THE RECOVERY OF EXPERIMENTAL DIETARY CIRRHOSIS

II. Turnover Changes of Hepatic Cells and Collagen

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The functional and structural features of the changes occurring in rats in which previously induced experimental dietary cirrhosis is regressing under treatment with various isomethionine diets, which are low or high in protein and with or without choline-supplementation, have been reported in a previous study.[1]

Available biochemical and autoradiographic techniques offer the possibility of evaluating in this model the regenerative capacity of hepatocytes, as well as the proliferative characteristics of other cells in a heterogeneous population.[2,3] Kinetic studies on hepatic collagen may also provide important clues for the understanding of certain dynamic aspects of the lesions of experimental dietary cirrhosis. However, there are only a few reports directed at this aim. Neuberger and Slack[4] first showed that collagen in liver and in bones of young normal (growing) rats have more accelerated turnover rates than does the collagen in other organs. In fibrotic livers of ethionine-treated rats, Hutterer, Rubin, and Popper[5] found a rapid turnover rate of the alkali-soluble collagen, while slow turnover of collagen in irreversible cirrhosis produced by carbon tetrachloride has recently been reported by Rubin, Hutterer, and Popper.[6]

In the present experiment, turnover rates of hepatic cells and collagen were studied in cirrhotic rats treated with various therapeutic regimens which, however, all contained equal amounts of methionine (isomethionine).

Material and Methods

The experimental design was the same as previously reported.[1] One group of rats was maintained for 5 months (Phase I) on a basal diet low in protein, choline, and Vitamin B_{12}. Another group was pair-fed, the basal diet supplemented with 0.35 gm. of choline chloride per 100 gm. diet from the beginning; these animals were used as

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noncirrhotic controls. At the end of Phase I, choline-deficient rats developing cirrhosis (as ascertained by laparotomy) were allotted to four groups so that variations in the degree of the lesions were equally represented in each group. These rats received a low-protein, choline-deficient diet (Group 2A); a low-protein, choline-supplemented diet (Group 2B); a high-protein, choline-deficient diet (Group 3A); or a high-protein, choline-supplemented diet (Group 3B). The noncirrhotic controls were allotted to two groups and received a low-protein, choline-supplemented diet (Group 1A) and a high-protein, choline-supplemented diet (Group 1B). The total methionine contents of all diets employed in Phase II were equal (methionine in the protein plus any supplement of amino acid methionine).

At the beginning of this therapeutic period (Phase II), the rats of each group received intraperitoneal injections of 0.1μc. of uniformly labeled ^{14}C-proline * (sp. act. 8.2 mc./mM) per gram of body weight, 0.8μc. of ^{3}H-thymidine † (sp. act. 0.36 c./mM) per gram of body weight, or both.

For the study of hepatic cell turnover rates, 3 rats from each group were killed by decapitation at 1, 3, 6, 15, and 30 days after injection, while for collagen turnover an additional 3 rats from each group were also killed at 60 and 90 days after injection.

Autoradiographs from the median hepatic lobes were prepared following the technique of Messier and Leblond [7] (except for rats killed at 30 days). Labeled hepatocytes and "other cells" (which include ductular and mesenchymal cells) were identified. Numbers of grains overlying at least 200 labeled cells were counted and the ratio of hepatocytic grains to the total number was determined. This ratio was multiplied by the specific activity of DNA to determine the activity of DNA in hepatocytes.

Hepatic remnants were frozen in dry ice and stored in the electric freezer at −20° C. for later biochemical analyses.

According to the method of Gerber, Gerber, and Altman,[8] the hepatic DNA was isolated as follows: livers were homogenized in a Waring Blender® with 20 volumes of cold 0.1 M NaCl-0.05 M sodium citrate solution and then centrifuged for 10 min. The turbid supernatant was discarded and the sediment was resuspended in the same solution and recentrifuged. This procedure was repeated over 10 times until the supernatant was clear. The final sediment was suspended in cold 10% NaCl solution and kept overnight in a refrigerator at −4° C. An insoluble residue was removed by centrifugation. The DNA in the supernatant solution was precipitated by the addition of two volumes of cold ethanol and finally dissolved in distilled water. DNA concentration was determined by the method of Webb and Levy.[9] Counting of ^{3}H-activities in DNA was done in a liquid scintillation counter in which internal standards for quenching corrections were used. A solution containing 1000 ml. of dioxane, 100 ml. of absolute ethanol, 100 gm. of toluene, 0.5 gm. of 1-4-bis-2 (5-phenyloxazolyl) benzene, and 7.0 gm. of 2,5-diphenyloxazole was used as scintillation solution. Results were expressed as counts per minute per milligram of DNA.

Alkali-soluble collagen was isolated from hepatic homogenates by the methods of Singer *et al.*,[10] except that further treatment with 5% hot trichloroacetic acid was performed according to the technique of Fitch, Harkness, and Harkness.[11] The insoluble residue in 0.1 N NaOH was then washed with distilled water several times and autoclaved for 3 hr. with 3 ml. of distilled water. After centrifugation, the supernatant was prepared for the estimation of hydroxyproline as insoluble collagen. After drying, both alkali-soluble and alkali-insoluble fractions were hydrolyzed with 6 N HCl at 140° C. for 3 hr. Hydroxyproline content and ^{14}C-activities in hydroxyproline of both collagens were estimated according to the procedure of Prockop, Udenfriend, and Lindstedt.[12]

