CORRELATION OF STRUCTURAL AND FUNCTIONAL RECOVERY FROM CIRRHOSIS IN RATS TREATED WITH LIPOTROPIC DIETS

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After delineation of the pathologic events leading to the development of cirrhosis in choline-deficient rats by Hartroft,[1] numerous investigators have studied by multidisciplinary approaches the progressive changes of this disease at relatively early stages. Although some histologic and biochemical features have also been reported during the recovery phase in experimental dietary cirrhosis under a variety of therapeutic regimens,[2-10] many aspects have not yet been elucidated. Reabsorption of hepatic collagen and reactive features of hepatocytes and ductular cells have not been sequentially studied and few reports have been published concerning hepatic function tests during treatment.

The present study is concerned with the therapeutic effects of two diets, one low and the other high in protein, but both supplemented with choline, fed to rats in which cirrhosis had been produced by choline deficiency.

MATERIAL AND METHODS

Wistar albino male rats (100 to 120 gm initial weight) were maintained for 6 months on a basal diet low in protein, choline and vitamin B_{12}. This group was initially composed of 120 rats but during the 6-month period more than half were killed or had died, reducing the number to only 55. Seven of this choline-deficient group were then randomly killed to ascertain the extent of multilobular cirrhosis in this sample.

Another 20 rats had been pair-fed from the beginning the basal diet, supplemented with 0.35 gm per cent of choline chloride. They were used as a control group without cirrhosis (group 1).

At the beginning of the seventh month, the previously choline-deficient rats were allotted to 3 groups, each of 16 animals. One group (group 2) was used as an untreated control by continuing its members on the basal choline-deficient diet. The other 2 (groups 3 and 4) were transferred to one or the other of 2 choline-supplemented diets. Rats in group 3 were fed the basal (low protein) diet supplemented with 0.35 per cent choline chloride; rats in group 4 were given a similarly supplemented diet which, however, contained 25 per cent casein (see Table I).

All animals were housed in wire-bottomed individual cages in air-conditioned rooms supplied during the day with continuous soft music. Body weights were recorded twice weekly. All 4 groups were fed their respective diets for 7 months. At the end of this

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period, mortality rates were 75 per cent in group 2 and 12.5 per cent in group 3. None of the rats in groups 1 and 4 died during the same period. Rats in groups 2 and 3 which succumbed during the course of the experiment (after institution of therapeutic diets) were necropsied along with their respective controls which were killed by decapitation.

TABLE I

COMPOSITION OF THE DIETS

(GM PER CENT)

	Basal diet (group 2)	Therapeutic diets (groups 1 & 3)	(group 4)
Casein	6	6	25
Alpha soya protein	6	6	—
Sucrose	44.5	39.15	26.15
Alphacel	5	5	5
Lard	20	30	30
Corn oil	10	5	5
Vitamin powder *	4	4	4
Salt mixture W.	4	4	4
L-Cystine	0.5	0.5	0.5
Choline chloride	—	0.35	0.35

* Without choline and vitamin B_{12}.

Two weeks after commencing the therapeutic diets all rats were given intraperitoneal injections with 0.3 μc of tritiated thymidine (^{3}H TdR, sp. activity: 36 c per mM) per gm body weight. The injection was repeated every 2 weeks in order to obtain autoradiographs from those animals dying during the experiment as well as from their controls. The autoradiographs from the median hepatic lobes were prepared following the technique of Messier and Leblond,[11] using Kodak nuclear track emulsion type NTB$_3$. Labeled hepatocytes and "other cells" (which included ductular and mesenchymal cells) were identified and counted separately in sections. Results were expressed as a percentage of the total number of nuclei counted (3,000 per section).

Additional paraffin sections were stained with hematoxylin and eosin, oil red O[12] for detection of ceroid, or with Masson's trichrome stain[13] to aid in visualizing even small amounts of fibrous tissue. Fat was stained in frozen section with oil red O (ORO).

For electron microscopy, small blocks, also taken from the median lobe of each liver, were fixed by immersion in Dalton's solution[14] and embedded in Epon 812.[15] Ultrathin sections were stained with lead using Karnovsky's method,[16] and photographed in a Philips 200 electron microscope at established initial magnifications.

Hepatic remnants were stored in the electric deep freeze (−20° C) for biochemical analyses.

Six weeks after initiating the therapeutic phase (phase II) of the experiment and biweekly thereafter, levels of activity of glutamic-oxaloacetic transaminase (GOT) and serum alkaline phosphatase (ALPase) were measured in samples of sera drawn from all rats.[17,18]

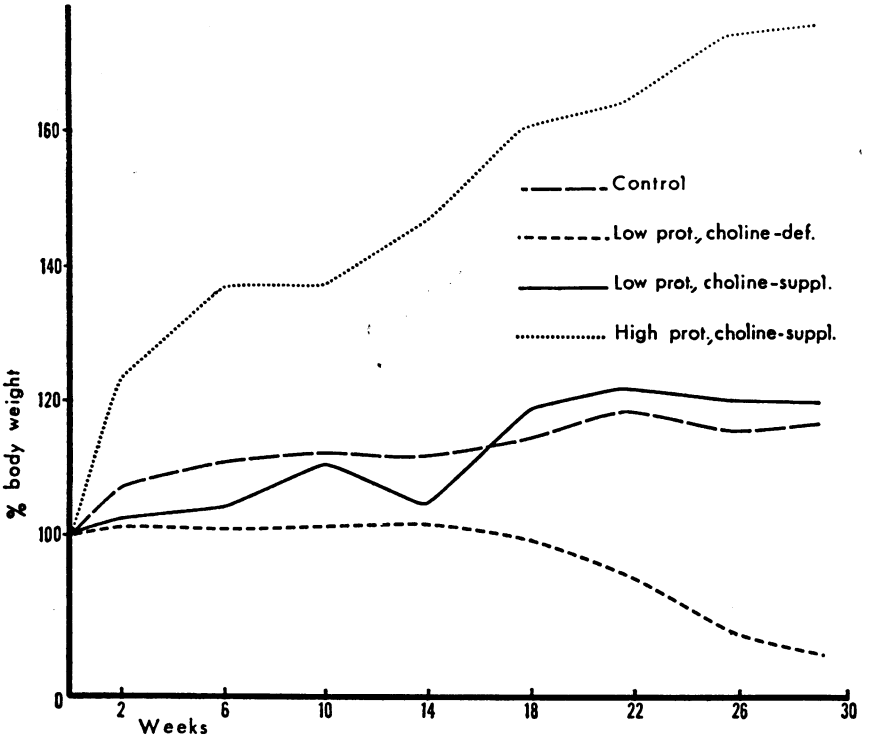
Seven months after phase II was instituted all the survivors were killed. Blocks of hepatic tissue were taken for light and electron microscopic studies and for biochemical determinations. The latter were performed simultaneously with those from livers of rats dying during the experiment. The total hepatic lipid was estimated by Folch's method.[19] Hepatic DNA content was determined by the method of Webb and Levy.[20] The alkali-soluble and non alkali-soluble collagens of the livers were extracted according to the method of Singer.[21] Hydroxyproline content in both collagen frac-

tions was estimated by the method of Neuman and Logan.[22] Total collagen content was expressed as hydroxyproline concentration.

Results

General Findings

Body Weights. Changes in body weights were expressed as percentages of the initial values in plotting their growth curve (Text-fig. 1). Rats in

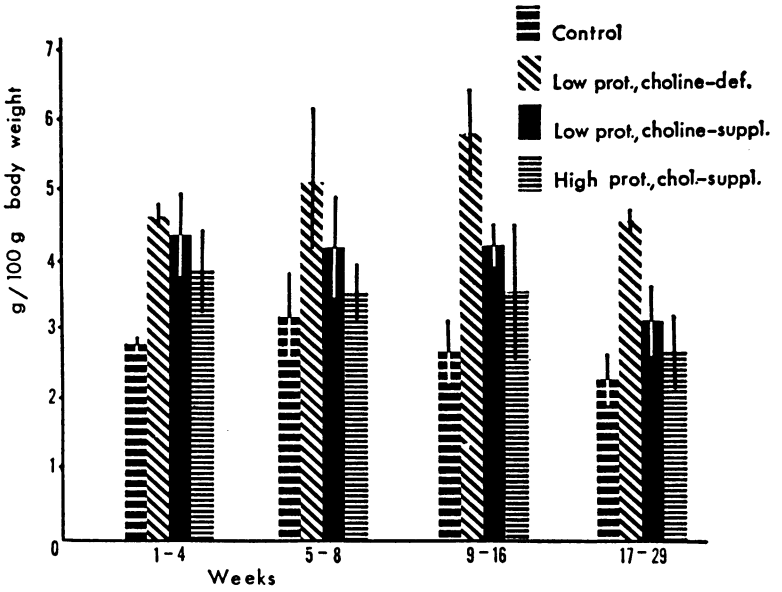


Text-fig. 1. Growth curves for each group expressed as percentages of initial body weights.

group 4 (high protein, choline-supplemented) gained rapidly during the first week of therapy and more slowly but steadily during the remainder of phase II attaining finally a total increase of some 160 per cent of their weights at the beginning of treatment. Animals in group 3 (low protein, choline-supplemented) increased almost 20 per cent in their average body weight by the 22nd week on administration of the therapeutic diet. This increase closely paralleled that of group 1 (non-cirrhotic control animals). Group 2 (cirrhotic, choline-deficient) continued to lose weight (average of 10 per cent over the 22 weeks of phase II.

