TREATMENT OF EXPERIMENTAL DIETARY CIRRHOSIS OF THE LIVER IN RATS*

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PLATE 3

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Diffuse hepatic fibrosis is a regular occurrence in rats kept for a prolonged period (100 to 150 days) on a diet low in lipotropic factors (1). Cystine and, among the fats, lard, and especially cod liver oil, have an enhancing effect on the production of hepatic cirrhosis (2). Conversely, rations rich in lipotropic factors (choline and its precursors, such as methionine or, methionine-containing protein) free from cod liver oil and with a vegetable shortening replacing lard may consistently prevent the development of diffuse hepatic fibrosis in rats (2).

In general, dietary factors which assure prevention of a pathologic condition may not necessarily be beneficial in its treatment. Regarding cirrhosis, prevention is synonymous with the maintenance of normal architecture and function of the liver. In contrast, therapeutic factors have to exert their influence on a pathologically changed liver exhibiting various degrees of parenchymatous lesions (fat infiltration, necrosis, etc.) and of fibrosis. Furthermore, long standing experimental dietary cirrhosis is often associated in rats with secondary, but independent, pathologic changes involving parts of the endocrine apparatus, especially the gonads, and their secretion (3). In the presence of such pathologic changes it is by no means certain that the utilization of dietary factors in therapy could proceed in the same manner as in preventive experiments on normal animals.

There are only two reports in the literature, comprising about 40 rats, on the dietary therapy of experimental cirrhosis. From the observations recorded (4) it appears that lipotropic factors may accelerate regeneration of liver cells, reduce fat infiltration, and improve the histological appearance of the liver parenchyma. The dietary therapy, limited to the use of lipotropic factors (choline, casein), had, however, no recognizable effect on the

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basic, specific, underlying process of cirrhosis; *i.e.*, the diffuse fibrosis (4). This latter restriction is of special interest, not only because of its possible practical implications, but also with reference to the apparently well established fact that in toxic (chemical) cirrhosis of the liver, in rats, such as is seen after administration of carbon tetrachloride (5), or after para-dimethylaminoazobenzene (5 *b*, 6), the fibrosis undergoes a reversal when the administration of the hepatotoxic agent is discontinued. It has recently been demonstrated by Best and his associates (7) that the regression of hepatic fibrosis in rats previously exposed to carbon tetrachloride was due to dietary factors; *i.e.*, to the seemingly specific effect of lipotropic factors contained in the diet used.

In view of these discrepancies, as well as of the scarcity of available data in the literature, and of the possible importance of experimental findings of this type for the therapy of human cirrhosis, treatment by dietary means was carried out on 223 rats with dietary cirrhosis of the liver. One group of animals was included in this series in which dietary (methionine) and possible endocrine (reduction of thyroid activity by thiouracil) factors were used in combination.

Experimental Methods and Findings

The experiments comprised two periods: (*a*) period of production, and (*b*) period of treatment. The technique followed for the production of diffuse hepatic fibrosis has been previously described (2). This period lasted in general 150 days, and only rarely 120 days. The rations used were those with the designation C I and C III (2). Ration C I contained casein 8, crisco 38, cod liver oil 2; C III contained casein 8, crisco 30, peanut oil 8, but no cod liver oil. The rest of the ration, in both C I and C III, was made up by sucrose, 48 and 50 respectively, and salt mixture 4. The diets were supplemented daily throughout both periods (production and treatment) with 20 micrograms of thiamine, 25 micrograms of riboflavin, 20 micrograms of pyridoxine, and 100 micrograms of calcium pantothenate, dissolved together in 1 ml. of water. The fat-soluble vitamins, A and D, were given to rats fed the ration C III, which is free from cod liver oil, as percomorph oil (3 drops once a week), or as carotene and drisdol (2). Further supplements in the period of production were represented by cystine (50 mg. daily) and carrots (1 gm. daily).

In the second period the animals received treatment either in the form of supplements of methionine (50 mg. daily), cystine (50 mg. daily) *plus* choline (20 mg. daily), liver extract (0.2 gm. daily of a crude liver extract by mouth), thiouracil (0.1 per cent in the diet) to the original basal diet, or by the substitution of a diet (S I) rich in casein and low in fat (1 *b*) for the original basal diet. Treatment was extended, if possible, to 150 days (or even longer), and only rats which survived at least 75 days of this second period were regarded as having been sufficiently exposed to the influence of the dietary factors in question.