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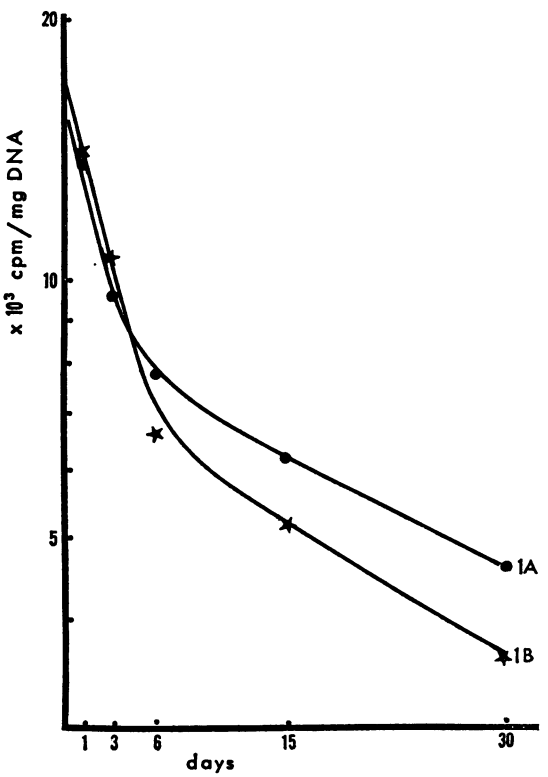
All data were statistically analyzed. Regression lines for the logarithm of ^{3}H-activities and ^{14}C-activities against postinjection times were computed by the least-square method. Half-life times of DNA and collagen were calculated from their respective regression coefficients.

Results

Turnover of Hepatic Cells

DNA. The specific activities of hepatic DNA in all cirrhotic rats (Groups 2A, 2B, 3A, and 3B) were significantly higher than in the noncirrhotic controls of Groups 1A and 1B (Table I). Between groups of cirrhotic animals these activities were slightly higher in those receiving high-protein diets (Groups 3A and 3B) than in those fed low-protein diets (Groups 2A and 2B) at the beginning of the experiment.

The highest activities of hepatic DNA were observed 1 day after the isotope injection in control groups (1A and 1B) and 3 days after injection in the cirrhotic rats, except in the case of those treated with high-protein, choline-deficient diets (Group 3A). The activities in all groups decreased with time.



Text-fig. 1. Time gradients of ^{3}H-specific activities of DNA in control noncirrhotic Groups 1A (low-protein, choline-supplemented diet) and 1B (high-protein, choline-supplemented diet).

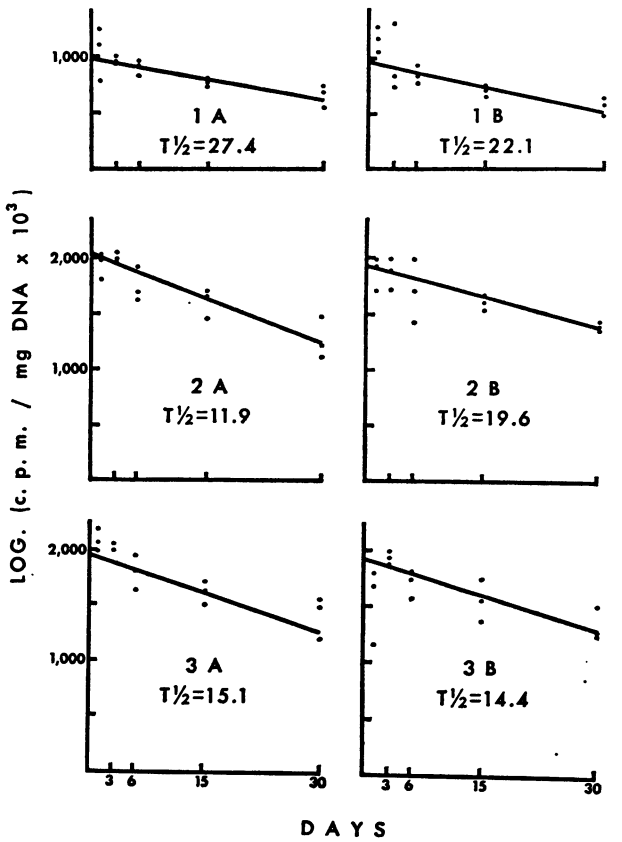
TABLE I

SPECIFIC ACTIVITIES OF HEPATIC DNA

Days after injection	*Group*					
	1A	*1B*	*2A*	*2B*	*3A*	*3B*
1	12.80 ± 5.16	14.20 ± 3.26	81.80 ± 19.99	73.00 ± 20.53	119.20 ± 19.94	82.10 ± 40.05
3	9.30 ± 0.60	10.60 ± 6.55	99.20 ± 10.35	78.50 ± 18.93	106.90 ± 8.62	84.60 ± 11.02
6	7.80 ± 0.88	6.90 ± 0.96	65.30 ± 12.09	56.90 ± 27.66	63.80 ± 18.15	51.20 ± 10.63
15	6.20 ± 0.13	5.10 ± 0.47	40.60 ± 9.07	39.00 ± 3.44	41.50 ± 7.77	38.00 ± 13.33
30	4.60 ± 0.79	3.60 ± 0.49	19.60 ± 7.12	25.50 ± 1.31	25.80 ± 7.71	21.10 ± 8.15

Expressed as: specific activity $\times 10^3$ cpm/mg. DNA ±S.D.

Time-gradients of specific activities of DNA in normal groups are shown in Text-fig. 1. In this graph the average values of the logarithm of ^{3}H-activities of 3 rats from each group were plotted against post-injection times. The rates of decay of activities seemed to be nearly curvilinear with time, but curvilinearity was not confirmed statistically. Therefore, the regression lines were computed, disregarding the values at Day 1. The regression lines of hepatic DNA in the different groups are shown in Text-fig. 2.



TEXT-FIG. 2. Regression lines and half-life times of ^{3}H-specific activities of hepatic DNA. Groups and diets: 1A: low-protein, choline-supplemented; 1B: high-protein, choline-supplemented; 2A: low-protein, choline-deficient; 2B: low-protein, choline-supplemented; 3A: high-protein, choline-deficient, 3B: high-protein, choline-supplemented.

