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Alkali-Soluble and Insoluble Collagen in Infant, Adult and Cirrhotic Livers.* (25309)

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Recent investigations emphasized the importance of the distinction between insoluble and soluble collagen, the latter being tentatively considered a precursor of insoluble collagen(1,2,3,4). The radioactive - isotope studies of Harkness indicated that alkali-soluble collagen rather than acid-soluble collagen is the true precursor(3). During evolution of experimental hepatic fibrosis produced by ethionine, the increase in alkali-soluble collagen precedes that of insoluble collagen and relative rate of increase of soluble collagen greatly exceeds that of the insoluble(5). A similar fractionation of collagen was performed, therefore, on human livers in which collagen was increasing either under physiologic circumstances, as in the infant, or under pathologic conditions, as in cirrhosis. Concentration of hydroxyproline as a measure of collagen was compared with histologic distribution of collagen and reticulum fibers.

Materials and methods. All livers were obtained at autopsy. The adult control group was derived from patients between ages 33 and 90, who died from diseases without known influence on hepatic connective tissue. The infant group comprised 7 premature newborn infants and 8 full-term infants, varying in age from 1 day to 9 months. The cirrhotic group consisted of 10 postnecrotic cirrhoses, one case

TABLE I. Alkali-Soluble and Insoluble Collagen in Infant, Adult and Cirrhotic Livers.

		Total liver		Alkali-soluble fraction			
	No. of cases	Hydroxy-proline mg %*	Collagen† mg %	Hydroxy-proline mg %	Collagen mg %	Sol. collagen as % of total %	Histological grading‡ Collagen / Reticulum
Control adult	6	.376 ±.122§	2.80	.039 ±.009	.29	10.58 ± 2.62	++ / ++
Infant	15	.155 ±.036	1.15	.037 ±.013	.27	24.10 ± 9.00	+ / +
Cirrhosis	12	1.391 ±.590	10.37	.313 ±.081	2.33	22.57 ± 5.90	++++ / +++++

* mg % of dry, defatted liver. † Collagen content calculated on the assumption that both soluble and insoluble collagen contain 13.4% hydroxyproline. ‡ Collagen and reticulum were graded histologically 1+ to 5+. § Stand. dev.

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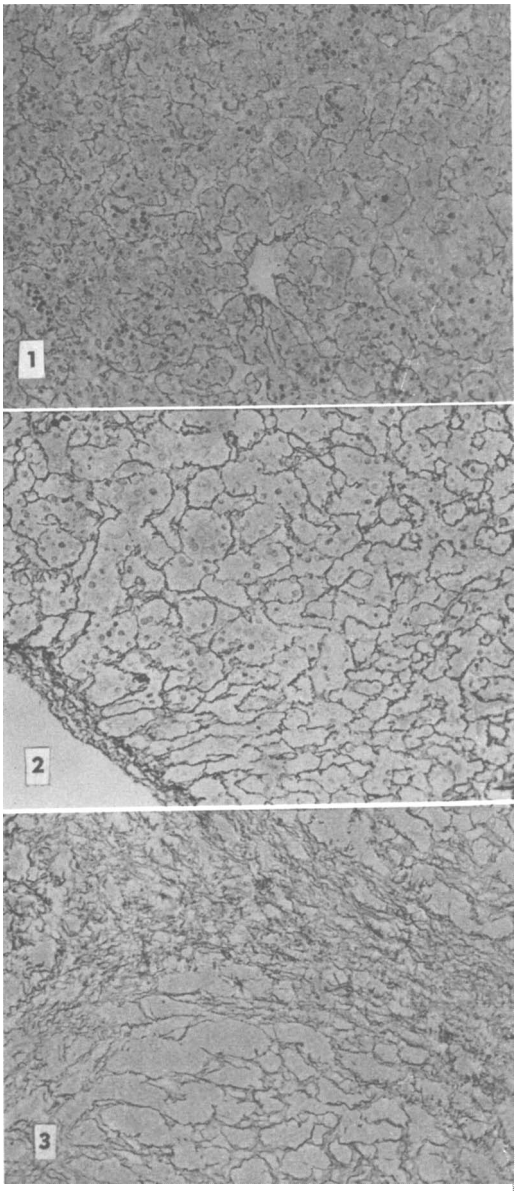

FIG. 1. Liver of premature infant. Hydroxyproline concentration .120 mg/100 mg dried, defatted liver. Alkali-soluble collagen 28.5%. Gomori silver impregnation, 120×.