Liver Weights. In the initial 1- to 4-week period of phase II, liver weight of samples taken from the 3 groups with cirrhosis (2, 3 and 4) increased significantly (expressed per 100 gm of body weight) over the

control group 1 (Text-fig. 2). In group 4, however, the average significantly decreased in the initial 1- to 4-week period when compared with the cirrhotic control group 2, and approximated the range in group 1 (normals) in the 17- to 29-week period of treatment. Livers of group 3 decreased significantly in weight after 9 to 16 weeks, but were still heavier than those of group 1 at the end of the experiment.

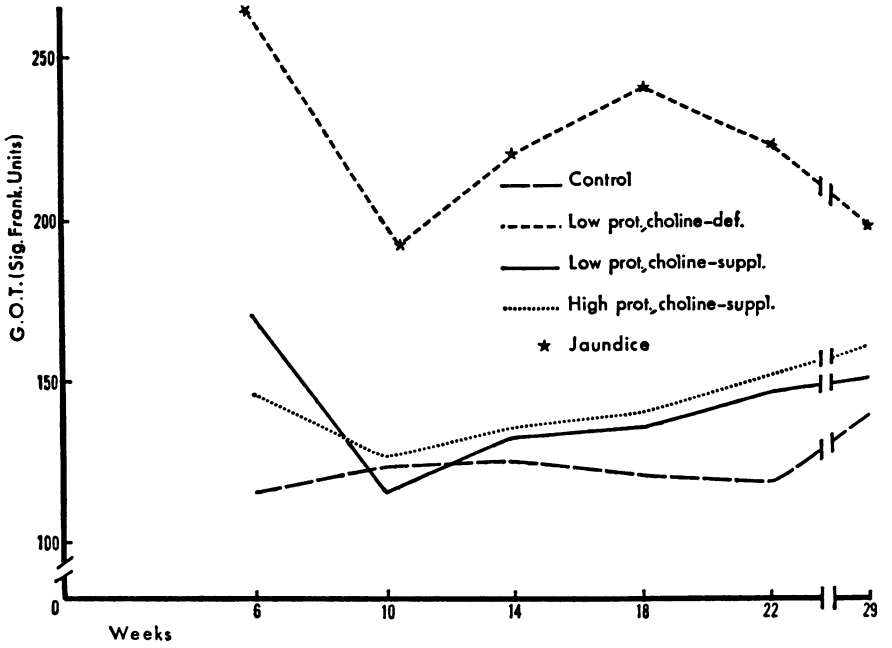


TEXT-FIG. 2. Changes in liver weights, expressed per 100 gm body weight, at different periods of the experiment (phase II).

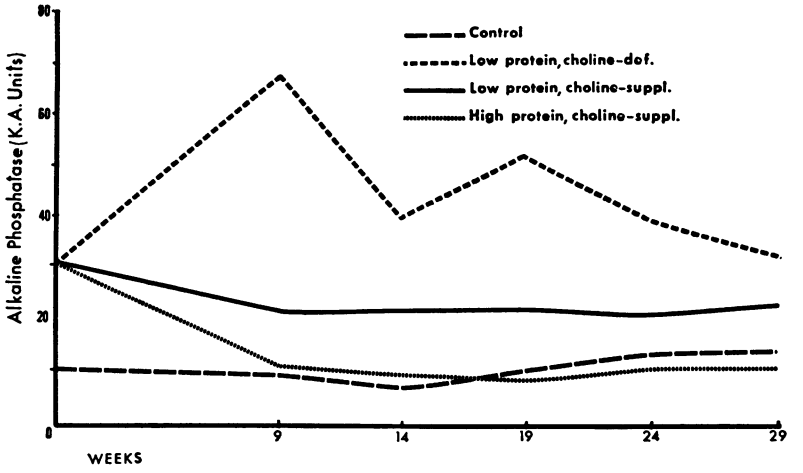
Liver Function Tests. Significant decreases of GOT activity in groups 3 and 4 were found within 6 weeks of phase II. By the tenth week, levels of GOT activity in the 2 treated groups (3 and 4) and in the control group (1) did not differ. Group 2 consistently showed the highest activity throughout (Text-fig. 3). Icteric sera were noted only in group 2 (Text-fig. 3). Changes in levels of alkaline phosphatase activity paralleled those of GOT, except that group 3 constantly showed slightly higher activities of ALPase than group 4 (Text-fig. 4).

Biochemical Analyses of the Livers

Total Hepatic Lipids. Fat content, expressed per 100 gm of body weight, in group 4 showed values close to the levels of the control group within the first 4 weeks of treatment and maintained these values to the end of the experiment (Text-fig. 5). Although significant decreases of fat content occurred in group 3 during the first month on the therapeutic

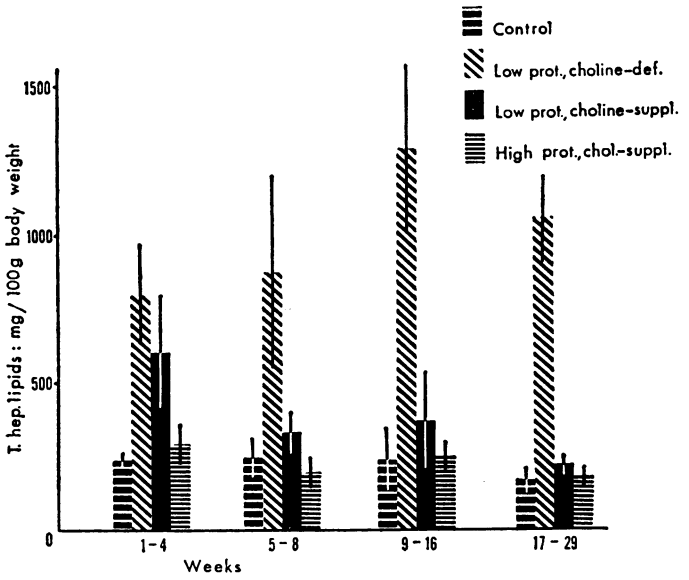


TEXT-FIG. 3. Changes in serum glutamic-oxalacetic transaminase activity, expressed in Sigma-Frankel units, during phase II of the experiment. Icteric serum was noted grossly only in the low protein, choline-deficient rats.

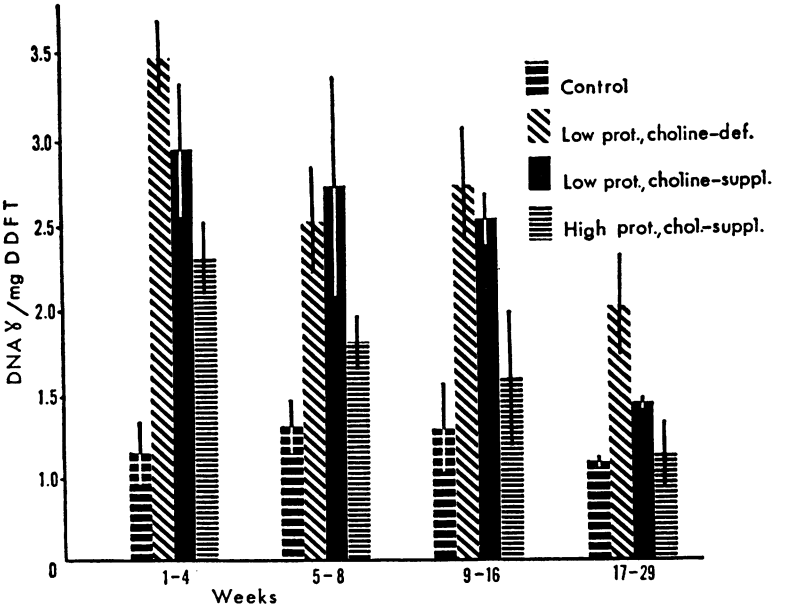


TEXT-FIG. 4. Changes in serum alkaline phosphatase activity, expressed in King-Armstrong units, during phase II of the experiment.

diet, the values were significantly higher than those in group 4. After the 5 -to 8-week period, no significant differences in fat content were found between group 3 and groups 1 or 4. Group 2 showed the highest value of fat content throughout the experiment.



TEXT-FIG. 5. Changes in total hepatic lipids, expressed in 100 gm body weight.

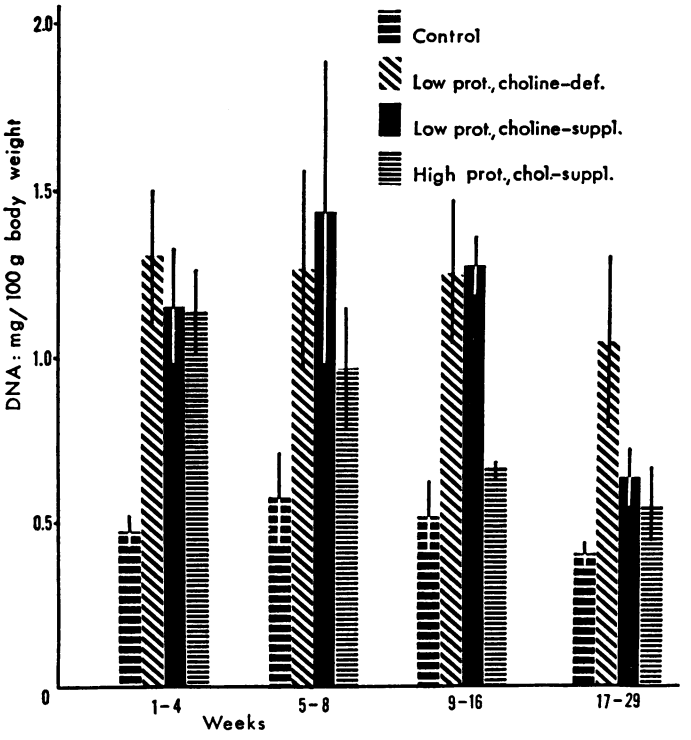


TEXT-FIG. 6. Changes in the hepatic DNA content expressed in γ per mg of dry defatted tissue (DDFT).