In the first group of 76 animals the results of treatment were assessed by comparing the histological changes in the liver found at necropsy, after termination of the treatment period, with those seen in a specimen of the same liver obtained by biopsy, at the end of the preparatory period. Laparotomy was done under ether anesthesia, and a small piece of the liver was removed from the anterior border of one of the main lobes. Although an attempt was made carefully to select for biopsy that part of a lobe which, in the gross, seemed to exhibit the most distinct cirrhotic changes, yet in many instances this result was not achieved. In several rats that died within the first 24 hours, or within a few days or weeks following biopsy, the histological examination of the entire liver at autopsy revealed a greater degree of fibrosis

than was found in the specimen obtained by biopsy. The estimation of cirrhotic changes in the biopsy specimen and at necropsy was carried out objectively by one of us without knowing to which animals the biopsy and necropsy sections corresponded. The common occurrence of discrepancies in histological findings in specimens of liver obtained by biopsy and necropsy, when the latter followed the former within a short period of time, has also been stressed in the past by Lowry, Ashburn, and Sebrell (4 *b*). It obviously makes a good comparison of findings before and after treatment very difficult. For instance, if no appreciable change takes place in the cirrhotic process under the influence of a therapeutic regime, as revealed by the comparison of findings at biopsy and necropsy, this does not necessarily indicate a failure of the therapeutic procedure applied. Even an apparent aggravation of the diffuse hepatic fibrosis from biopsy to necropsy is not a reliable sign of an uninhibited progression of the underlying pathologic process, but may be explained by the removal of an only mildly cirrhotic specimen for biopsy from an otherwise severely cirrhotic liver. On the other hand, a definite, general histological improvement found at necropsy, when compared with findings at biopsy, may be considered an unmistakable and reliable sign of a positive therapeutic effect.

In our experience, biopsy, when carried out in the presence of severe cirrhotic changes in the liver, entailed a high rate of mortality. Out of 76 animals of this group 37 animals died within 14 days, and an additional 9 animals within 60 days following biopsy. Among these 46 animals, biopsy and postmortem findings were roughly identical in 10 animals; the fibrotic changes were less pronounced in the specimens obtained by biopsy than at necropsy in 18 rats, whereas the reverse was observed in none of the animsls. Comparisons were not carried out in the remaining 18 animals that died immediately after biopsy. Table I contains a few examples for the illustration of comparisons between findings secured by biopsy and necropsy.

The therapeutic results in 28 rats that survived biopsy for longer than 75 days (24 animals in this group were killed 150 days after biopsy), are summarized in Table II. A few representative samples of improvement achieved through therapy are given in Table III. The beneficial effect of therapy was measured mainly by the extent of fibrosis present and by the peculiar redistribution of ceroid in the connective tissue.

The first and most common sign of beneficial change in the condition of cirrhotic liver under the influence of lipotropic factors, such as methionine and casein (Table III), was the reduction of fat infiltration, as judged from the histological picture. Reduction of fat content, however, was by no means regularly accompanied, or even followed, by other more specific manifestations of progressive repair. The extent of fibrosis may remain unchanged for the whole period of observation, from biopsy to necropsy, although the fat content usually becomes normal soon after the initiation of therapy. One change noted in the sections of liver from some of the treated animals, in which a considerable deposit of ceroid was present in the biopsy sections, was a peculiar agglomeration of the ceroid which will be referred to as *clumping*. Although the actual amount of ceroid may not be reduced, yet an apparent decrease occurs, because the globules of ceroid seem to wander from some portions of the bands of connective tissue to form dense clumps in other parts, usually close to the central veins, but occasionally close to a portal region (Figs. 1 and 2). Another indication of a favorable therapeutic effect, frequently observed, was the restoration of the parenchyma to normal. In sections of biopsy specimens, taken before treatment, there was usually a variable amount of degeneration of liver cells. Also, necrotic individual liver cells, or cords of liver cells, were often present enmeshed in the connective tissue and between the macrophages filled with ceroid. In the sections of biopsy material, accompanying the degenerative changes, there were also frequently signs of regeneration, indicated by variation in the size, shape, and staining qualities of the nuclei, some large hyperchromatic nuclei, and a considerable number of binucleate cells, as well as occasional normal mitoses. In the sections taken after the treatment was completed, these signs of regeneration as well as necrotic individual cells ("focal"

