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Hepatic Fibrosis in the Persistently Non-Fatty Liver of the Hypophysectomized Dog.

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The frequent association of hepatic lipoidosis and necrosis with cirrhosis in man and experimental animals has led to the view that fatty and other forms of liver-cell degeneration induce cirrhosis. Where fatty changes did not precede hepatic fibrosis, hemorrhagic or some other form of hepatocellular necrosis has usually been considered to be the precipitating lesion.

That some factor or factors other than liver-cell damage may possibly be responsible for the initiation and progress of hepatic fibrosis is brought out in the present investigation. Fibrosis was found in livers of hypophysectomized dogs in which the fatty-acid content never exceeded 4%, *i.e.*, normal concentrations, and in which evidence of extensive liver-cell destruction was not detected histologically.

Experimental Procedures. For the first 3-4 weeks after their arrival at the laboratory the dogs were fed a diet high in protein and adequate in all respects. Each dog received twice daily, at 8:00 a.m. and at 4:00 p.m., 15 g of lean meat per kilo, 10 g of sucrose, 4 g of bone ash, and 1 g of Cowgill's salt mixture.[1] Vitamin supplements were provided by the addition of 3 cc Sardilene† and 5 cc of Galen B.† After the dogs had received this diet for 2-3 weeks, they were hypophysectomized by a method previously described.[3] In the procedure used in this study no cautery was applied to the base of the brain. In most cases the appetites of the dogs were excellent after hypophysectomy.

At the end of the periods of observation recorded in Table I, the whole livers of the dogs were excised while they were under nembutal anesthesia. Several sections of each liver were removed for histological study, and the rest was ground and sampled for lipid analysis as described in a previous paper.[4]

Serial sections were made of the base of the brain of each dog and every fifth section carefully examined. No cells of the anterior, posterior, or intermediate lobes of the pituitary gland were found in the dogs recorded in this study; nor was there any evidence of damage to the hypothalamic region.

Results. The chemical and histological findings in the livers of these animals have been summarized in Table I. In an earlier study the fatty-acid contents of dogs' livers were examined at intervals of 2-4 months after hypophysectomy;[5] no deviation from the normal was found at these time-intervals. The results recorded in Table I leave no doubt that hypophysectomy *per se* does not influence the fat content of the liver, even 32 months after it has been performed.

Histologically, the liver cells did not show any evidence of fatty change or necrosis. The only abnormal findings in the liver cells were firstly an unusual distinctness of the cell walls with clumping of the chondriosomes (plant-like cells), and secondly an eosino-

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1 Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.

† Each cubic centimeter of Sardilene contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A. The vitamin content of Galen B has been recorded elsewhere.[2]

2 Montgomery, M. L., Entenman, C., Chaikoff, I. L., and Nelson, C., *J. Biol. Chem.*, 1941, **137**, 693.

3 Dandy, W. E., and Reichert, F. L., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 1.

4 Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

5 Chaikoff, I. L., Gibbs, G. E., Holtom, G. F., and Reichert, F. L., *Am. J. Physiol.*, 1936, **116**, 543.

TABLE I. Pathological Findings and Fatty Acid Content of Livers of Hypophysectomized Dogs.

Dog	Body wt			Sacrificed after hypophysectomy, mo.	Liver findings					Total fatty acids, %
	Initial, kg	Max., kg	Final, kg		Pathological			Eosinophilic hyalinization	Wt, g	
					Fibrosis*	Cirrhosis†	Plant-like cells			
H25	7.2	10.0	9.7	24	3+	? early	2+	2+	220	2.2
H26	7.6	8.6	8.1	24	0	0	2+	2+	170	1.9
H27	7.2	8.1	7.8	24	patchy slight	0	2+	2+	152	4.9
H28	6.2	8.2	8.2	24	4+	+	2+	+	176	2.7
H29	6.4	10.6	9.1	24	3+	+	2+	+	196	3.9
H32	4.1	8.3	8.3	32	0	0	3+	0	265	3.8
H33	5.5	6.7	6.7	32	0	0	3+	2+	212	3.2

* *Hepatic fibrosis* or precirrhosis indicate the presence of abnormal amounts of fibrous tissue in the liver without architectural distortion of the lobular pattern.

† *Cirrhosis* indicates the supervention of architectural distortion in association with hepatic fibrosis.

Histological grading of "plant-like" cells and eosinophilic hyalinization:

Assessment of degree of fibrosis:

+ = Scattered foci.
2+ = About ¼ of lobule affected.
3+ = About ½ of lobule affected.
+ = Present and in scattered foci.
3+ = About ½ of lobules affected.
4+ = About ¾ of lobules affected.

philic hyalinization of the liver cells. Both these changes were widespread in the livers of *all* the dogs and apparently occurred independently of the incidence of fibrosis. While the eosinophilic hyalinization occurred in all the livers, this reaction was most prominent around the non-fibrosed portal tracts (Fig. 1). Apart from these changes in the liver cells, the majority of livers appeared relatively avascular by virtue of the fact that most of sinusoids were closed and did not contain blood. This occlusion of the sinusoids and the close apposition of the individual liver cells to one another gave the liver a highly cellular appearance (Fig. 1).