DNA. DNA content of livers in the 3 cirrhotic groups (2, 3 and 4) was highest during the initial 4 weeks of phase II. A significant decrease of hepatic DNA content, expressed as γ per mg dry defatted tissue (DDFT), occurred in group 4 during the first 4 weeks and gradually

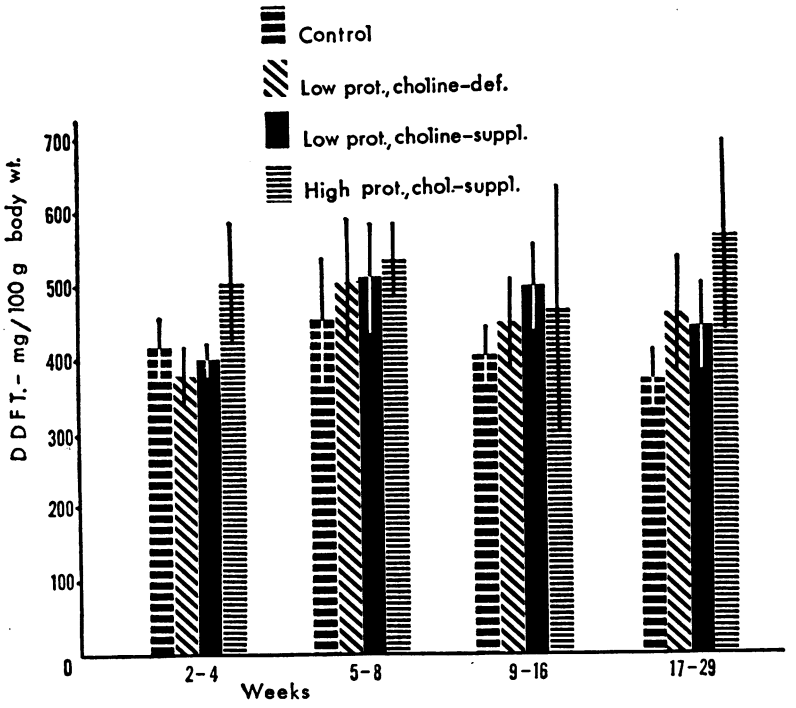
fell to control levels by the end of the experiment (Text-fig. 6). The difference of DNA content, expressed as mg per 100 gm body weight (Text-fig. 7), between group 4 and the other two cirrhotic groups was not statistically significant until the ninth week and after. DNA content in group 3 was as high as for group 2 by the 16th week, but a significant decrease could be demonstrated in the 17- to 29th-week period.

Dry Defatted Tissue (DDFT). The content of fat-extracted dry tissues corresponded to the content of crude protein in the liver.[21] There was no significant difference in DDFT content expressed as per 100 gm of body weight among the 4 groups, except between groups 1 and 4 at the 17- to 29th-week period (Text-fig. 8). However, a significant increase in DDFT content, expressed as mg per mg of DNA, after the 4-week period in group 4, and at the 5- to 8th-week period in group 3 was found (Text-fig. 9). After the eighth week, the difference between groups 2

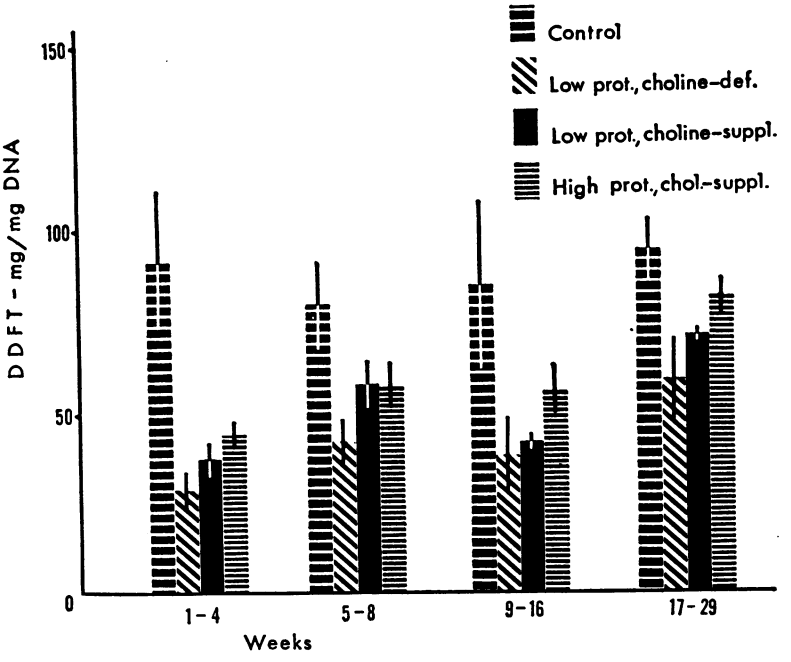


TEXT-FIG. 7. Changes in the hepatic DNA content, expressed in mg per 100 gm body weight.

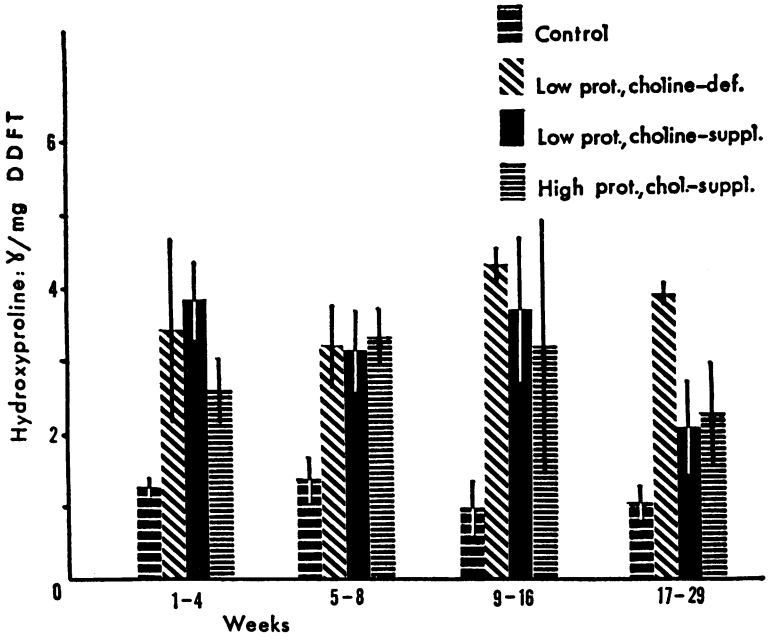
and 3 was not statistically significant because of the wide spread of values in group 2. At the end of the experiment, DDFT levels in both groups 3 and 4 were still significantly lower than in group 1 (Text-figs. 8 and 9).



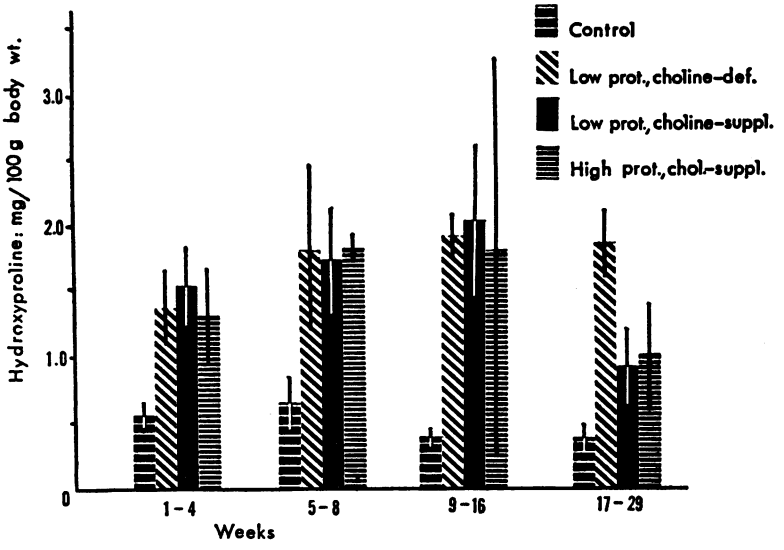
TEXT-FIG. 8. Changes in the hepatic content of fat-extracted dry tissue, expressed in mg per 100 gm body weight.



TEXT-FIG. 9. Changes in the hepatic content of fat-extracted dry tissue, expressed in mg per mg hepatic DNA.



TEXT-FIG. 10. Changes in the hydroxyproline content of hepatic collagen, as expressed in γ per mg dry defatted tissue (DDFT).