TABLE I

Discrepancies in Histological Findings in Specimens of Liver Obtained Either through Biopsy or through Necropsy, the Latter Following the First within a Short Period of Time (Days or Weeks)

Protocol No. of animal	Experimental day of		Fat infiltration	Ceroid	Cirrhosis	Diet	
	Biopsy	Necropsy				Before biopsy	After biopsy
21211	77		+	+	+	C I	
		79	++	+	+++		C I + methionine
21209	77		+++	±	±	C I	
		90	+	++++	++++		C I + methionine
20870	143		++	++	++	C I + carrot	
		144	++	++++	++++		C I + carrot
20920	150		++	+	+ to ++	C I + carrot	
		152	+++	+++ to ++++	+++		C I + carrot
21409	150		+++	++	++	C I + carrot + cystine	
		157	++	+++	++++		S I
20464	142		+++	+ to ++	+ to ++	C I + carrot + cystine	
		179	±	++++	++++		S I
20901	98		+	±	±	C III + carrot	
		106	++	±	++		C III + carrot + methionine
20948	122		+++	−	±	C III + carrot	
		128	+++	±	++		C III + carrot + methionine
21207	63		+++	+	+	C III + carrot + cystine	
		68	+++	+	+++		C III + carrot + methionine
20916	83		+++	−	−	C III + carrot + cystine	
		136	+++	±	+++		C III + carrot + methionine

necrosis) were usually absent (Table III), with the exception of binucleate cells which were frequently abundant. Reconstruction of architecture and seeming decrease of fibrosis have also been observed, but it is difficult to establish whether reduction in the amount of connective tissue is absolute, or only relative, due to attenuation caused by growth of the parenchyma.

TABLE II

Therapeutic Results in Rats in Which Biopsy Preceded Treatment

Diet		No. of animals	Result of treatment				Average weight		
Before biopsy	After biopsy		Definite improvement	Slight improvement	"Clumping" of ceroid	No improvement	At start	At biopsy	Final
C I	S I + methionine	4	3	1	4	0	158	153	191
"	S I	1	0	0	1(±)	1	223	158	197
"	C I + methionine	1	0	0	0	1	190	118	196
C I with carrot	S I + methionine	1	0	0	0	1	200	141	144
	C I + methionine with carrot	1	1	0	1	0	190	185	215
C I with carrot and cystine	S I + methionine	3	3	0	2	0	190	221	208
	S I	4	2	1	2	1	231	225	243
	C I with carrot and methionine	4	1	1	1	2	216	223	233
C III with carrot	S I + methionine	3	3	0	1*	0	198	168	165
	S I	1	0	0	0	1	155	115	198
C III with carrot and cystine	S I + methionine	4	4	0	4	0	144	147	216
	S I	1	0	0	0	1	185	225	368
C I or C III	S I + methionine	15	13	1	11*	1	171	167	192
	S I	7	2	1	3	4	212	200	249
	C I or C III with methionine	6	2	1	2	3	207	199	224

* Additional two rats were free from ceroid.

Definite improvement of the cirrhotic changes, with reduction of fibrosis and clumping of the ceroid, was a prominent feature in this group of animals which received as a therapeutic measure a combination of normal proteins and supplements of methionine (Tables II and III). In contrast, the therapeutic results achieved by the protein diet alone or by supplements of methionine added to the original alipotropic (low protein, high fat) diet were statistically significantly inferior to those observed with the combination of protein diet and methionine.

In 9 rats in the total series of 28 rats of the above group a moderate or severe degree of ascites was observed during the performance of the biopsy. In contrast, at autopsy, following treatment, no ascites was found in any of the surviving animals, regardless of the therapeutic procedure.

