. B_{12}* % lipid	Vit. B_{12}* Wt increase (g)
356	♀	14.0	78	9.2	68
	♀	19.7	89	—	—
399	♀	11.1	94	3.4	81
	♂	5.6	125	5.0	136
403	♀	16.8	103	11.6	99
416	♀	21.2	120	13.0	121
	♂	10.1	170	6.4	146
417	♀	5.6	128	3.0	79
	♀	8.3	104	3.4	96
	♀	—	—	4.7	94
	♀	—	—	11.3	79

* 3 μg vit. B_{12} inj. intraper. once weekly.

Summary. Rats subsisting on a diet containing 76% corn and 3% or less casein, developed a fatty liver characterized by initial and preponderant accumulation of lipid in the portal areas. Rats of the same strain on a diet in which the only protein was casein, developed a centrolobular type of fatty liver. Methionine, choline, and vit. B_{12} were able to decrease the liver lipid. However, methionine was rarely capable of preventing the deposition of some excess fat while, under certain conditions, the lipotropic action of vit. B_{12} was unpredictable.

¶ Cooked banana, an important constituent of the diets of children in Uganda, among whom Kwasiorkor occurs.

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The Adequate Stimulus for Shivering.* (20861)

L. D. CARLSON. (With the assistance of Wade Sherwood and Robert Elsner.)
From the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle.

In man, acute exposure to cold is a stimulus for a heat conservation mechanism (vasoconstriction) and a heat production mechanism (increased metabolism). The response of the vasoconstrictor mechanism to decreasing environmental temperature has been repeatedly demonstrated by both skin temperature(1) and blood flow measurements(2). Although the adequate stimulus can be either local or general body cooling, it is usually local skin cooling, vasoconstriction occurring before deep body temperature changes. Previous exposure to cold, however, modifies this response(3-5). The mechanism inducing increased metabolism upon acute exposure has been the subject of controversy for almost a century. The argument is still much as Cannon *et al.*(6) stated it: whether there is a true chemical regulation as Rubner(7) defined it in addition to the metabolic response to increased muscular activity in the form of involuntary shivering. The work from Cannon's laboratory gave evidence that a cold stimulus causes increased metabolism when there is no muscular activity. This was ascribed to the release of adrenalin. Subsequent work has failed to confirm these results, whether the negative heat load was from the external environment(1) or from internal cooling(8). Cannon had hinted that acute rapid application of the negative heat load gives rise to early shivering which masks the chemical regulation, but the data of Hardy and DuBois(1) and Pinson and Adolph(8) do not bear out this suggestion. A steady state was approached in both series of experiments, in the former with a lowered skin temperature and a maintained rectal temperature, and in the latter with a lowered rectal temperature and less change in skin temperature. However, during long-term exposure of animals to cold, shivering seems to decrease while the increase in metabolism is maintained (9). Initiation of shivering has been attributed to a fall in internal body temperature (rectal)(10,11) or in skin temperature(12). Hensel's(13) recent account of the thermal sense organs gives a basis for predicting that shivering is the result of the rate (depending on steepness of thermal gradient) at which cold sensitive end organs are discharged, the number (depending on area and density of end organs) being stimulated and the thermal state of the temperature regulating center.

The following experiments were conducted to determine if a true chemical regulation could be demonstrated and if shivering is controlled according to Hensel's description.

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