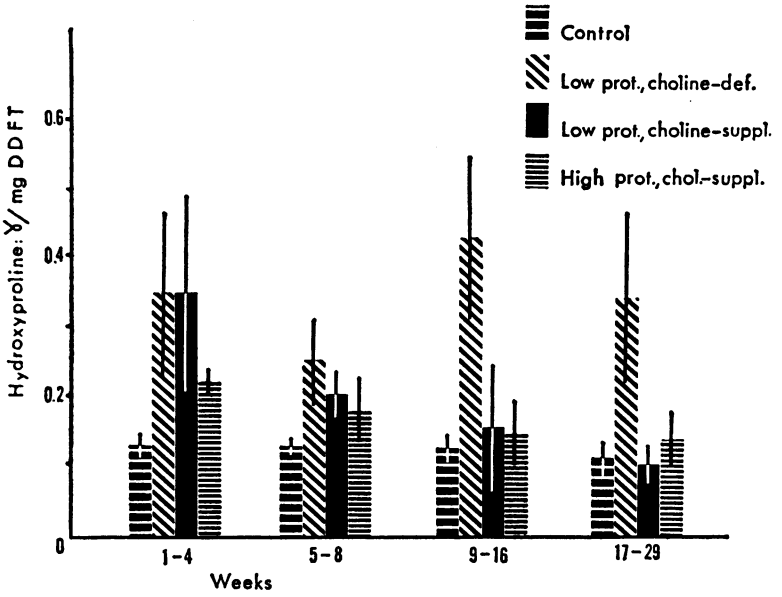


TEXT-FIG. 11. Changes in the hydroxyproline content of hepatic collagen, as expressed by mg per 100 gm body weight.

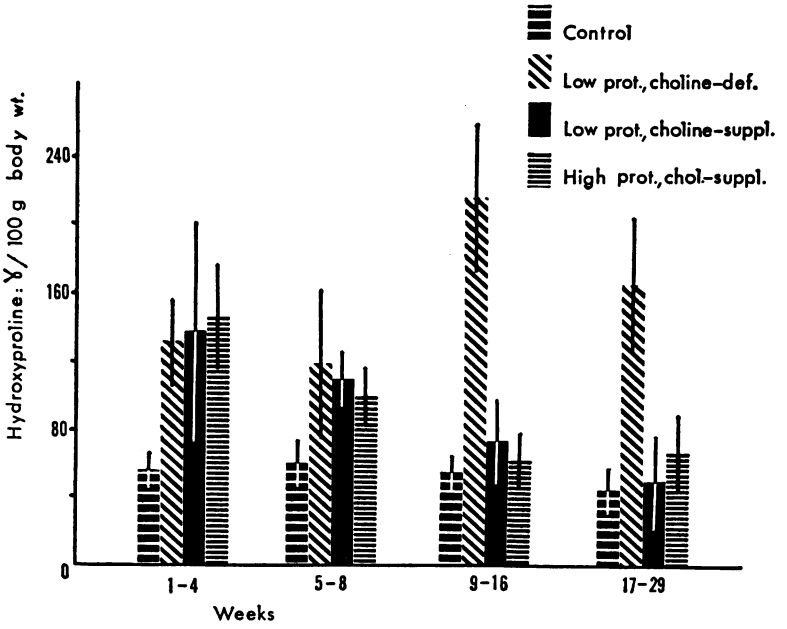
Collagen. Total collagen content in the 3 cirrhotic groups was significantly higher than in the control group 1 (Text-figs. 10 and 11). There was no significant difference in total collagen content among the 3 cir-

rhotic groups until the 16th week but in the 17- to 29th-week period, total collagen content in group 3 and group 4 significantly decreased.

The 3 cirrhotic groups also showed significantly higher contents of alkali-soluble collagen than did the non-cirrhotic group (Text-figs. 12



TEXT-FIG. 12. Changes in the hydroxyproline content of hepatic soluble collagen, as expressed in γ per mg dry defatted tissue (DDFT).



TEXT-FIG. 13. Changes in the hydroxyproline content of hepatic soluble collagen, as expressed in γ per 100 gm body weight.

TABLE II

HISTOLOGIC EVALUATION OF HEPATIC FATTY CHANGES

Weeks following therapeutic diets	*Group 1*			*Group 2*			*Group 3*			*Group 4*		
	Con.	Dist.	Cys.	Con.	Dist.	Cys.	Con.	Dist.	Cys.	Con.	Dist.	Cys.
1–4	+	C	−	++++	D	+++	+	*Sc	+	+	Sc	++
	+	C	−	++++	D	+++	++	*D–PN	++	++	PN	++
	+	C	−	++++	D–C	+++	+++	D	++	++	PN	++
	++	D	−	++++	D	+++	+++	D–C	++	++	D–C	++
5–6	+	C	−	++++	D–PC	+++	+	*D	+	+	*D	+
	+	C	−	++++	D–PC	+++	+	Sc	++	+	D	±
	+	C	−	+++	D–PC	++	++	*D–C	+	+	*C	+
	+	P	−	++++	D	+++	++	*Sc	++			
	++	C	−									
9–17	+	C	−	++++	D–PC	+++	+	Sc	±	+	*D–Sc	±
	+	C	−	++++	D–C	+++	++	C	±	++	*D–C	+
	+	C	−	++++	D	+++						
17–29	+	D	−							−		−
	+	C	−	++++	D	+++	+	D	±	+	Sc	±
	++	C	−	++++	D	+++	++	D	−	++	C	+
	+	C	−							±	Sc	±
	+	P	−	+++	D	++	±	Sc	±	+	D	±

Con., Content.
Dist., Distribution.
Cys., Fatty cysts.

−, No change.
±, Very slightly increased.
+, Slightly increased.
++, Moderately increased.
+++, Markedly increased.
++++, Abundant.

D, Diffuse.
P, Periportal.
C, Pericentral.
PN, Perinodular.
D–C, Diffuse and some centrally located.
*, Islet-like fatty nodules.
Sc, Scattered.

and 13). Treated groups 3 and 4 showed a significant decrease in soluble collagen content after the eighth week. At the end of the experiment, levels of soluble collagen in the treated cirrhotic groups had returned to normal (groups 3 and 4).

Light Microscopy

Fatty Changes. The degree of fatty change seen in frozen sections stained with ORO paralleled, with few exceptions, the levels of total lipids biochemically obtained. A semiquantitative histologic evaluation of the hepatic fatty change and its lobular or nodular distribution was attempted (Table II).

Minimal amounts of stainable fat in the form of small intracytoplasmic droplets were observed in centrilobular hepatocytes of rats in the control group 1 (Fig. 1). The amount of fat in sections from the cirrhotic group 2 was in sharp contrast, for fatty cysts were here abundant (Fig. 2). The extent of fatty changes in different nodules of these livers varied greatly, however, some exhibiting little or none. In a few instances, a zonal nodular distribution was also observed. In such circumstances localization of the fatty changes were variously peripheral, central, or both (Fig. 3). No relation could be established between the content of fat and the nodule size.

The extent of fatty changes in livers in groups 3 and 4 was found greatly reduced when compared with that in group 2, particularly in late stages (Fig. 4). Hepatocytes containing small or medium-sized droplets of fat were found scattered in nodules (or lobules in the non-cirrhotic livers). Small numbers of fatty cysts of erratic distribution were also encountered in these livers. Occasionally, in livers with minimal fatty changes, one or two nodules would be paradoxically loaded with fat (islet-like fatty nodules).

In a few animals in group 2, nodule necrosis was occasionally found. The amount of fat in these necrotic nodules was only moderate.

Ceroid. No ceroid was detected in the livers in the control group 1. But in the majority of those in groups 2, 3 and 4, considerable amounts of this pigment were seen in trabecular macrophages and occasionally in hepatocytes (Table III). No appreciable differences could be found between cirrhotic groups. Ceroid was also found in the few non-cirrhotic, but still fibrotic livers of groups 3 and 4.

Diameter of Nodules and Width of fibrous Bands. In the first 1- to 4-week period there were no significant differences in the nodule diameters and width of fibrous bands among cirrhotic groups (Table IV). In group 2, the two parameters increased with time, while in group 3 they decreased. The diameters of nodules in group 4 were slightly larger than

those in group 3 in the 5- to 8-week period, but no difference was found thereafter. In late stages (17- to 32-week period), the width of fibrous bands in groups 3 and 4 was clearly narrower than in group 2 (Figs. 5 and 6).

TABLE III

HISTOLOGIC EVALUATION OF CEROID

Weeks following therapeutic diets	Group 1	Group 2	Group 3	Group 4
1–4	−	+	++	+†
	−	++	+++	+
	−	++	+++	++
	−	+++	+++	+++
5–8	−	−	++	+
	−	++	+++†	++
	−	+++	+++	+++
	−	+++	+++	+++
	−	+		
	−			
9–16	−	+	+	±†
	−	+	++	+
	−	+	+++	++
17–32	−	+	+	−*
	−	+	++	±†
	−	+		+
	−			++
				++

−, No ceroid.
±, Very little.
+, Little.
++, Moderate.
+++, Abundant.

* No fibrosis.
† Fibrosis, no cirrhosis.

After the eighth week, 3 of 8 animals in group 4 showed fibrosis but architectural distortion had been corrected. They were no longer considered cirrhotic, as were all livers in animals in groups 2 and 3, because we do not regard the presence of fibrosis without architectural distortion as fulfilling the criteria for cirrhosis.

Within the nodules in cirrhotic livers, fibrous bands which subdivided them were frequently observed in group 2 throughout the experiment but were rarely seen in groups 3 and 4 after the fourth week.

Other Findings. Proliferation of ductular cells and the presence of other interstitial cells were prominent features in livers from group 2 during the course of the experiment. Although at early stages they were also abundantly found in groups 3 and 4 their number decreased sharply after the 16th week in the former and after the eighth week in the latter.