Half-life values computed from these time gradients, summarized in Table II, were shorter for cirrhotic groups than for control groups. The differences of half-life times between the rats fed low-protein diets (control Group 1A) and those in the choline-deficient, cirrhotic groups (2A and 3A) were statistically significant, but insignificant between

TABLE II

HALF-LIFE TIMES OF HEPATIC DNA, HEPATOCYTES, AND "OTHER CELLS"

Group	*Hepatic DNA*	*Hepatocytes*	*"Other cells"*
1A	27.4	15.0	7.1
1B	22.1	—	5.2
2A	11.9	9.8	6.0
2B	19.6	—	6.7
3A	15.1	6.5	6.5
3B	14.4	8.5	7.4

Expressed in days.

TABLE III

TOTAL ^{3}H ACTIVITIES OF HEPATIC DNA, HEPATOCYTES, AND "OTHER CELLS"

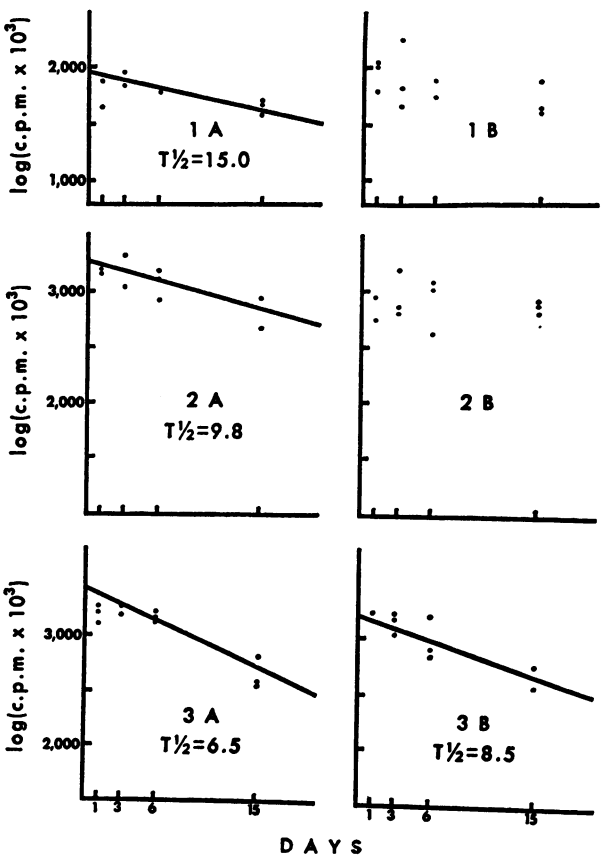
Days after injection	*Hepatic DNA*	*% of grains in hepatocytes*	*Hepatocytes*	*"Other cells"*
		GROUP 1A		
1	161.0	24.0	39.9	122.4
3	160.1	43.2 *	79.2 *	102.9 *
6	135.5	51.8 *	64.6 *	58.1 *
15	74.6	58.3	44.7	29.9
		GROUP 1B		
1	221.3	44.0	92.0	129.3
3	175.4	62.0	97.1	135.2
6	88.5	66.7 *	67.4 *	23.6 *
15	68.1	78.1	53.1	15.0
		GROUP 2A		
1	3045.2	35.9 *	1485.5 *	2282.5 *
3	2973.3	50.6 *	1597.4 *	1557.0 *
6	2327.3	55.3	1191.0	1177.6
15	1326.8	61.3 *	670.8 *	430.1 *
		GROUP 2B		
1	2051.9	22.7	505.3	1424.5
3	1771.6	48.2	966.4	1005.2
6	1510.4	58.7	890.5	620.0
15	1041.5	71.8	738.4	303.1
		GROUP 3A		
1	3544.8	44.8	1557.2	1654.2
3	3141.1 *	53.5 *	1680.4 *	1460.6 *
6	2146.0	62.7	1483.6	662.4
15	969.8	55.3	523.5	446.3
		GROUP 3B		
1	1782.9	30.6 *	732.9 *	1218.4 *
3	2207.3	55.7	1259.4	981.2
6	1602.9	73.3	1016.2	586.7
15	1045.2	63.5 *	462.7 *	291.2 *

Values (in cpm $\times 10^3$) are the means from 3 rats, except those followed by asterisk (*) which are means from 2 rats.

control groups (1A and 1B) and the cirrhotic rats fed choline-supplemented diets (Groups 2B and 3B).

Among the cirrhotic groups, rats treated with low-protein, choline-deficient diets (Group 2A) showed the shortest half-life; the longest was found in Group 2B (low-protein, choline-supplemented diet). Cirrhotic rats treated with high-protein diets (Groups 3A and 3B) had slightly shorter times than those treated with low-protein, choline-supplemented diets (Group 2B). The differences of half-life time among cirrhotic groups were not statistically significant.

Hepatocytes. Percentages of hepatocytic grains in autoradiographs increased with time in every group (Table III). The activities of DNA in hepatocytes decayed with time (Text-fig. 3). For computing regression lines, the values at Day 1 were also disregarded. A statistically significant regression coefficient was not obtained either in the rats fed high-protein diets (control Group 1B) or in those given low-protein,