The high mortality from the biopsy procedure made it desirable to eliminate it in the experiments dealing with the therapy of diffuse hepatic fibrosis. In analogy to the remarkable regression of the cirrhotic process often seen in rats previously exposed to hepatotoxic agents (5–7), it was expected that similar far reaching repair of the hepatic parenchyma may become evident also in the case of dietary experimental cirrhosis as soon as the cirrhosis-producing dietary factors are eliminated and effective therapeutic measures, perhaps in the form of adequate diet, are instituted. Thus, in one group of rats, after 150 days of a preparatory period, the treatment of experimental dietary hepatic cirrhosis was instituted without simultaneous controls, and without previous biopsy, simply by replacing the cirrhosis-producing diet for all rats of the group by special rations containing lipotropic factors (casein, methionine, choline). This treatment was continued for 150 days. Two rats receiving the basal diet (C I) supplemented with choline *plus* cystine were observed for 200 and 240 days respectively.

TABLE III

Selected Examples for Comparison of Findings at Biopsy before Treatment and at Necropsy after Treatment

Rat No.	Treatment	Biopsy				Necropsy			
		Fat infiltration	"Focal" necrosis*	Ceroid	Cirrhosis	Fat infiltration	"Focal" necrosis	Ceroid	Cirrhosis
21444	S I + methionine	+++	±	+	++	−	−	±(clumping)	±
21424	S I + methionine	++		++ to ++++	++ to ++++	−		+(clumping)	+
21435	S I + methionine	+++		+	+	−		+(clumping)	−
21436	S I + methionine	++++		++++	++++	−		+ to ++	+ to ++
21416	S I + methionine	+++		+	+	−		+(clumping)	±
20980	S I	+++	−	++ to ++++	++ to ++++	−	−	+(clumping)	+ to ++
21421	S I	+++	±	++	++	− to +	−	± to ++	± to ++
20900	S I	+++		±	+	−		−	+
20918	Methionine	+++		+++	++	−		−	−
20899	Methionine	+++	±	± to +	± to ++	±	−	±(clumping)	+

* "Focal" necrosis includes individual necrotic cells and necrotic cell cords.

The results are summarized in Table IV. They appear to be much less favorable than those obtained in the first group (Tables II and III). Among the 25 rats of the series, 19 still exhibit severe cirrhotic changes (++ to ++++) at the end of the experiment. In 17 rats of the same group the amount of ceroid present in the liver was still very high (++ to ++++), with "clumping" in only 7 rats. Even fat infiltration persisted to a considerable extent in 13 of the 25 experimental animals. On a protein diet (S I) with its low fat content, the results were slightly better than with supplements of methionine or choline plus cystine added to the original basal experimental diet (C I or C III). In 2 rats receiving supplements of choline and cystine for 200 and 240 days, respectively, the liver showed severe cirrhosis (++++), at necropsy, with a very large amount of ceroid (++++).

In the third group of experiments the effort was made to avoid the production of too severe, and therefore possibly irreversible, cirrhosis, and controls were included for proper evaluation of a therapeutic regime. These goals were achieved (*a*) by the use in the preparatory period of an experimental ration (C III with carotene and drisdol as sources for vitamins A and D), the cirrhosis-producing effect of which was known to be mild (2); (*b*) by limiting the preparatory period to only 120 days, and (*c*) by maintaining a control group of animals

on the basal cirrhosis-producing diet throughout the whole experiment. During the 120 days of the preparatory period the ration was supplemented with cystine (50 mg. daily), in addition to vitamins. After 120 days, rats of the control group received only the basal diet and vitamins. The various therapeutic groups obtained supplements of methionine (50 mg.

TABLE IV

Results of Attempted Treatment of Experimental Dietary Hepatic Cirrhosis in Rats without Simultaneous Controls

Diet		No. of rats	Fat infiltration			Ceroid				Cirrhosis			Average weight at		
Before treatment	After treatment		0	+	++ to +++	0	+	++ to +++	Clumping	0	+	++ to +++	Start	150th Day	End
C I	S I	7	1	4	2	1	3	3	3	1	2	4	268	199	250
C I	C I + methionine	3			3			3	1			3	202	143	193
C I	C I + cystine and choline	7		3	4	1		6	2	1		6	246	191	225
C I + cystine	S I	2		1	1		1	1			1	1	171	145	288
" + "	C I + methionine	3	1		2			3				3	175	129	152
C I + carrot	S I	1	1					1				1	170	141	220
C III + carrot	C III + carrot + methionine	1			1		1					1	150	170	170
C III + carrot + cystine	S I	1	1				1		1		1		145	139	215
C I, C III	S I	11	3	5	3	1	5	5	4	1	4	6	230	178	251
C I, C III	Methionine	7	1		6		1	6	1			7	183	141	172
C I, C III	Choline and cystine	7		3	4	1		6	2	1		6	246	191	225