Electron Microscopy

Within the first 1 to 4 weeks the cytoplasm of hepatocytes in the noncirrhotic control rats (group 1) contained small droplets of fat of various electron densities (mostly pale). They were not surrounded by any definite continuous membrane, but on occasion endoplasmic membranes par-

TABLE IV

NODULE DIAMETER AND WIDTH OF FIBROUS BANDS

Weeks following therapeutic diets	Nod.	Fib.	Nod.	Fib.	Nod.	Fib.
1–4	Med. (1.92)	Nar.	Med. (1.5)	Med.		Nar.†
	Med. (1.72)	Med.	Med. (1.84)	Broad *	Small (1.25)	Nar.*
	Med. (1.75)	Broad	Med. (1.66)	Broad *	Med. (1.84)	Med.
	Large (2.05)	Med.*	Large (2.22)	Med.*	Large (2.4)	Med.
5–8	Small (1.25)	Nar.*		Nar.†	Med. (1.63)	Med.
	Med. (1.72)	Broad *	Small (0.82)	Nar.	Med. (1.66)	Med.
	Med. (1.73)	Broad *	Small (1.48)	Nar.	Med. (1.96)	Med.
	Large (2.34)	Broad	Med. (1.67)	Nar.	Large (2.26)	Broad
9–16	Small (1.25)	Med.	Small (1.23)	Nar.		Nar.†
	Med. (1.79)	Broad *	Med. (1.59)	Med.*	Small (1.0)	Nar.
	Med. (1.88)	Broad *	Large (2.15)	Nar.	Large (2.18)	Broad
17–32	Med. (1.84)	Nar.	Small (1.42)	Nar.	Normal	
	Large (2.0)	Broad *	Med. (1.50)	Nar.*		Nar.*
	Large (2.58)	Broad *	Med. (1.73)	Nar.	Small (1.17)	Nar.
					Small (1.42)	Nar.
					Med. (1.65)	Nar.

( ), Average diameter on 5 largest nodules (mm).
* Intranodular fiber.
† No cirrhosis.
Med., Medium.
Nar., Narrow.

tially surrounded them. The overall size and configuration of mitochondria were normal (Fig. 7), although some were moderately enlarged with alterations of their cristae including dilatation and occasional intracristal deposits of helical filaments. Although the ergastoplasm in this control group was usually normal, a tendency to small vesiculation and microsomal dispersion was found in a regular number of hepatocytes. All these features remained unchanged in late experimental stages.

Profound ultrastructural alterations were observed in most hepatocytes in cirrhotic group 2 and they were equally detected at early and late stages. Mitochondria in these were enlarged and spherulated (Fig. 8). Their matrices were usually less electron-dense than normally with frequent areas of rarefaction, although in other instances the mitochon-

drial matrix was even more granular and dense. Diminution in number and shortening of cristae were evident in the majority. Some cristae, particularly those separated from the inner mitochondrial membranes and dispersed throughout the matrix, were dilated and contained groups of helical filaments (Fig. 9). In other altered mitochondria, rounded, concentrically laminated bodies were observed in their matrices. In some hepatocytes, deterioration of mitochondria was still more severe exhibiting stages of actual disintegration. Cisternae of the rough ER were dilated occasionally giving a vacuolated aspect to the cytoplasm. Ribosomes were either still attached to these membranes or dispersed throughout the cytoplasm. Although glycogen granules were generally less abundant, there were exceptions to this rule, particularly in the case of those hepatocytes in which the Golgi apparatus was prominent. Swollen microvilli were numerous even in otherwise normal or only slightly dilated bile canaliculi, but this alteration was less prominent along the sinusoidal border. Large droplets and globules of fat were found in almost all hepatocytes; fatty cysts were numerous. Cyst walls were without exception formed by greatly stretched and thinned hepatocytes. Striking morphologic features were encountered in Kupffer cells and other macrophages. They contained globules of fat of varying densities mixing with vesiculated or shrunken erythrocytes and extruded ferritin granules (Fig. 10). This intermixing of fat and red cells favored the formation of ceroid which appeared as heterogeneous granules that became engulfed by lysosomes (residual bodies).

Shortly after transfer to the therapeutic regimes, (group 3) ultrastructural configuration of hepatocytes improved. At early stages many mitochondria were still abnormal and bizarre (club-shape, doughnut-shape) (Fig. 11), but to a less extent this was true for the untreated controls (group 2). After prolonged treatment (22 weeks), only a few mitochondria were even slightly enlarged or spherulated and the ER was normal (Fig. 12).

Improvement was complete and rapid in the livers of rats in group 4 (high protein, choline-supplemented) in which ultrastructural alterations could not be found in cytoplasmic organelles at either early or late stages (Fig. 13).

Autoradiographic Observations

Although the small number of autoradiographic specimens obtained in this experiment precluded a more complete sequential study on the proliferation of cells, some interesting features could be traced (Table V). The number of labeled hepatocytes in the cirrhosis groups 2, 3 and 4 was clearly increased as compared with the control group 1. Among cir-

rhotic animals, values for rats in group 2 were much higher than those in groups 3 and 4 (except at the sixth week in group 4). In early stages of the experiment, group 3 showed lower values for labeled cells than group 4, but in the late stages they became higher.

TABLE V

PERCENTAGE OF TdR-^{3}H LABELED HEPATIC CELLS

Weeks following therapeutic diets	Days following TdR-^{3}H injection	Group 1	Group 2	Group 3	Group 4
5	6	H 0.60	H 3.10	H 0.70	H 1.94
		O	O 1.30	O 0.53	O 0.93
6	3	H 1.11	H 3.33	H 1.85	H 4.00
		O 0.50	O 1.07	O 0.33	O 2.11
8	12	H 0.40	H 2.33	H 1.02	H 1.05
		O 0.27	O 0.78	O	O 0.43
14	10	H 0.64	H 2.56	H 1.25	H 1.66
		O 0.43	O 1.25	O 0.53	O 0.20
22	14	H 0.34	H	H 1.15	H 1.50
		O 0.20	O	O 0.53	O 0.20
29	1	H 1.07	H 6.40	H 2.90	H 2.33
		O 1.50	O 3.23	O 1.10	O 1.77
"	"	H 0.70	H 5.50	H 2.70	H 1.47
		O 1.50	O 3.70	O 1.73	O 1.90
"	"	H 0.70			H 1.30
"	"	O 1.60			O 1.50
"	"	H 0.50			H 1.43
"	"	O 1.24			O 3.80
"	"	H 0.60			
"	"	O 1.35			

H, Hepatocytes.
O, Other cells.

Seeking information on the regenerative capacity of nodules in relation to their sizes, the values of labeled hepatocytes in small nodules of cirrhotic livers were compared with those in the large ones (Table VI). In general, small nodules showed values higher than did large nodules.

COMMENTS

The prompt disappearance of abnormal amounts of fat from cirrhotic livers following the institution of therapeutic dietary regimens, as found biochemically in our experiments, is in general agreement with previous reports based on histologic observations.[2–4,6–8,10] While the levels of hepatic total lipids of treated animals reverted to the levels of the non-cirrhotic controls within the first month, however, our histologic studies indicated that a complete disappearance of stainable fat did not occur, even in late stages of the experiment. One of the reasons for this apparent discrepancy may be found in the fact that livers in control rats did show

moderate amounts of visible fat histologically. At any rate, although few fatty changes and other pathologic alterations were observed in treated cirrhotic rats at the end of the experiment, a significant improvement of their liver function tests was detected at relatively early stages.

TABLE VI

PERCENTAGE OF TdR-^{3}H LABELED HEPATOCYTES IN LARGE AND SMALL NODULES

Group	Weeks following therapeutic diets	Days following TdR-^{3}H injection	Large nodules	Small nodules
2	5	6	2.60	2.80
2	6	3	2.90	2.70
4	6	3	2.87	4.70
2	29	1	3.10	6.60
2	29	1	6.53	6.36
3	29	1	2.20	3.52
3	29	1	0.60	3.70
4	29	1	2.68	2.40

The importance of the regression of fatty changes on the "functional" recovery of this type of cirrhosis could not clearly be established from our experiment.

The reabsorption of hepatic collagen in rats with cirrhosis treated with lipotropic diets has been reported by others [9,10] and is confirmed now in our experiments. Certain important characteristics of the collagen fractions, recently elucidated [23,24] helped in the interpretation of the events occurring in our treated animals. The alkali-soluble fraction has been recognized as the "young" and active collagen which corresponds apparently to the content of reticulin and intralobular connective tissue.[24] A decrease in this soluble fraction might suggest then a decrease in the formation of new collagen. Our data indicated that a significant decrease in new collagen formation did occur at the late stages of the experiment. Changes in soluble collagen preceded those of insoluble collagen.

A close correlation between ductular ("oval") cell proliferation and hepatic fibrosis has been emphasized by Popper, Paronetto, Schaffner and Perez.[25,26] In addition, it has been found in our laboratories that the proliferation of these "oval" cells is a prominent feature of the cirrhosis produced by low-methionine, choline-deficient diets.[27] In the present experiment, despite the use of casein in the diet (which is high in methionine) as the source of protein, some of the results appear to indicate that such correlation exists. The decrease in hepatic DNA content along with the increase in the content of dry defatted tissue per mg of DNA found in groups 3 and 4 suggests a decrease in the number of cells with little cytoplasmic protein. Ductular cells possess this characteristic. The DNA

and DDFT changes were observed at a relatively early stage of the experiment and preceded the changes in soluble collagen.

