HISTOCHEMICAL INVESTIGATION OF THE ADRENERGIC INNERVATION OF THE LIVER

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The livers of puppies, kittens, rats, and guinea pigs were investigated histologically by the Falk–Hillarp method. Adrenergic nerve fibers were detected in Glisson's capsule and in the adventitia of the arteries. There were fewer fluorescent fibers in the walls of the hepatic veins, but they were all arranged in the same layer. Adrenergic fibers were seen most distinctly in the hepatic lobule of guinea pigs as thin fibers with tiny varicosities. The walls of the bile ducts contained fewer adrenergic fibers.

The autonomic innervation of the liver has not yet been adequately studied. Nearly all information about its innervation has been obtained by classical neurohistological methods [1-6, 10, 12, 13] which do not enable the true nature of the nerve endings to be established. Neither electron microscopy nor histochemical methods have yet been widely used in this field. Only isolated papers describing the study of the adrenergic innervation by these methods have been published.

Yamada [15], for instance, using the electron microscope, observed direct contact between the liver cells and terminal branches of nerve fibers which he classed as adrenergic. Ungvary and Donath [14] used Falk's fluorescence-histochemical method [9] and showed that adrenergic nerve fibers are mainly located in the walls of the hepatic blood vessels. However, they did not obtain convincing proof of the innervation of hepatocytes. Sisto and Erobecchi [11], using the same method, showed that there are considerably more adrenergic fibers around the arteries, veins, and bile ducts than in the connective tissue accompanying the triads. Only in one or two specimens were very thin adrenergic fibers with swellings along their course found between the hepatic trabeculae.

Hence, although in principle it has now been established that there is an adrenergic innervation of the blood vessels and ducts of the liver, so far as the parenchyma of the liver is concerned the number of observations is not yet adequate. Yet information in this field is vital to the understanding of the histophysiology of the liver. This was the motivation behind the investigation described below.

EXPERIMENTAL METHOD

The livers of puppies aged 2-4 weeks, of kittens of the same age, and of sexually mature rats and guinea pigs were studied by the fluoresence-histochemical method [9]. To strengthen the histochemical reaction for catecholamines, the animals were given an intraperitoneal injection of 200 mg/kg iproniazid 18 h before sacrifice. The control animals received reserpine in a dose of 10 mg/kg 24 h before sacrifice. The reaction observed was regarded as due to catecholamines only if it was completely exhausted by reserpine. As an additional control, pieces of liver not treated with paraformaldehyde were used. The animals were killed by thoracotomy under pentobarbital anesthesia. Material was taken from the region of entry of vessels and nerves into the liver and also from the various lobes of the liver. Serial sections were studied in the luminescence microscope. Photographs were taken on RF-3 film. Identification of catecholamines was

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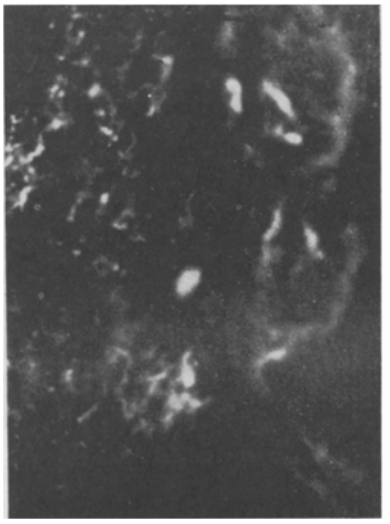

Fig. 1

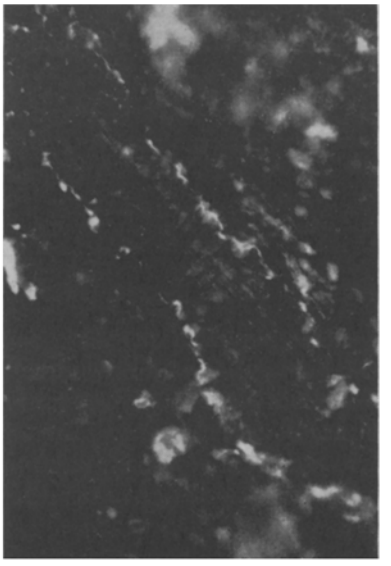

Fig. 2

Fig. 1. Adrenergic nerve fibers in Glisson's capsule. Arrow indicates edge of lobe of liver. Guinea pig. Falk's reaction, 200 ×, Homal.