daily), or liver extract (0.2 ml.), singly or in combination. In two groups (II and III, Table V), the basal ration was changed to the usual semisynthetic protein diet (S I) with or without liver extract. One final group (VI) was treated with the combination of methionine and thiouracil, the latter being known to have a beneficial effect on the prevention of dietary hepatic cirrhosis (8). The period that followed the preparatory period of 120 days, was extended to 150 days. Only rats which survived at least 100 days in any group receiving

therapy were considered as sufficiently treated. Animals which died within 30 days of the therapeutic period were added to the control group and regarded as untreated. In the control groups, many rats died during the first few days or weeks of the second period and only three survived 150 days following the preparatory period.

The findings obtained at necropsy in the animals of the treated groups (II to VII) were compared with those in the rats of the control group. It was assumed that in approximately

TABLE V

Results of Treatment of Experimental Dietary Cirrhosis without Preceding Biopsy

Experimental group	No. of animals	Sex	Treatment	Average daily food intake in consecutive 40 day peroids, *gm.*	Average weight at			Cirrhosis		
					Start	120th day	End	0	+	2+ or more
I *a*	16	M	None	7.1;6.2;5.6 4.8; 5.4; 4.2	215	175	155	0	3	13
I *b*	12	F		6.9;6.0;5.7 5.1; 5.2, 5.0	220	200	158	4	1	7
II *a*	8	M	Casein	7.3;6.7;6.6 10.4; 9.1; 9.5; 8.8	222	214	256	0	4	4
II *b*	5	F		6.3;6.0;5.9 7.9; 7.9; 8.7; 8.8	188	189	195	5	0	0
III *a*	10	M	Casein and liver	8.0;6.8;6.0 9.8;10.3;11.3;11.0	219	203	312	4	5	1
III *b*	4	F	extract	6.8;5.7;5.9 9.3; 9.0; 8.7; 8.5	184	179	234	3	1	0
IV *a*	8	M	Liver extract	7.2;6.1;5.8 5.9; 5.8; 6.5; 6.1	202	205	206	2	5	1
IV *b*	5	F		7.4;5.9;6.0 7.2; 6.8; 6.6; 7.3	221	207	181	3	1	1
V *a*	7	M	Methionine	7.7;6.9;6.8 6.7; 6.9; 7.4; 7.4	221	223	246	3	3	1
V *b*	7	F		6.3;5.7;5.7 5.4; 5.1; 6.0; 5.6	200	201	207	6	1	0
VI *a*	10	M	Methionine +	7.5;6.5;5.8 5.1; 5.1; 6.1, 5.9	241	219	219	4	2	4
VI *b*	4	F	thiouracil	6.5;5.8;5.6 5.3; 5.5; 5.0; 5.4	211	192	197	3	1	0
VII *a*	8	M	Methionine +	7.7;7.1;6.2 6.6; 6.9; 7.5; 7.7	250	235	300	6	0	2
VII *b*	6	F	liver extract	7.0;5.5;5.8 6.2; 5.8; 6.0; 6.4	229	228	218	5	1	0
I *a*	16	M	None	7.1;6.2;5.6 4.8; 5.4; 4.2	215	175	155	0	3	13 *
II*a*–VII*a*	51	M	Yes	7.8;6.6;6.1 7.0; 7.0; 7.8; 7.7	227	216	257	19	19	13 *
I *b*	12	F	None	6.9;6.0;5.7 5.1; 5.2; 5.0	220	200	158	4	1	7 *
II*b*–VII*b*	31	F	Yes	6.7;5.8;5.9 6.9; 6.6; 6.9; 7.0	206	201	205	25	5	1 *

In Group I, eight males and four females have shown ascites (or pleural effusion). In no other group was ascites found at necropsy.

* $p < 0.0001$.

all rats cirrhosis, although of a mild degree, was produced in the preparatory period. This assumption was borne out by the presence of cirrhosis in rats which died soon after the 120 days of the preparatory period. In the surviving rats of the control group, the cirrhosis may have progressed further during the second period, whereas in all other groups exposed to the influence of therapeutic factors cirrhosis should have been arrested or have undergone regression toward complete repair. Thus, the comparison was more of a statistical, indirect nature and was not based on the direct method of biopsy.