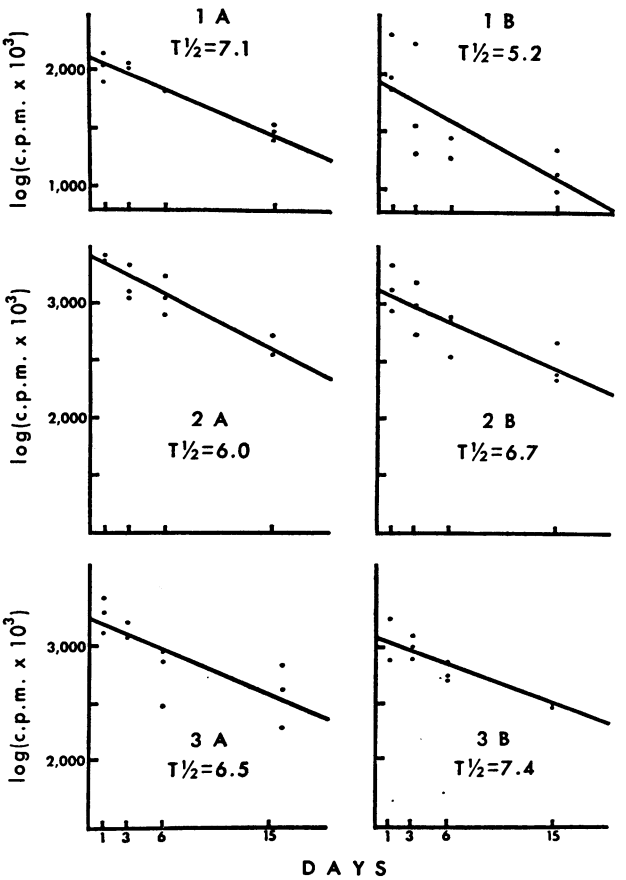


TEXT-FIG. 3. Regression lines and half-life times of total ^{3}H-specific activities in hepatocytes of groups treated as indicated in Text-fig. 2.

choline-supplemented diets (Group 2B) because of large individual variations.

The calculated half-life times are shown in Table II. Cirrhotic groups had shorter values than the noncirrhotic controls. Longer life span of hepatocytes was found in the cirrhotic rats fed low-protein diets (Group 2A) than in any of those treated with high-protein diets (Groups 3A and 3B). On the other hand, values for the cirrhotic group (3B) fed choline-supplemented diets were higher than for the choline-deficient group (3B). The differences of half-life times among each group were not statistically significant, except between control Group 1A (low-protein diet) and Group 3A (high-protein, choline-deficient diet).

"Other Cells." The ^{3}H-activities of DNA in "other cells" decayed with time. The regression lines of ^{3}H-activities and calculated half-life times of the different groups are shown in Text-fig. 4 and Table II. The



TEXT-FIG. 4. Regression lines and half-life times of total ^{3}H-specific activities in "other cells" of groups treated as indicated in Text-fig. 2.

TABLE IV

SPECIFIC ACTIVITIES OF ^{14}C-HYDROXYPROLINE IN SOLUBLE COLLAGEN

Days after injection	Group					
	1A	1B	2A	2B	3A	3B
1	100.1	77.6	23.2	22.7	32.0	37.6
3	85.8	70.2	28.2	63.5	46.8	30.6
6	52.8	64.6	72.1	26.4	39.6	29.9
15	46.3	34.6	29.9	29.7	18.2	17.6
30	10.8	8.3	11.6	7.4	2.7	2.5
60	2.1	1.2	1.4	1.1	0.6	1.0
90	0.4	0.3	—	0.8	0.2	0.2

Values are the means from 3 rats and are expressed in cpm $\times$ 10^3.

times in the control rats fed low-protein diets (Group 1A) and the rats given a high-protein, choline-supplemented diet (Group 3B) were slightly longer than in the control rats given a high-protein diet (Group 1B) and other cirrhotic groups (2A, 2B, and 3A), respectively. However, the differences of life spans among these groups were not statistically significant.

Turnover of Collagen

Alkali-soluble Collagen. The maximum specific activities of ^{14}C-hydroxyproline in alkali-soluble collagen were obtained at 1- to 3-day periods, except in Group 2A. The activities decreased with time (Table IV).

The regression lines and half-life times of soluble collagen in the different groups are shown in Text-fig. 5 and Table V. The shortest life span was found in Group 2A (low-protein, choline-deficient diet) and the longest in Group 3B (high-protein, choline-supplemented diet). Cirrhotic groups, except Group 3B, showed shorter life spans than the control groups. Groups given choline-supplemented diets showed longer life spans than choline-deficient groups, but these differences were not statistically significant.

Alkali-insoluble Collagen. The highest specific activities of ^{14}C-hydroxyproline in insoluble collagen were observed within 15 days and the activities decreased with time (Table VI). The regression lines and

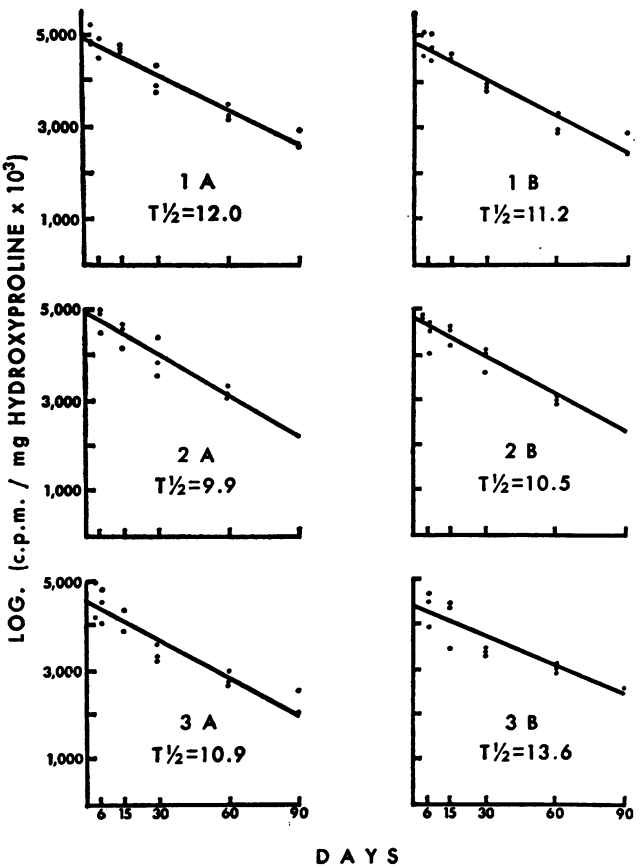
TABLE V

HALF-LIFE TIMES OF COLLAGEN

Solubility in alkali	Group					
	1A	1B	2A	2B	3A	3B
Soluble	12.0	11.2	9.9	10.5	10.9	13.6
Insoluble	121.4	82.5	190.8	72.2	89.6	73.6