FIG. 2. Liver of a control adult. Hydroxyproline concentration .348/100 mg dried, defatted liver. Alkali-soluble collagen 10.7%. Gomori silver impregnation, 120×.

FIG. 3. Liver of adult with postnecrotic cirrhosis. Hydroxyproline concentration 1.970 mg/100 mg dried, defatted liver. Alkali-soluble collagen 21.4%. Gomori silver impregnation, 120×.

of hemochromatosis, and one of myeloid metaplasia with excessive fibrosis but without cirrhotic transformation. Purification and partition for chemical analysis were carried out according to method previously described(5). For extraction of soluble collagen, 0.1 N NaOH was employed. The hydroxyproline content of dry defatted liver tissue was determined by modification of the method of Neuman and Logan(6). For histologic study, 5μ-thick sections were stained with H and E or chromatrope –2R aniline blue(7), or subjected to Gomori's silver impregnation. Amount of collagen, as reflected by aniline blue staining and that of reticulum as indicated by silver impregnation, were arbitrarily graded 1 to 5 +.

Results. The defatted and dehydrated portion of the control (normal adult) liver contained approximately 0.4% hydroxyproline, which corresponds to 3% collagen (using factor of 7.46(6)). In cirrhotic liver, total hydroxyproline concentration was approximately 3.7 times greater than that of the control, without any overlap. In contrast, the infant liver contained only about 40% of total hydroxyproline concentration of the control liver. There was, however, approximately 8 times as much hydroxyproline in the soluble fraction of cirrhotic liver as in the control liver, whereas hydroxyproline concentration in the soluble fraction of control and infant liver was virtually the same. In the control liver, the hydroxyproline of the alkali-soluble fraction comprised about 10% of total hydroxyproline, while in both infant and cirrhotic livers, this fraction accounted for approximately 25% of total hydroxyproline (Table I). No marked differences were found between livers of premature and full-term infants. Livers of premature infants had a mean total hydroxyproline concentration of 0.128 g/100 g of dry defatted liver with 26.7% in the soluble fraction, while the group of full-term infants had a mean total hydroxyproline concentration of 0.179 g/100 g of dry defatted liver with 21.8% in the soluble fraction.

Histologically, both collagenous and reticulum fibers were significantly increased in cirrhosis. In infant livers, considerably less reticulum was found than in control adults (Fig. 1, 2, 3). Liver cells were frequently arranged in more than one cell-thick plates, and

therefore, the sinusoidal surface on which the reticulum fibers were aligned was relatively less extensive. Moreover, along the surfaces were fewer fibers than in the adult liver. The difference in amount of stainable collagen fibers between infants and control adults was in great measure accounted for by the lesser amount in the portal tracts of infant livers. No reliable differences in the listed histologic characteristics between premature infants and babies could be demonstrated.

Discussion. The increase in total collagen concentration in cirrhosis, as measured by hydroxyproline concentration, has been repeatedly demonstrated(8,9). The range found in our study is consistent with that reported by Kent *et al.*(9). In the infant liver, collagen concentration is considerably lower, corresponding to the range reported for adult rat (5,9). Lower concentration of hydroxyproline is reflected in the amounts both of stainable collagen and of silver impregnated reticulum, which according to electron microscopic studies appear to be identical. The relatively lower collagen concentration in the infant liver is a reflection of the more intensive growth of parenchyma as compared to stroma. This situation also obtains in hepatic regeneration after hepatectomy(10) and is reflected microscopically in plates that are several cells thick. To this quantitative difference must be added a qualitative alteration of the collagen of the human infant liver, the proportion of soluble collagen being much higher. The fact that the ratio between soluble and total collagen in infant liver is of similar magnitude as in the cirrhotic liver, although the latter contains almost 10 times as much total collagen, tends to confirm the assumption of Harkness *et al.*(3) that the alkali-soluble collagen is a precursor of the insoluble collagen, and is evidence that this is independent of the conditions under which the fibers are formed. Increased amounts of soluble collagen were previously reported in uterus, tail tendon, aorta, and skin of young rats(11) and in lathyritic chick embryo(12).

Summary. Concentration of hydroxyproline in the liver, as a measure of collagen, is lower in human infants than in adults, and histologically-stainable collagen and silver impregnated reticulum fibers are fewer. While in cirrhosis, total collagen concentration is 10 times greater than in infant livers, in both cirrhotic and infant livers the ratio of alkali-soluble collagen to total collagen is similar and considerably higher than in control adults. This suggests that formation of new collagen fibers, under both physiological and pathological circumstances, is associated with a relative increase of alkali-soluble collagen.

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