This experiment comprised 110 rats distributed among the various groups and subdivided in each group according to sex. From the findings recorded in Table V the following conclusions may be drawn. In confirmation of previous observations (2) regarding the greater

resistance of female rats to experimental dietary cirrhosis, female rats seemed to respond more readily to treatment with dietary factors. The effect of these therapeutic dietary factors (including the combination of methionine and thiouracil) was clearly demonstrable in all experimental groups, in female as well as in male rats. It is of interest that, whereas in the control group all male rats exhibited signs of moderate or severe cirrhosis, yet in the groups receiving treatment, a large number of male rats showed no cirrhosis at necropsy. The difference is statistically significant. The effect of therapy was also demonstrable histologically by the disappearance of fat infiltration of the liver. In view of the special experimental diet used (C III, without cod liver oil or other source of unsaturated fatty acids), ceroid was absent both in the control (2) and treated groups.

Comparison of the various dietary therapeutic procedures revealed no distinct superiority of any of the factors used. It appeared that crude liver extract, given by mouth, enhanced the effect of casein and methionine. The beneficial effect of crude liver extract manifested itself also by the fact that only in the groups receiving liver extract with casein or methionine did all rats survive the entire experimental period, whereas in all other groups some (1 to 3) of the rats died within 100 days of treatment. Thiouracil, with its distinct *preventive* effect on experimental hepatic cirrhosis (8), did not show any synergistic *therapeutic* activity when added to methionine. On the contrary, the results achieved with this combination were slightly less favorable than those with methionine alone. The only rat with acute hepatic necrosis was one treated with methionine and thiouracil. This necrosis was unusual, inasmuch as it occurred within the nodules and lobules of liver parenchyma associated with advanced cirrhosis.

Food intake during the preparatory period was characterized by progressive decline. In groups receiving effective treatment in the second period, and especially in animals fed a normal protein diet (S I), food consumption showed a steady increase which did not occur in the control group.

DISCUSSION

The beneficial effect of lipotropic dietary factors (casein, methionine, choline), singly or in combination, applies not only to the prevention, but, at least to some extent, also to the therapy of experimental dietary cirrhosis. In confirmation of previous findings (4) dietary lipotropic factors brought about a reduction of the fat content of the diseased liver and restored hepatic cells to the normal state. In extension of past observations (4) repair of the cirrhotic process seemed also to include regression of the diffuse fibrosis. Thus, the final result of therapy of the experimental dietary cirrhosis did not differ from that observed in toxic cirrhosis (5–7). In the latter, the diminution of fibrosis was measured and determined by chemical methods (5 *b*), in addition to other more circumstantial evidence (6).

In the present experiments, dealing with dietary cirrhosis, the question whether the regression of fibrosis, as determined by the histological appearance in sections obtained at necropsy, was real or due more to stretching and "thinning out" of the originally wide fibrotic bands in the cirrhotic liver, as a result of growth of liver parenchyma, cannot be answered definitely. The smooth surface and almost normal architecture of the liver, at autopsy, and the lack of correlation between weight of liver, gain of body weight, and extent of demonstrable fibrosis speak in favor of real regression of the cirrhotic process.

The same consideration may also apply to the fate of ceroid during the process of repair. Here, progressive healing is first represented by "clumping" and by seemingly true reduction of the amount of ceroid in the liver.

The reduction and even complete disappearance of histologically demonstrable fat infiltration may occur very soon after treatment is started. This is in complete analogy to similar observations with the use of lipotropic factors in the therapy of human cirrhosis (9). The initial improvement, however, is by no means always followed by regression of the basic, specific, fibrotic process. For instance, in our second series of experiments, treatment, even when continued from 200 to 240 days, left the diffuse fibrosis apparently uninfluenced. From the three groups of experiments here reported it appears that lipotropic factors reduce fibrosis only when the latter is not too severe and widespread. Similar observations were made by Best and his associates (7) with regard to the dietary therapy of hepatic cirrhosis caused by carbon tetrachloride in rats. Even in the case of mild, dietary cirrhosis in rats, successful therapeutic use of lipotropic factors requires 3 months or longer.