Expressed in days.

half-life times of insoluble collagen in the different groups are shown in Text-fig. 6 and Table V. The half-life time of insoluble collagen in Group 2A (low-protein, choline-deficient diet) appeared to be about 200 days;



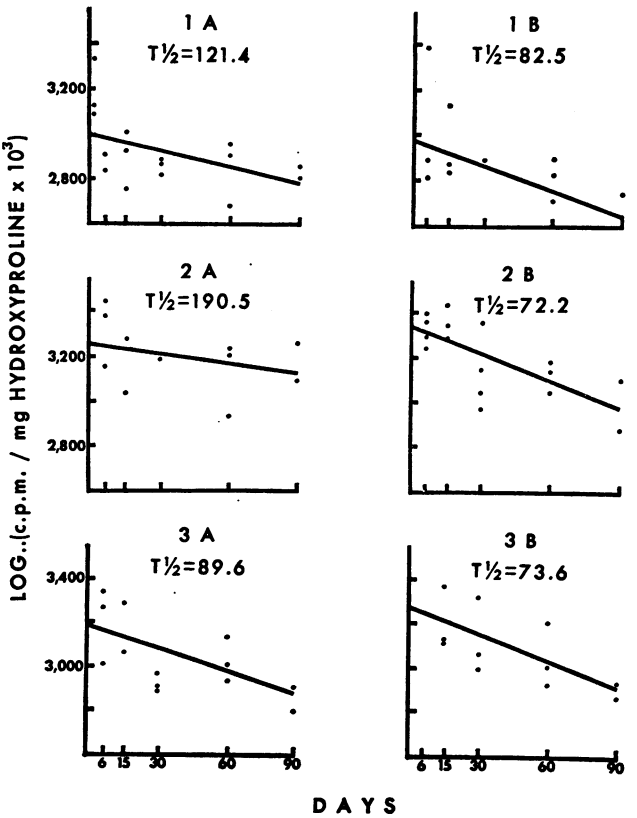
TEXT-FIG. 5. Regression lines and half-life times of ^{14}C-hydroxyproline in alkali-soluble collagen of groups treated as indicated in Text-fig. 2.

TABLE VI

SPECIFIC ACTIVITIES OF ^{14}C-HYDROXYPROLINE IN ALKALI-INSOLUBLE COLLAGEN

Days after injection	*Group*					
	1A	*1B*	*2A*	*2B*	*3A*	*3B*
1	1.57	0.75	1.80	2.51	1.85	1.44
3	0.84	1.08	1.96	1.36	2.28	1.64
6	1.01	1.30	2.20	1.99	1.66	1.16
15	0.80	0.94	1.51	2.28	1.52	1.73
30	0.72	0.53	1.23	1.61	0.88	1.36
60	0.72	0.65	1.40	1.33	1.12	1.15
90	0.65	0.46	1.53	1.01	0.74	0.79

Values are the means from 3 rats expressed in cpm $\times$ 10^3.



TEXT-FIG. 6. Regression lines and half-life times of ^{14}C-hydroxyproline insoluble collagen of groups treated as indicated in Text-fig. 2.

however, no statistically significant coefficient was obtained in this group. The cirrhotic groups treated with choline (2B and 3B) showed shorter life spans than the choline-deficient groups. The cirrhotic and normal groups treated with the high-protein diet showed shorter life spans than the corresponding groups treated with low-protein diets, except those cirrhotic groups given choline-supplemented diet. However, the differences were not statistically significant.

DISCUSSION

Variable half-lives of hepatic cells have been reported by several workers.[2,3,8] The replacement time of total hepatic DNA in normal rats was calculated as 20 days by Gerber, Gerber, and Altman.[8] MacDonald[2] estimated the half-life time of hepatocytes as 195–453 days in normal rats and 27 days in choline-deficient cirrhotic rats. Rubin *et al.*[3] have reported that half-life times of hepatic DNA and ductular cells were

about 10 days and 7.7 days, respectively, in hepatic fibrosis produced by ethionine intoxication. These variations may be attributed to the differences in techniques of determination and in the experimental conditions. Our results are more in agreement with those of Gerber, Gerber, and Altman [8] and Rubin *et al.*[3] Although linear decay curves of hepatic DNA activities have been reported by these groups of workers, it is difficult to reconcile them with the fact that ductular cells have different life spans from the "other" hepatic cells as shown in the experiments of Rubin *et al.*[3] However, curvilinearity of decay curves of hepatic DNA activities could not be confirmed statistically in our experiments. The changes of hepatic DNA would rather correspond to those of hepatocytes than those of the "other cells" because the latter have faster life spans than hepatocytes and the values at Day 1 were disregarded on calculating the life span of hepatic DNA.

The changes of life span in hepatic cells reflect the sum of cell death and/or the sum of cell division which caused a relative decrease of DNA activity by dilution with newly formed cells. All cirrhotic groups showed higher ^{3}H-activities of DNA in hepatocytes than the control groups throughout the experiment. Shorter life spans of hepatocytes were observed in cirrhotic groups than in the corresponding normal controls. Since all cirrhotic groups showed higher SGOT activities,[1] it appears that shortening of life spans of hepatocytes in choline-deficient cirrhosis is associated not only with an increase in the rate of new formation of hepatocytes, but also with an increase in the number of dying cells.