The decrease in ductular cells in treated groups could not be ascertained from the small number of autoradiographs obtained. On the same basis, however, clearer information regarding the proliferation of hepatocytes was secured. The prominent hepatocytic proliferation, characteristic of group 2, throughout the experiment, decreased in groups 3 and 4 (both received therapeutic diets). This decrease was more conspicuous in the high protein group 4 than in the low protein group 3 at late stages of the experiment, although such was not the case earlier. It would appear that the high protein diet produced a biphasic effect; initial increase and final decrease in hepatocytic proliferation.

Attempts to summarize in schematic form the data obtained in this experiment resulted in a series of graphics included in Text-figure 14. Here, the changes in different factors studied are expressed as a percentage of the changes found in group 2 at the 1- to 4-week period. The area delimited by the lines of groups 2 and 3 would represent the effect of choline supplementation on the regression of hepatic changes, while that limited by the lines of group 3 and 4 shows the effects of high protein. The changes in group 4 were more prominent and occurred generally earlier than in group 3.

The sequences of changes in group 4 could be placed in the following order: 1) decrease in fat; 2) increase in hepatocytic regeneration; 3) decrease in proliferated ductular and other mesenchymal cells; 4) decrease in hepatocytic proliferation; 5) decrease in collagen formation, and 6) increase in collagen absorption.

Since casein, a protein with relatively high proportions of methionine was used in this experiment, the differences between groups 3 and 4 cannot be attributed solely to the high amount of protein in the diet of the latter group. Further studies on the possible role of amino acid composition are indicated.

The presence of islet-like fatty nodules in some animals in groups 3 and 4 and of nodular necrosis in group 2 suggests that possible disturbances in the circulation supplying cirrhotic nodules, might play a role in the progression and regression of cirrhosis.

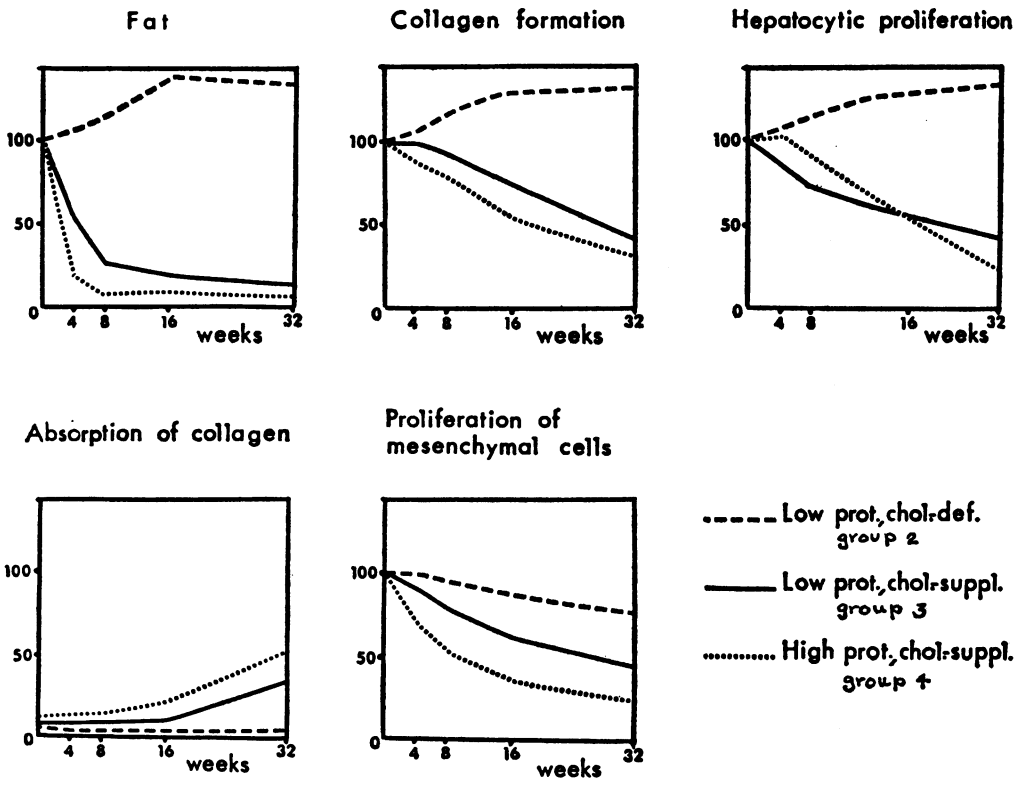
Summary

The recovery process of cirrhosis in choline-deficient rats was studied following the administration of two therapeutic diets supplemented with choline, one low and the other high in protein.

The regression was initiated by a prompt decrease in hepatic fat and followed by a decrease in proliferated ductular and other interstitial

mesenchymal cells, decrease in hepatocytic proliferation, decrease in "new collagen" formation and finally by the reabsorption of collagen.

The sequential changes were qualitatively similar in animals treated with the two different therapeutic diets but were more prominent in those



TEXT-FIG. 14. A graphic summary of the data in phase II of the experiment, expressed as a percentage of the base line taken for group 2 (untreated). The difference between the curves for groups 2 and 3 represents the effect of choline supplementation on the regression of hepatic changes. The difference between the curves for groups 3 and 4 shows the effect of dietary protein.

treated with high protein. Remarkable improvement in body weight, liver weight and hepatic function tests were found in both groups, particularly that receiving the high protein diet. Despite suggestions to the contrary in some of our earlier studies,[28] high protein diets consumed by rats with cirrhosis did stimulate recovery of hepatic function even when large hepatic nodules were still present.

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[*Illustrations follow*]

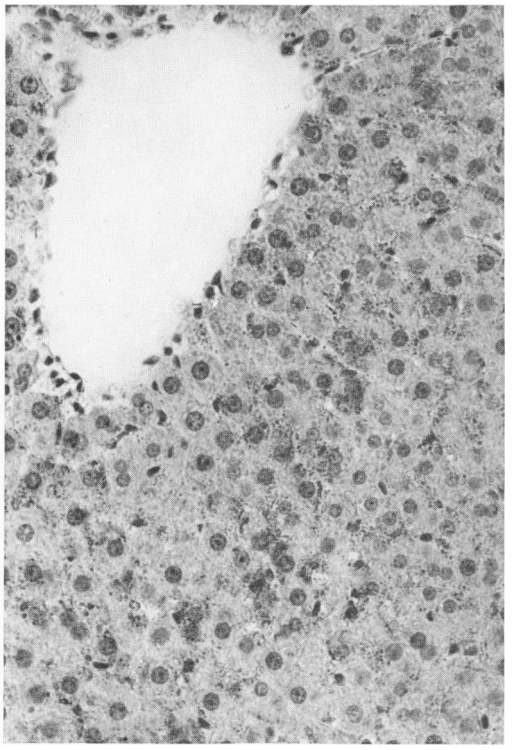
Legends for Figures

Fig. 1. Small droplets of fat appear in the centrilobular hepatocytes in otherwise normal parenchyma. Control rat (group 1). Oil red O (ORO) stain. × 350.

Fig. 2. Droplets and globules of fat are seen in hepatocytes as well as in numerous fatty cysts in the cirrhotic liver (multilobular type). Rat, group 2 (low protein, choline-deficient). ORO stain. × 150.

Fig. 3. Fatty changes occur with perivascular distribution in nodules in a rat with cirrhosis from group 2. ORO stain. × 35.

Fig. 4. The liver in a rat in group 4 (high protein, choline-supplemented) 22 weeks after commencing the therapeutic diet is fibrotic. Visible fat is completely absent in the parenchyma. ORO stain. × 150.



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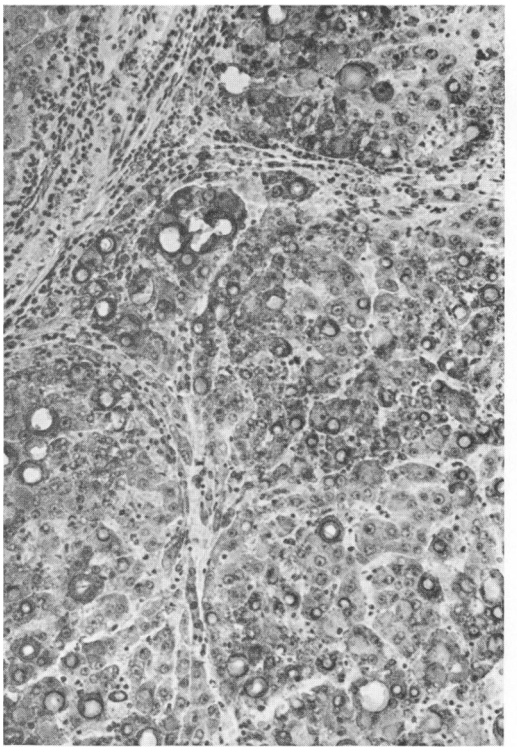

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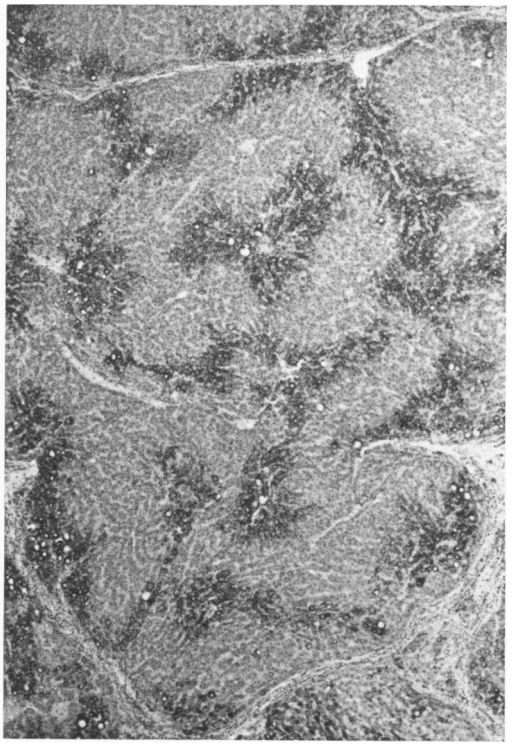