In our therapeutic experiments the various possible combinations of potentially effective lipotropic factors have not been exhausted. In the case of severe cirrhosis, such as existed in our second group of experiments (Table IV), supplements of methionine or choline (plus cystine) to the experimental, cirrhosis-producing, low protein, high fat diet, seemed to be less effective than a diet high in protein and low in fat (S I). On the other hand, in the first group of experiments (Table II), in which the results of treatment were compared with previous biopsy findings in the same animal, the best results were obtained by the combination of a diet high in protein (S I) supplemented with methionine. Finally, the last group of experiments (Table V) seems to indicate that crude liver extract (given by mouth) may enhance the effect of casein or of methionine. The possible application of these experimental findings to the treatment of human cirrhosis remains to be demonstrated.

In summary, the results of dietary therapy, in our experiments, show that the regimen used was certainly far from being uniformly effective. It is possible, although barely probable, that the addition of some other dietary factors to the therapeutic rations will improve their efficiency. Vitamin E, the rôle of which in the etiology of acute hepatic necrosis (2) has recently been elucidated, was well represented in our diet forms which contained a large proportion of a vegetable shortening (crisco), rich in vitamin E. The effect of much larger doses of vitamin E and, among other dietary factors, of purines, especially of xanthine (10), as well as of inositol, should be studied in separate experiments.

As bearing on the factors which may be involved in the pathogenesis of hepatic cirrhosis, the present experiments have again clearly demonstrated that the female sex fares the better. Both in the prevention and in the treatment of hepatic cirrhosis female rats showed a more favorable response than male rats.

It may also be pointed out that in the third group of our experiments (Table V) rats of the control group showed at the end of the experiment pronounced pathologic changes in the gonads, whereas in the treated animals such injury was almost uniformly absent (3). The good results of treatment in this group may be connected with these findings.

The fallacy of applying the results of preventive experiments to treatment is well illustrated by the rôle of thiouracil in the prevention and treatment of dietary hepatic injury. In preventive experiments thiouracil has been found highly beneficial (8), but in therapeutic experiments it has proved to be, if anything, slightly injurious. This observation may be interpreted as follows: In the prevention of hepatic injury, lowering of the basal metabolic rate, under the influence of thiouracil, also lowers the requirement for lipotropic factors, but treatment of hepatic cirrhosis requires increased regeneration of hepatic parenchyma and high cellular activity for the regression of fibrosis—which are not likely to be favored by lowering of thyroid activity.

SUMMARY

Dietary cirrhosis of the liver was produced in 223 rats, and then therapy of the condition attempted. Administration of lipotropic factors (casein, methionine, choline) was followed not only by reduction of fat infiltration and by regeneration of hepatic parenchyma but, by a reduction of the degree of the fibrosis. In one group of rats, comparison of sections obtained by biopsy, before treatment, with findings at necropsy, after completed therapy, indicated apparent reduction of the fibrosis and of the amount of ceroid and considerable restoration of architecture. This improvement, however, was obtained neither with complete regularity nor in a short time. In very severe cirrhosis, as a rule, the effect of a lipotropic diet was disappointing, even after prolonged treatment up to 200 to 240 days. It is assumed that factors determining prevention are beneficial only to a limited extent in treatment. The therapy of very severe cirrhosis may require the interaction of further beneficial factors (nutritional and hormonal).

Best therapeutic results were obtained by the combination of an adequate amount of casein with methionine or liver extract, and by the combination of methionine with liver extract.

Methionine and thiouracil, both of which, singly, are effective in the prevention of dietary hepatic cirrhosis in rats, have proved to be less effective for the therapy of cirrhosis, when administered together, than methionine given alone for the same purpose.

Under identical conditions, female rats have shown greater resistance to the production of dietary hepatic cirrhosis and a more favorable response to therapeutic dietary factors, than male rats.