The fibrotic reaction to be described below was detected, in varying severity, in 4 of the 7 hypophysectomized dogs. This connective-tissue reaction was most unusual in its form, location, and distribution in the affected livers. In the first place not all the lobes of the same liver were affected by this reaction and, had we not had sections from several parts of each liver, we would clearly have missed the change in at least 2 of the dogs which manifested this lesion. It is possible that the patchy distribution of this lesion was due, in several of our dogs, to the fact that it had begun only a short time before death. It is probable that, had the dogs been maintained for longer periods of time, the fibrosis would have become both more extensive and more severe.

When present, the fibrosis which we detected was most frequent and most severe in and around the portal tracts, although by no means confined to these regions (Fig. 2). The fibrosis took two forms, which possibly represented different stages in the evolution of the lesion. In its most striking form it appeared as a highly cellular connective-tissue proliferation (Fig. 2, 3, and 4). This cellularity was due primarily to the large numbers of fibroblast and fibrocyte nuclei. The portal tracts, both large and small, were made very prominent by the accumulation around them of round cells and numerous closely packed, spindle-shaped, young-fibrous-tissue nuclei (Fig. 2 and 3). Often, when sections were cut obliquely through what, by location, seemed to be portal tracts, sheets of this highly

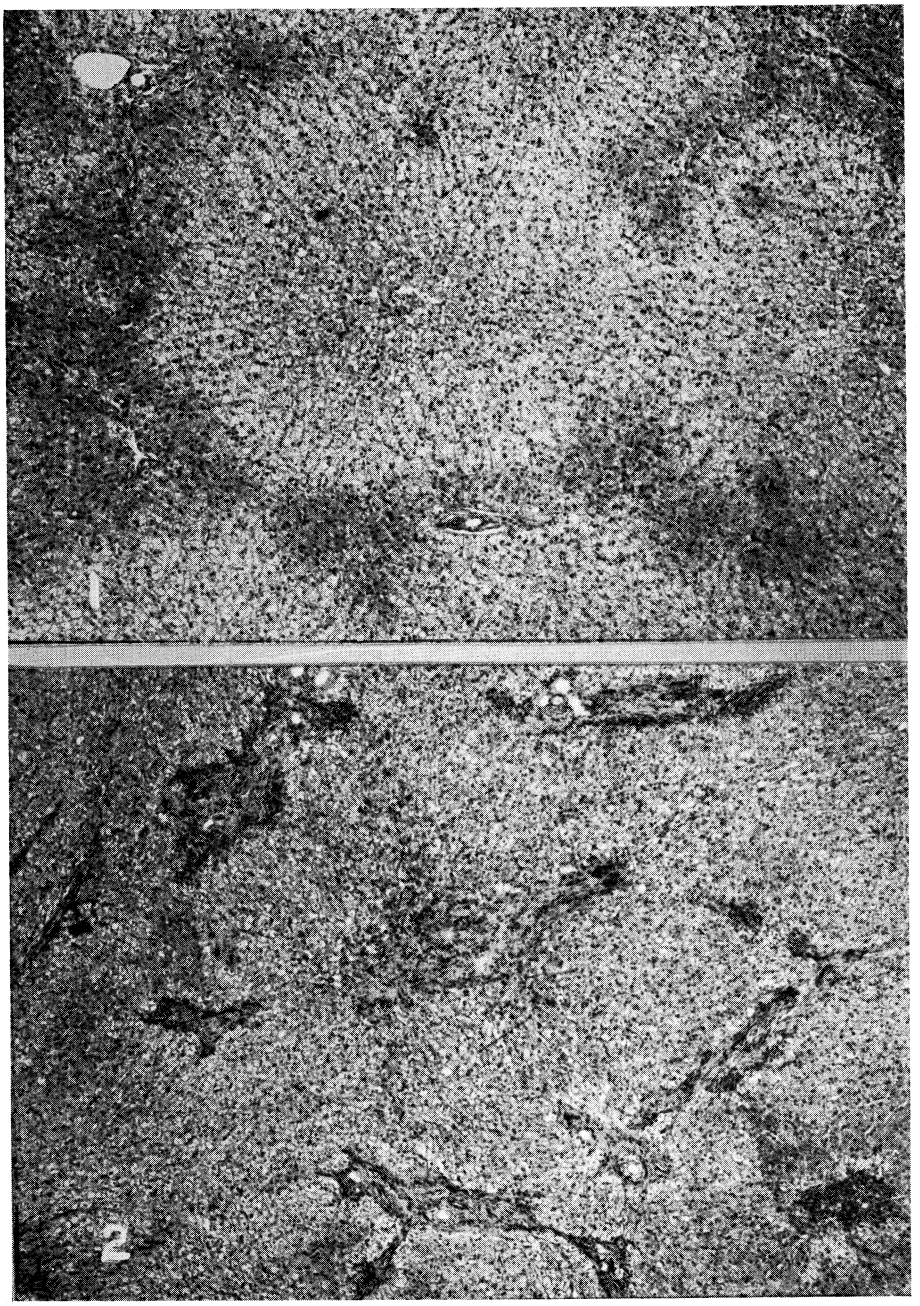

FIG. 1.

(H32) The periportal localization of the eosinophilic hyalinization with nuclear hyperchromasia of the liver cells is clearly shown. Note also "plant-like" cells in the rest of the lobule and the avascularity of the liver. Note also the absence of fatty change. Hematoxylin and Eosin × 45.

FIG. 2.

(H28) The cellular periportal fibrosis and the less highly cellular reaction in the central portion of the lobule are typical of the changes seen in these livers. Hematoxylin and Eosin × 70.

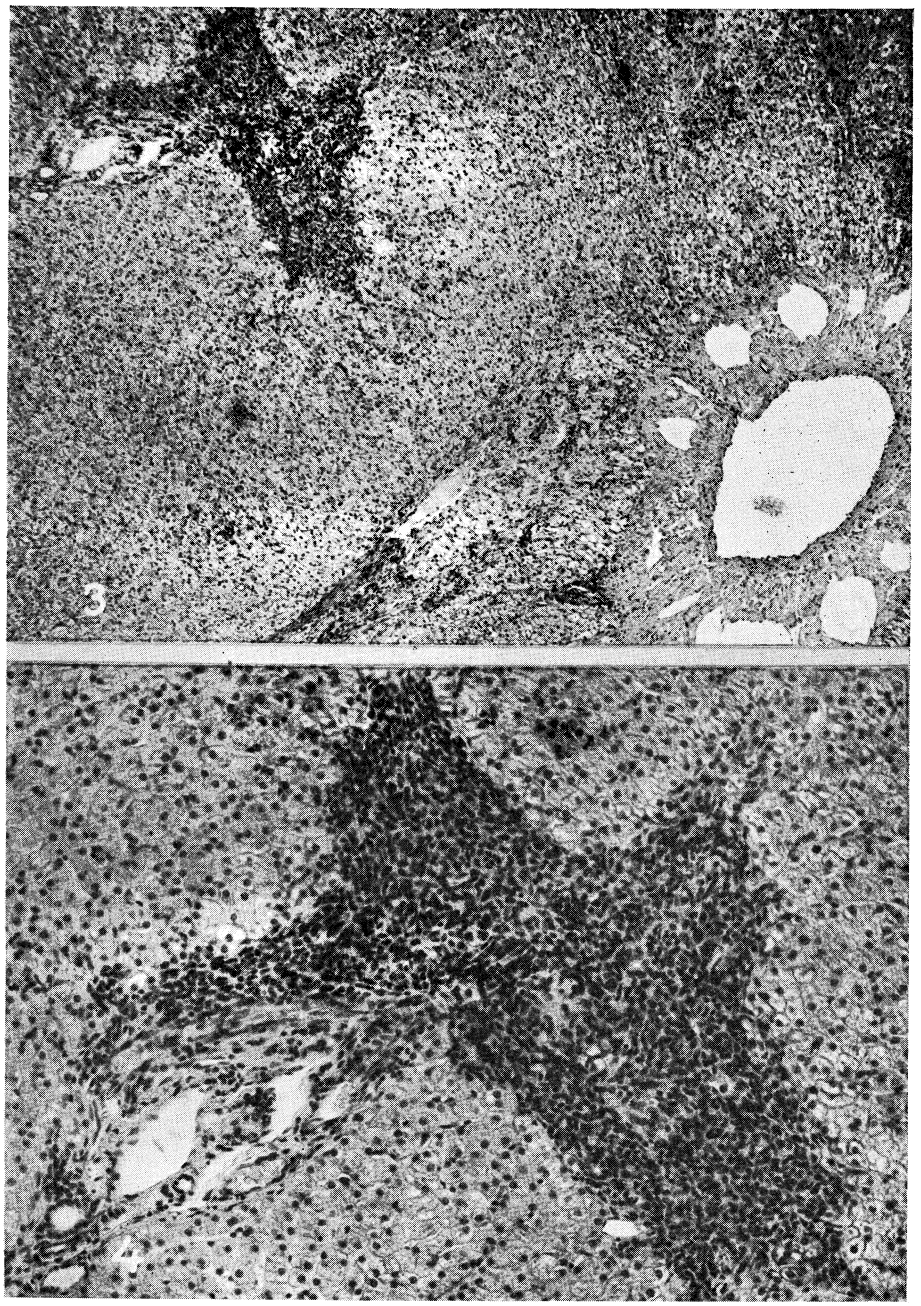

FIG. 3.