A shorter life span of hepatocytes was found in choline-deficient rats treated with the high-protein diet (Group 3A) than in the corresponding group (2A) given a low-protein diet. Shorter life spans of hepatocytes in normal and cirrhotic rats treated with high-protein diets than in the corresponding groups given low-protein diets were suggested from the changes of hepatic DNA. In addition, the high-protein-treated groups (3A and 3B) showed higher ^{3}H-activities of DNA in hepatocytes than in low-protein-treated groups (2A and 2B) at the beginning of the experiment. These results would indicate that high-protein diets accelerate the proliferation of hepatocytes. On the other hand, hepatocytes of the choline-supplemented cirrhotic group (3B) showed a longer life span than those of the choline-deficient group (3A). Longer life spans in choline-supplemented groups than in choline-deficient groups were also suggested from the changes of hepatic DNA. Although choline-deficient groups showed higher serum glutamic oxalacetic transaminase activity than in choline-supplemented groups during the experimental period, necrosis was histologically found only in a few cases of choline-deficient groups.

Choline-deficient groups (2A and 3A) showed higher activities of DNA in hepatocytes than choline-supplemented groups at the beginning of the experiment. These results suggested that prolongation of half-life times in choline-supplemented groups may be attributed to a decrease in hepatocytic regeneration rather than to a decrease in total cell death. Decreased hepatocytic regeneration in cirrhotic rats treated with choline-supplemented diets was also suggested by our previous results.[13]

Nodular regeneration of hepatocytes is a characteristic feature of hepatic cirrhosis. However, the magnitude and the pathogenic significance of hepatocytic regeneration in the recovery phase of cirrhosis is still controversial. Cameron and Karunaratne[14] considered that the regenerative capacity of hepatocytes is impaired in cirrhosis produced by carbon tetrachloride. On the other hand, increased regeneration of hepatocytes of rats with dietary cirrhosis treated with lipotropic regimens has been observed by several workers.[15–18] Ohta, Zaki, and Hoffbauer[19] showed that the regenerative nodules continued to increase in size even though fat disappeared and the fibrous trabeculae became increasingly thin and stretched during treatment with lipotropic diets. Using mice, Quinn and Higginson[20] reported that nodular hyperplasia in cirrhosis produced by various means continued independently of the inciting stimulus, while fibrosis, necrosis, inflammation and ductular cell proliferation decreased on withdrawal of stimuli. However, in the above-mentioned reports, regeneration of hepatocytes was evaluated only by histologic findings or by changes in liver weight and hepatic protein content.[14–20] These methods are not highly accurate and may invite debatable conclusions. At any rate, increased hepatocytic regeneration was demonstrated by autoradiographic studies using ^{3}H-TdR in choline-deficient rats.[21,22] In addition, Pechet and MacDonald[23] reported that hepatocytes in cirrhotic livers showed the same regenerative capacity as in normal livers following partial hepatectomy.

In our present experiment, significant shortening of half-life times of hepatocytes in cirrhotic rats was observed, suggestive of increased hepatic cell regeneration. On the other hand, choline-supplemented diets prolonged the half-life times of hepatocytes with a consequent decrease in hepatic cellular pathologic regeneration or degeneration. This decrease in hepatocytic regeneration was correlated with the improvement of hepatic function tests and decreases in hepatic fat and collagen. Furthermore, an increase in hepatocytic regeneration was observed in the groups fed high-protein diets. This increase was also correlated with restoration of cirrhosis. Sellers, Lucas, and Best[24] showed that lipotropic factors which facilitate hepatic regeneration had a beneficial

effect on the recovery from cirrhosis produced by carbon tetrachloride. These phenomena would suggest that the increase in hepatocytic regeneration in cirrhosis differs in nature from that observed under the influence of high-protein diets. While the former may be due to deterioration of hepatic function and structure, the latter may be attributed to the improvement of these parameters. Consequently, choline-supplemented diets may decrease the rate of abnormal regeneration of hepatocytes responding to previous hepatic damage and the high-protein diets may increase the rate of regeneration more directly.

It is very difficult to interpret the results of the "other cells," because this designation included several different types. We cannot deal with the data of normal groups in the same way as we do the data of cirrhotic groups. In normal rats, these cells were mainly littoral with a few ductular ones in which the ratio of grains to total grains was under 5% in every case. Therefore, the regression lines in normal rats may correspond to changes in littoral cells. It seemed that a high-protein diet accelerated the turnover rate of littoral cells in normal rats.

In cirrhotic rats, the interpretation of the data is even more complicated because a predominant increase of ductular cells in fibrous tissue was found in this type of cirrhosis, and considerable numbers of grains were also encountered over littoral cells. Furthermore, a precise differentiation of ductular from other cells in fibrous tissue is almost impossible by light microscopy in this experimental condition. Life spans of "other cells" in cirrhotic groups resembled those of ductular cells, as reported by Rubin *et al.*[8] in ethionine-induced hepatic fibrosis. However, the group given the high-protein, choline-supplemented diet showed relatively longer half-life times. It might be possible to consider that this change is a manifestation of the change in the cellular content of fibrous bands. We have no definitive evidence to support this notion without further studies.

In the present study, the differences of half-life times among the different groups were not statistically significant, except in a few groups, because of the large individual variations of the values obtained. It would need further studies using larger numbers of animals to obtain more conclusive data; because of the time and expense involved in carrying them out, it was decided instead to report our observations as they stand since a number of conclusions can still be drawn.