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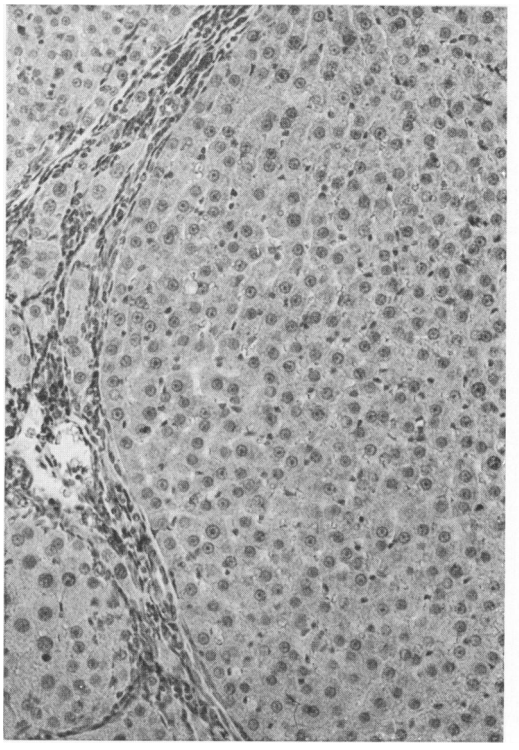

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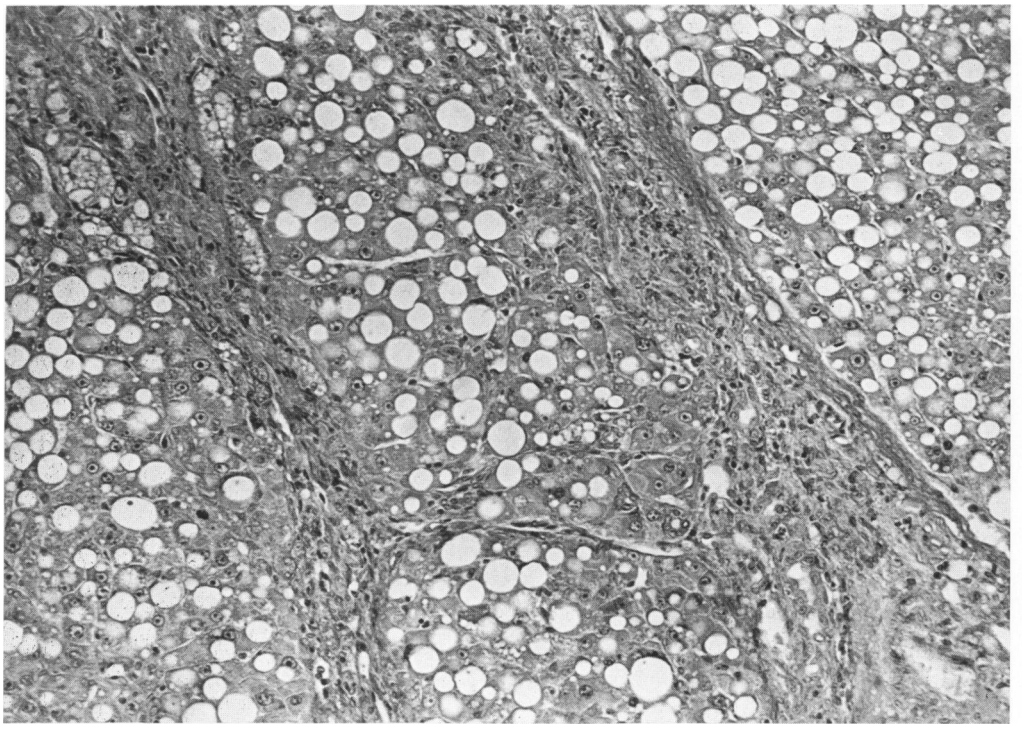
FIG. 5. Fibrous bands are evident in a cirrhotic fatty parenchyma in a rat in group 2 at the end of the experiment (32 weeks of phase II). Masson's trichrome stain. × 150.

FIG. 6. Residual narrow bands of connective tissue appear in the fibrotic liver in a rat from group 4 (high protein, choline-supplemented), 29 weeks after commencing the therapeutic regimen. Masson's trichrome stain. × 150.



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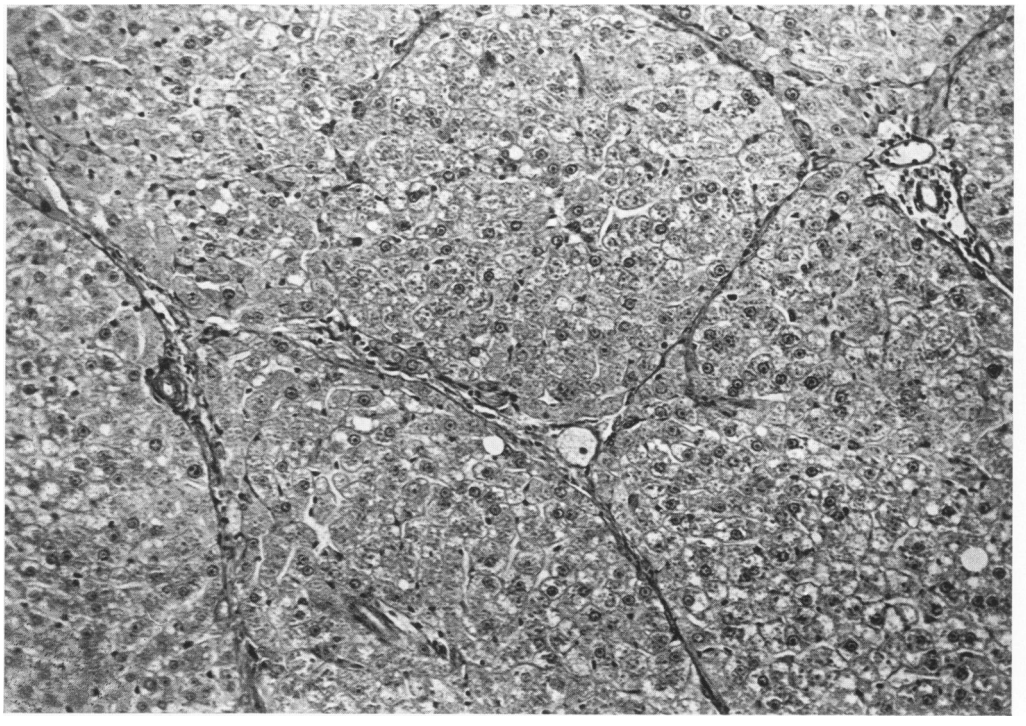

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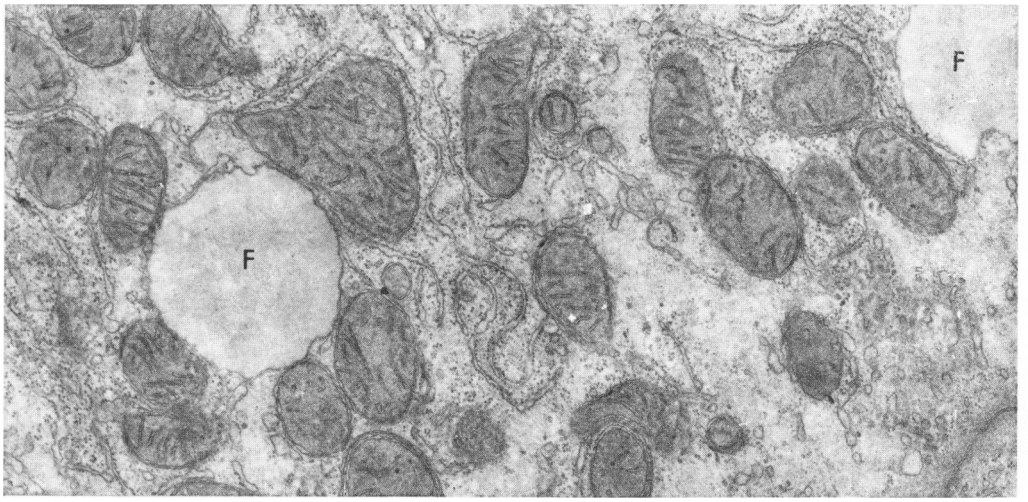
FIG. 7. The overall size and ultrastructural configuration of hepatocytic mitochondria are within normal range. Occasional droplets of fat (F) are found. Control rat (group 1). Lead stain. × 17,000.

FIG. 8. Mitochondria are profoundly altered and there is dilatation of the rough ER in hepatocytes in a cirrhotic rat from group 2 (low protein, choline-deficient). Some mitochondria (arrows) are disintegrating. There are many droplets and globules of fat (F). Lead stain. × 17,500.

FIG. 9. Mitochondria are altered in a hepatocyte in a rat in group 2. Helical filaments (arrow) can be seen in a dilated crista of a spherulated and enlarged mitochondria. Numerous laminated dense bodies are seen in other mitochondria. Lead stain. × 22,000.