(H29) The portal tract towards the top left corner of the picture is made prominent by the marked accumulation of cells. Scattered bands of fibrous tissue can also be detected in this picture. Hematoxylin and Eosin × 64.

FIG. 4.

(H29) Details of the nature of the cells infiltrating the portal tract depicted at the top left corner of Fig. 3. In addition to round cells there are large numbers of spindle-shaped fibrocytic nuclei. Hematoxylin and Eosin × 152.

cellular connective tissue seemed to be stretched across the liver lobules. However, in other areas these sheets of young connective tissue seemed to be related to radicles of the hepatic veins or occurred in the middle of apparently intact liver-cell lobules (Fig. 2). Such masses of young connective tissue occasionally joined together, thus outlining 2 sides of a lobule, or, when more extensive, it obliterated a liver lobule. This latter reaction when present was most common in the subcapsular regions, although in the more severely affected livers such obliteration of liver tissue was more widespread.

The great cellularity of this connective tissue was very similar to that detected by Graef *et al.*[6] in the fatty livers of their hypophysectomized dogs (*cf.* their Fig. 5 and 6 with our Fig. 2 and 3). However, in many of the fibrosing areas the cellularity was made less prominent by the fact that masses of dense, highly eosinophilic collagen fibers had been deposited among the still numerous fibroblast and fibrocyte nuclei (Fig. 2 and 3).

Associated with this fibrous tissue were numerous histiocytic cells containing very dark-brown coarse pigment granules. Similar pigmented histiocytes have been encountered in smaller numbers in the livers of normal dogs, but such pigmented cells were not detected in the non-fibrotic livers of hypophysectomized dogs.

Discussion. The relation of liver-cell damage to the onset of cirrhosis has been much discussed in recent years. Although the connective-tissue changes in response to hypophysectomy are not so pronounced as those encountered in livers in which extensive fatty infiltration or necrosis has been induced, the present findings leave no doubt that the stimulus to connective-tissue proliferation in the liver need not be hepatocellular degeneration, either fatty or necrotic. The greatest amount of fibrous-tissue proliferation occurred in the livers of dogs H25, H28, and H29; in these, fatty change or degeneration of the liver cells was not detected.

The mechanism by which hypophysectomy brings about fibrous-tissue proliferation in the liver is not clear at present. Graef *et al.* stated that "it appears reasonable to conclude that hypophysectomy itself . . . . had little if anything to do with the hepatic lesions encountered in these dogs. The hypothalamic lesions disclosed by histological examination seemed to have a pivotal role in the hepatic changes." Careful histological studies of the hypothalamic regions in *our* animals, using the serial-section technic mentioned above, failed to reveal any evidence of damage to this important center. This fact, together with the absence of fat from the livers of our hypophysectomized dogs, indicates that the pathogenesis and possibly the etiology of the hepatic fibrosis in our animals differed somewhat from those described by Graef *et al.*

The protein content of the diets of our animals was high, and consequently low-protein *intake* could not be blamed as a factor in the etiology of the hepatic fibrosis in them. However, some interference in the absorption process has been shown to follow excision of the pituitary gland.[7,8] Hence despite the ingestion of a high-protein diet by our dogs, it is conceivable that hypophysectomy induced some disturbance in the digestion or absorption of certain dietary substances which play a critical role in maintaining a normal liver. The possibility that hypophysectomy promotes an increased need for such substances is, of course, not ruled out.

Summary. 1. Fibrous-tissue proliferation is shown to occur in the livers of hypophysectomized dogs. Its development was not preceded by an increase in the fat content of the liver.

2. Hypothalamic damage as a cause of the fibrous-tissue proliferation was ruled out.

3. The development of hepatic fibrosis in these dogs fed a diet adequate in all respects and rich in proteins suggests that hypophysectomy induces either (a) a derangement in digestion and/or absorption, or (b) an increased need for certain dietary substances essential for the maintenance of a normal liver.

[6] Graef, I., Negrin, J., and Page, I. H., *Am. J. Path.*, 1944, **20**, 823.

[7] Russell, J. A., *Am. J. Physiol.*, 1938, **121**, 755.

[8] Althausen, T. L., *Essays in Biology in Honor of H. M. Evans*, University of California Press, Berkeley, 1943, p. 13.