For the calculation of half-life times of hepatic collagen, the values for specific activities of ^{14}C-hydroxyproline were not corrected for changes in body weight. It was assumed that changes in collagen content and organ weight were proportional to those in body weight. The validity of this assumption seems somewhat doubtful in cirrhotic livers, although

Group 3B showed an 80% increase in body weight during the experiment. In control groups, the increase in body weight was relatively small and remained within 15%.

Relatively large variations in ^{14}C activity for each of the points on all the curves were observed, especially in those of cirrhotic groups. When these points were incorporated into composite curves, however, they fell into distinct patterns.

Before the introduction of isotopic techniques for the study of protein metabolism, collagen was considered by some to be a metabolically inert protein. Neuberger and Slack [4] using ^{14}C-glycin showed that collagens in various organs are continually, but slowly, replaced and they suggest that collagen in liver and bone have higher turnover rates than those in other organs. Half-life times in skin or total body collagen of normal rats were calculated by several workers [25–29] as 28–150 days in young rats and about 300 days in adult rats. Collagens in muscle, intestine, and bone showed shorter half-life times than in skin in the report of Gerber, Gerber, and Altman.[27] Half-life times of soluble collagen were computed to be 15–25 days in skin of young rats. Gerber, Gerber, and Altman [27] calculated half-life times of liver collagen as 30 days in young normal rats.

In our experiment, time gradients of ^{14}C-activity in total collagen were resolved into 2 components. The changes in faster components were paralleled by those in alkali-soluble collagen and the changes in slower component corresponded to those in alkali-insoluble collagen. In control groups, half-life times were 135–270 days for Group 1A (low-protein diet) and 75–115 days for Group 1B (high-protein diet). Half-life times of soluble collagen were 10 days in both control groups. These values are lower than those reported in the literature [27–29] but the differences may be due to the older age (5 months) of our rats. The control group given a high-protein diet showed a shorter life span of insoluble collagen than did the control group given a low-protein diet. This result would be supported by the experiments of Bavetta, O'Day, and Bekhor [30] who found an enhancement of collagen synthesis induced by large amounts of dietary casein.

In pathologic conditions, Hutterer, Rubin, and Popper [5] reported an extremely rapid turnover rate of soluble collagen for the livers of rats with hepatic fibrosis produced by ethionine, but the details of these data are not available. Rubin, Hutterer, and Popper [6] observed a half-life time of 20–30 days in reversible cirrhosis produced by carbon tetrachloride. These authors have calculated two different half-life times (30 and 160 days) in irreversible cirrhosis. In our experiment, both choline-supplemented cirrhotic groups (2B and 3B) showed shorter life spans

of insoluble collagen than did choline-deficient cirrhotic groups (2A and 3A). The soluble collagen in both choline-supplemented groups (2B and 3B) showed longer life spans than both choline-deficient groups (2A and 3A). Rats of Group 2A (choline-deficient, low-protein diet) showed the shortest life span of soluble collagen and the longest life span of insoluble collagen. Choline-deficient cirrhotic groups (2A and 3A) showed shorter life spans of soluble collagen, and longer life spans of insoluble collagen than did the control noncirrhotic groups (1A and 1B). In the choline-supplemented cirrhotic groups (2B and 3B), the life span of soluble collagen were almost the same or longer, and of insoluble collagen almost the same or shorter than that of control noncirrhotic groups (1A and 1B).

Our data suggest that new formation of collagen was accelerated and reabsorption of formed collagen was delayed in untreated cirrhosis. Absorption of collagen was prominently enhanced and new formation of collagen was retarded in cirrhotic livers of rats treated with diets supplemented with choline. On the other hand, high-protein diets (with choline) not only enhanced absorption of collagen, but also did not inhibit formation of collagen in cirrhotic and normal conditions.

The difference in specific activity of insoluble collagen between the nontreated cirrhotic group (2A) and the noncirrhotic control groups (1A and 1B) was remarkable and the differences in specific activity of soluble collagen were less prominent. These results suggest that a delay in collagen absorption is a more important factor in determining the rate of accumulation of hepatic collagen in this type of cirrhosis than is the increased formation of new collagen. Similar observations have been reported for skin collagen by Nimni and Bavetta.[29]

Summary

Turnover rates of hepatic cells and of collagens in cirrhotic livers of choline-deficient rats treated with choline-supplemented and high-protein diets were compared with the changes in the nontreated cirrhotic rats and in noncirrhotic control animals.

In cirrhotic rats, life spans of hepatocytes were shorter (9.8 days in the choline-deficient group given a low-protein diet, 6.5 days in the choline-deficient group given a high-protein diet, and 8.5 days in the group given a high-protein, choline-supplemented diet) than in control groups (15 days in the group given a low-protein, choline-supplemented diet). High-protein diets enhanced hepatocytic renewal rates in both normal and cirrhotic rats. Choline supplementation delayed the turnover rates of hepatocytes which were accelerated in hepatocytes of cirrhotic rats treated with choline-deficient regimens. It is suggested that there

are two types of hepatocytic regeneration: one type responding to the deterioration of hepatic structure and function accompanying the development of cirrhosis induced by choline deficiency, and the other reflecting hepatic improvement occurring as a more direct effect of treating the animals with high-protein diets.

Choline-supplemented diets enhanced absorption of collagens and delayed their new formation in cirrhotic rats. High-protein diets enhanced absorption of collagen in both cirrhotic and noncirrhotic animals.