FIG. 10. Droplets of fat and deteriorated erythrocytes are intermixed within this trabecular macrophage in a rat from group 2. Polymorphic ceroid granules appear in phagosomes in the cytoplasm in which ferritin granules are numerous. Lead stain. × 4,500.



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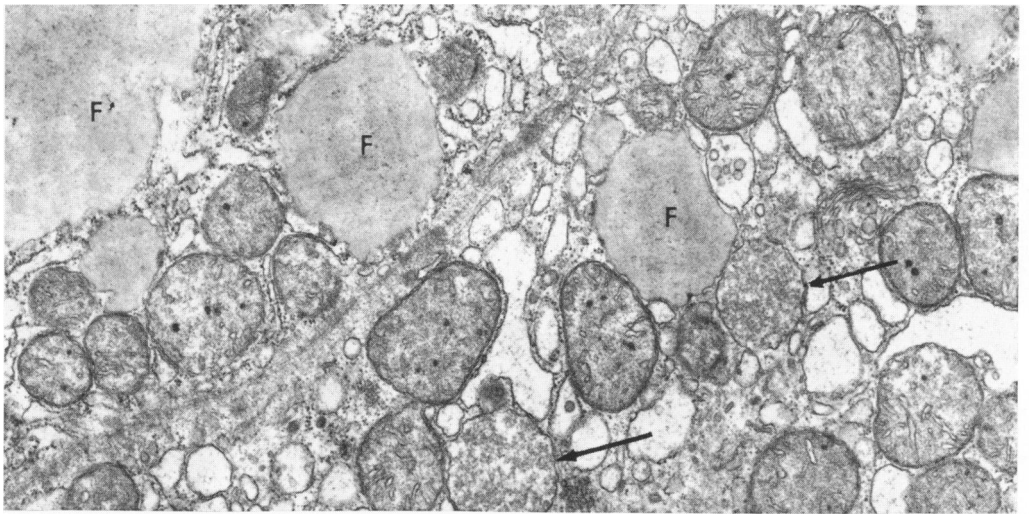

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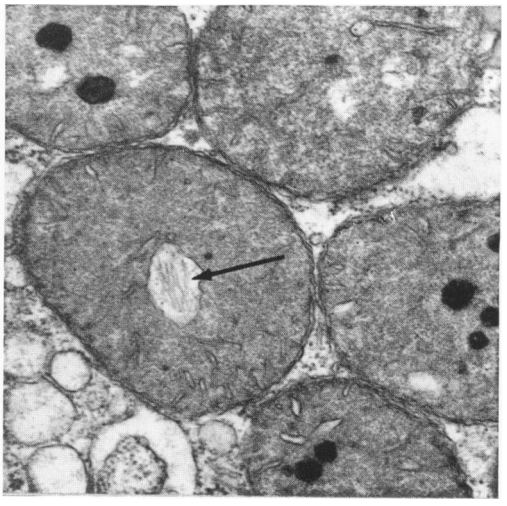

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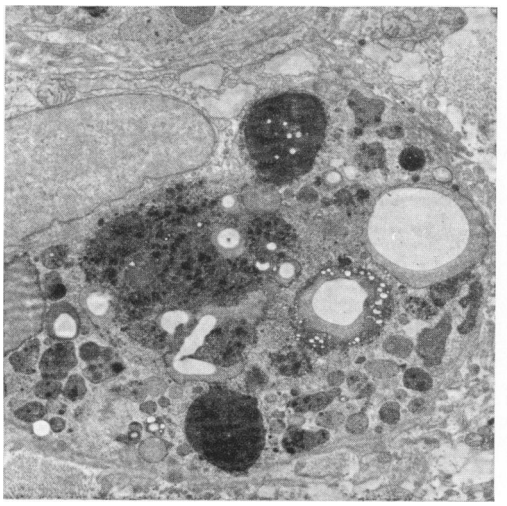

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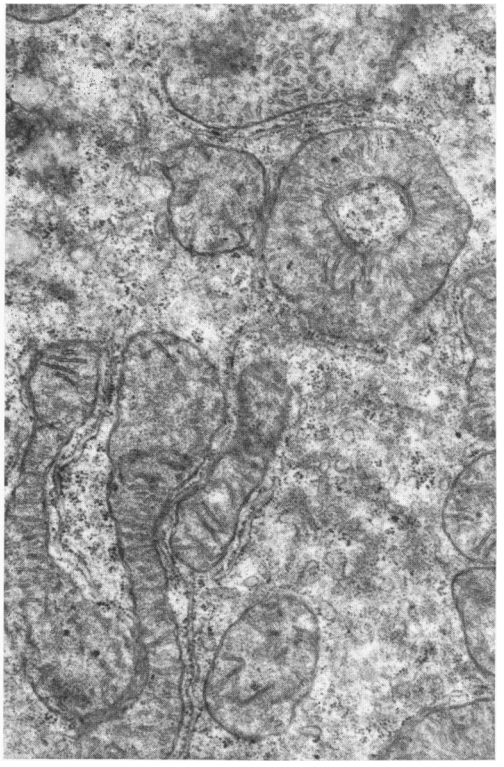
FIG. 11. Mitochondrial alterations are still apparent in this field taken from the cirrhotic liver in a rat in group 3 (low protein, choline-supplemented) after 2 weeks of treatment. Lead stain. × 17,500.

FIG. 12. Twenty-two weeks after the institution of the therapeutic regimen (group 3) ultrastructural appearances of hepatocytes in this rat are much improved. × 17,500.

FIG. 13. A hepatocyte in a rat in group 4 (high protein, choline-supplemented), killed after 29 weeks of treatment, is normal. Lead stain. × 17,500.



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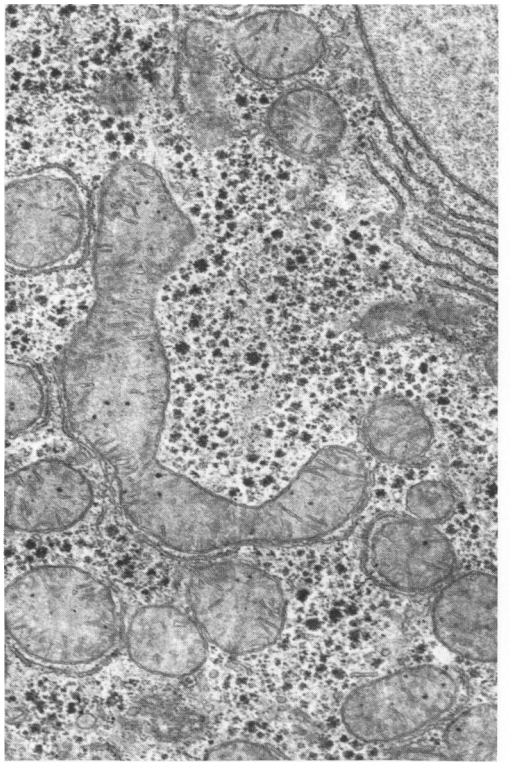

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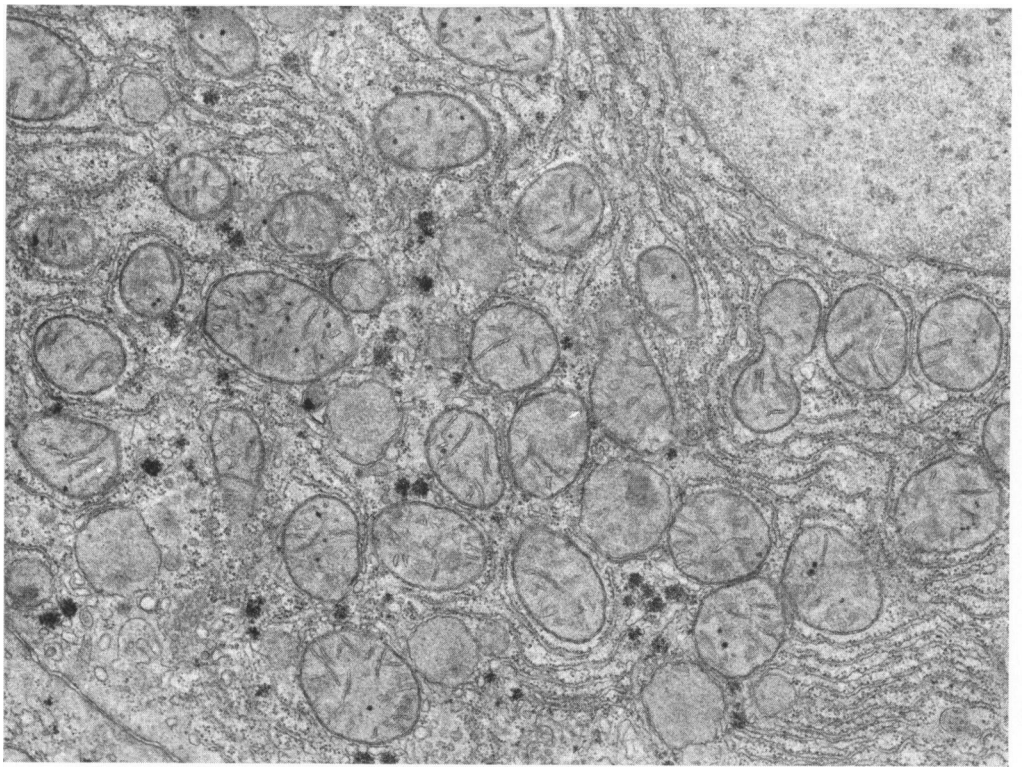

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