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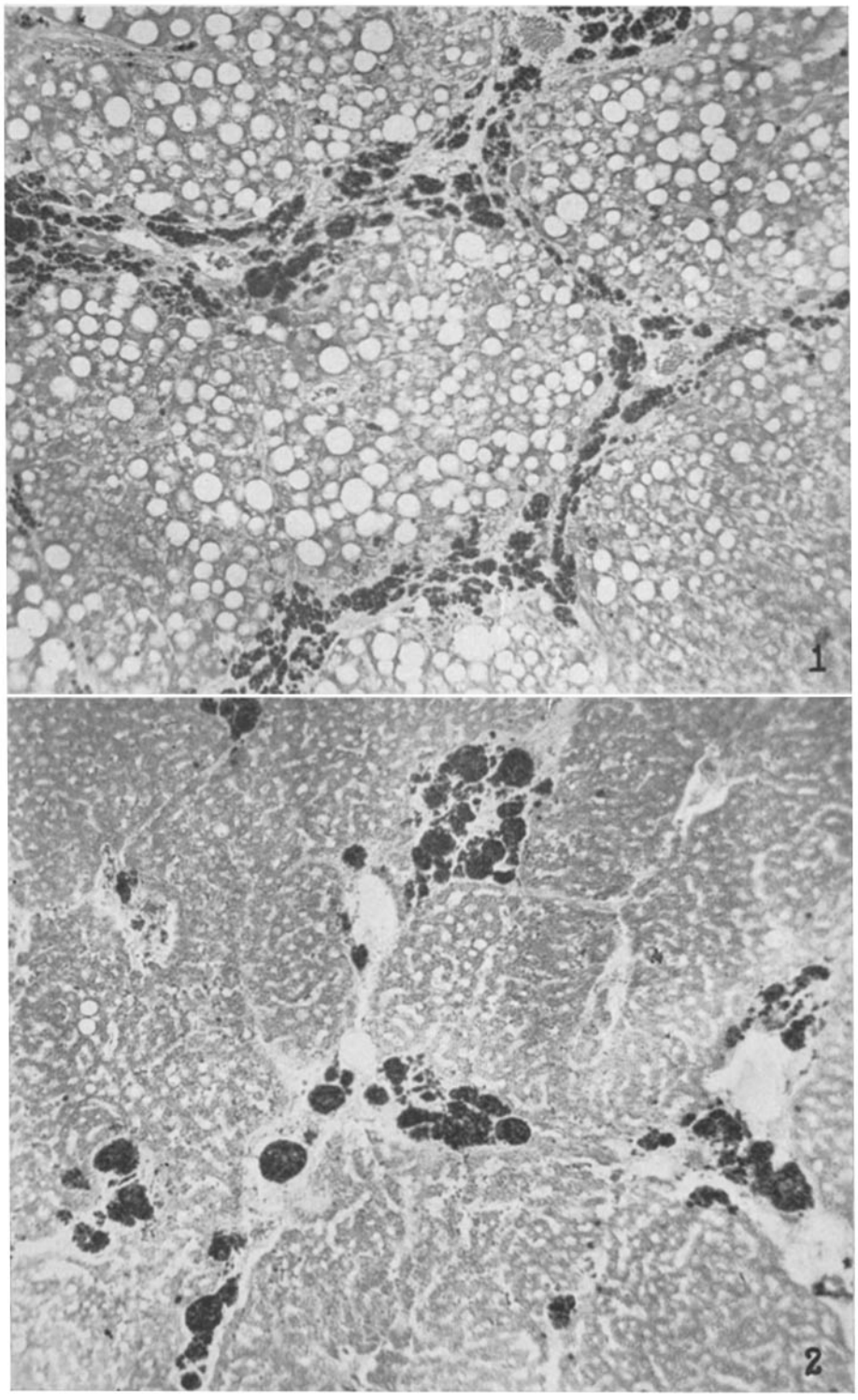
EXPLANATION OF PLATE 3

FIG. 1. Section of biopsy specimen of liver before treatment was begun, showing cirrhosis ++ and ceroid deposit ++. The granules of ceroid are smaller than in the section taken after treatment (Fig. 2), are diffusely distributed throughout the connective tissue, and are not agglomerated into large clumps. Fat infiltration of liver cells ++ to +++. Methyl green stain for ceroid. × 100.

FIG. 2. Ceroid, showing clumping. Most of the ceroid is in the neighborhood of central veins. By comparison with the amount of ceroid shown in Fig. 1 of a biopsy specimen removed before treatment was begun, the quantity of ceroid appears reduced. Although the individual particles of ceroid are still recognizable yet they are closely agglomerated into clumps. The absence of fat in the liver cells is another striking effect of treatment and the amount of fibrosis in this section appears to be less than was present in the section taken before treatment. Methyl green stain for ceroid. × 100.



(György and Goldblatt: Dietary cirrhosis of liver in rats)