REFERENCES

1. TAKADA, A., PORTA, E. A., and HARTROFT, W. S. The recovery of experimental dietary cirrhosis. I. Functional and structural features. *Amer J Path 51:*929–957, 1967.
2. MACDONALD, R. A. "Lifespan" of liver cells. Autoradiographic study using tritiated thymidine in normal, cirrhotic, and partially hepatectomized rats. *Arch Intern Med 107:*335–343, 1961.
3. RUBIN, E., HUTTERER, F., DANAN, G., and POPPER, H. Deoxyribonucleic acid turnover in a heterogeneous population. Life span of hepatic ductular cells. *Lab Invest 12:*657–662, 1963.
4. NEUBERGER, A., and SLACK, H. G. B. The metabolism of collagen from liver, bone, skin and tendon in the normal rat. *Biochem J 53:*47–52, 1953.
5. HUTTERER, F., RUBIN, E., and POPPER, H. Turnover of hepatic cells and fibers during the development of and recovery from hepatic fibrosis. (abst.) *Fed Proc 21:*303, 1962.
6. RUBIN, E., HUTTERER, F., and POPPER, H. Reversibility of cirrhosis related to collagen and interstitial cell turnover. (abst.) *Gastroenterology 48:*504, 1965.
7. MESSIER, B., and LEBLOND, C. P. Preparation of coated radioautographs by dipping sections in fluid emulsion. *Proc Soc Exp Biol Med 96:*7–10, 1957.
8. GERBER, G., GERBER, G., and ALTMAN, K. I. The catabolism of tissue nucleic acid in the rat. I. The replacement time of deoxyribonucleic acid. *J. Biol Chem 235:*1433–1436, 1960.
9. WEBB, J. M., and LEVY, H. B. A sensitive method for the determination of deoxyribonucleic acid in tissues and microorganisms. *J Biol Chem 213:*107–117, 1955.
10. SINGER, E. J., HUTTERER, F., KENT, G., ZAKI, F. G., and POPPER, H. Hepatic fibrosis. Chemical and histological studies during subacute ethionine intoxication. *Arch Path (Chicago) 68:*103–113, 1959.
11. FITCH, S. M., HARKNESS, M. L. R., and HARKNESS, R. D. Extraction of collagen from tissues. *Nature (London) 176:*163, 1955.
12. PROCKOP, D. J., UDENFRIEND, S., and LINDSTEDT, S. A simple technique for measuring the specific activity of labeled hydroxyproline in biological materials. *J Biol Chem 236:*1395–1398, 1961.
13. TAKADA, A., PORTA, E. A., and HARTROFT, W. S. Correlation of structural and functional recovery from cirrhosis in rats treated with lipotropic diets. *Amer J Path 49:*841–869, 1966.
14. CAMERON, G. R., and KARUNARATNE, W. A. E. Carbon tetrachloride cirrhosis in relation to liver regeneration. *J Path Bact 42:*1–21, 1936.

15. LOWRY, J. V., DAFT, F. S., SEBRELL, W. H., ASHBURN, L. L., and LILLIE, R. D. Treatment of dietary liver cirrhosis in rats with choline and casein. *Public Health Rep 56:*2216–2219, 1941.

16. GYÖRGY, P., and GOLDBLATT, H. Treatment of experimental dietary cirrhosis of the liver in rats. *J Exp Med 90:*73–84, 1949.

17. PLOUGH, I. C., PATEK, A. J., JR., and BEVANS, M. The relative effect of protein, choline and methionine in the treatment of experimental dietary cirrhosis in the rat. *J Exp Med 96:*221–231, 1952.

18. COHEN, S. I., SCHMATOLLA, E., BEVANS, M., and PATEK, A. J., JR. Amino acid mixture in the treatment of experimental dietary cirrhosis in the rat. *Arch Intern Med (Chicago) 101:*291–299, 1948.

19. OHTA, Y., ZAKI, F. G., and HOFFBAUER, F. W. Fatty cirrhosis in the rat. V. Regression upon return to normal diet. *Amer J Path 42:*729–741, 1963.

20. QUINN, P. S., and HIGGINSON, J. Reversible and irreversible changes in experimental cirrhosis. *Amer J Path 47:*353–369, 1965.

21. MACDONALD, R. A., SCHMID, R., and MALLORY, G. K. Regeneration in fatty liver and cirrhosis. Autoradiographic study using tritiated thymidine, *Arch Path (Chicago) 69:*175–180, 1960.

22. HARTROFT, W. S. "Experimental Reproduction of Human Hepatic Diseases. In *Progress in Liver Diseases* (Vol. 1). Grune, New York, 1961, pp. 68–85.

23. PECHET, G., and MACDONALD, R. A. Repair of nutritional cirrhosis. Autoradiographic and histological study after partial hepatectomy. *Cancer 14:* 963–970, 1961.

24. SELLERS, E. A., LUCAS, C. C., and BEST, C. H. The lipotropic factors in experimental cirrhosis. *Brit Med J 1:*1061–1065, 1948.

25. NEUBERGER, A., PERRONE, J. C., and SLACK, H. G. B. The relative metabolic inertia of tendon collagen in the rat. *Biochem J 49:*199–204, 1951.

26. LINDSTEDT, S., and PROCKOP, D. J. Isotopic studies on urinary hydroxyproline as evidence for rapidly catabolized forms of collagen in the young rat. *J Biol Chem 236:*1399–1403, 1961.

27. GERBER, G., GERBER, G., and ALTMAN, K. I. Studies on the metabolism of tissue proteins. I. Turnover of collagen labeled with proline-U-C^{14} in young rats. *J Biol Chem 235:*2653–2656, 1960.

28. KAO, K.-Y. T., HILKER, D. M., and MCGAVACK, T. H. Connective tissue. IV. Synthesis and turnover of proteins in tissues of rats. *Proc Soc Exp Biol Med 106:*121–124, 1961.

29. NIMNI, M. E., and BAVETTA, L. A. Collagen synthesis and turnover in the growing rat under the influence of methyl prednisolone. *Proc Soc Exp Biol Med 117:*618–623, 1964.

30. BAVETTA, L. A., O'DAY, P., and BEKHOR, I. Effect of dietary protein and ascorbic acid levels on biosynthesis of collagen. *Proc Soc Exp Biol Med 106:* 741–743, 1961.