Fig. 2. Longitudinal section through arterial wall. Adrenergic nerve fibers located only in adventitia. Rat. Falk's reaction, 120 ×, Homal.

Fig. 3. Adrenergic fibers in parenchyma of hepatic lobule and around central vein. Guinea pig. Falk's reaction, 120 ×, Homal.

based on the appearance of specific, intense emerald-green fluorescence of the structures. Altogether 48 animals were used.

EXPERIMENTAL RESULTS

Fluorescent nerve fibers were detected in Glisson's capsule as long structures with varicosities. They interwove often and sometimes radiated fanwise to form a tree-like figure which stood out sharply against the dark field (Fig. 1). Arteries of small and medium caliber as well as interlobular arteries were richly supplied with adrenergic fibers. They were particularly conspicuous in the tunica externa, where they formed thin ramifications (Fig. 2). There were considerably fewer fluorescent fibers in the walls of the hepatic veins, but they were arranged in the same layers. A positive reaction for catecholamines appeared in the walls of the central veins of the lobules only occasionally (in the transverse section) in very thin fibrils. Fibers were particularly numerous in the interlobular spaces. There they looked like straight threads running parallel to each other, sometimes fragmented and tapering, then reappearing as varicosities.

No adrenergic nerve fibers were observed in the hepatic lobule itself in puppies, kittens, and rats. However, they were demonstrated quite clearly in guinea pigs. On entering the lobule they ran along its border and then proceeded along the hepatic trabeculae as very thin fibers with small varicosities (Fig. 3). The walls of the bile ducts contained fewer adrenergic nerve fibers.

A noticeable feature of the hepatic parenchyma was the presence of mast cells, clearly distinguished by their intense yellowish-green fluoresence produced by their serotonin content. These cells were found chiefly around the interlobular vessels and central veins.

These observations are in agreement with those described by other workers [11, 14, 15], who showed that adrenergic nerve fibers and their endings are most numerous in the walls of blood vessels, and they

confirmed conclusions drawn from electron-microscopic and histochemical investigations to the effect that terminal adrenergic nerve fibers do not enter the media of the arterial wall, but come into close contact only with the outer surface of the smooth-muscle cells of the media [7, 14]. At the same time, the more numerous nerve fibers in the walls of the hepatic arteries than of the veins must be emphasized.

The scanty innervation of the bile ducts observed in these experiments conforms to the general view that the bile-duct system possesses a predominantly parasympathetic innervation [2, 14].

The discovery of adrenergic nerve fibers in Glisson's capsule and inside the lobules of the liver of guinea pigs is of the greatest interest because it is this aspect of the innervation of the liver which has been least studied. The results showing that the whole of Glisson's capsule is abundantly innervated by adrenergic nerve fibers constitute an important addition to the knowledge already possessed. The writers know of only one paper which describes adrenergic nerve fibers in Glisson's capsule, but only in those parts of it which are in the region of the hilum of the liver [11].

As regards the most difficult problem, the innervation of the hepatocytes, the results of the present investigation do not yet permit a definite conclusion that the adrenergic nerve fibers demonstrated terminate, in fact, on hepatocytes. According to one investigation [8], arterioles change into hepatic capillaries at different levels of the lobule, and since sphincters are found at these places the possibility cannot be ruled out that the adrenergic nerve fibers found in the lobules in the present investigation belong to the innervation of the muscle cells forming these sphincters and located in the walls of the arterioles. Further investigations are therefore required before this problem can be finally solved.

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