METHOD OF OBTAINING TOTAL CLEARED PREPARATIONS OF THE HEPATIC BLOOD VESSELS IN SMALL ANIMALS

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A technique of obtaining three- and two-dimensional total cleared preparations has been developed and used to investigate the intrahepatic architecture of the blood vessels in the liver of small animals. The method consists essentially of putrefaction of specimens of the liver after preliminary injection of its vessels with latex or nitrated enamel paints, followed by depigmentation, dehydration, and clearing. An advantage of the method is that the blood vessels of the liver can be studied in whole, total preparations in which the normal topographic relations are preserved.

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The methods of stereoroentgenography, clearing of thick sections, corrosion, and dissection are used at the present time to investigate the architecture of the intrahepatic blood vessels. Unfortunately, all these methods have their disadvantages, and these have been discussed by Parfent'eva [1].

The writer has developed and used a method of clearing total preparations of the albino rat liver after preliminary mono- and polychromatic injection of the intrahepatic blood vessels and bile ducts. Clearing is carried out as follows. After injection of the injection medium into the blood vessels, the liver is placed for 24 h in 10% formalin solution, and then transferred to water, in which putrefaction occurs. To prevent the liver from floating, it is wrapped in a layer of gauze. This also protects it against fly larvae. Putrefaction is allowed only until softening takes place, and not to the stage of tissue destruction. The correct time is established by noting when the specimen becomes jelly-like in consistency. After putrefaction, which takes from 1 to 4 weeks depending on the season of the year, the liver is carefully washed for 24 h without removing it from the vessel of water. It is then transferred for 24 h into a solution consisting of 5% hydrogen peroxide and 0.1% strong ammonia (10%) solution. The specimen is then again washed with tap water for 24 h, and then dehydrated and cleared in the usual way: in alcohols of increasing concentration (70, 80, 90, 96, and 100°), followed by a mixture of absolute alcohol and methyl salicylate, and finally, pure methyl salicylate, in which the specimen is kept, or alternatively it can be mounted in polystyrene. The specimen remains in each solution for 24 h.

By the use of this method it is possible to obtain both three- and two-dimensional preparations of the liver. To obtain two-dimensional preparations, after putrefaction the liver must be dried under a layer of gauze. In the course of this procedure it is important not to miss the moment when the liver has not quite lost all its moisture, and then to pour xylene over it, as otherwise cracks may appear. Depigmentation and dehydration are then carried out by the method described above.

The writer considers that this method is suitable for studying the architecture of the blood vessels in the pathological as well as the normal liver. He has used it to study the architectonics of the vascular system of the liver after partial experimental reaction of the organ. The results of these experiments show that the best media to use for injecting the vessels in conjunction with the method now described are nitrated enamel paints and latex. For x-ray control before and after clearing, orange or yellow lead oil paint was mixed with these media. The resulting preparations can be studied under the MBS-2 microscope, and they can also be photographed in incident and transmitted light.

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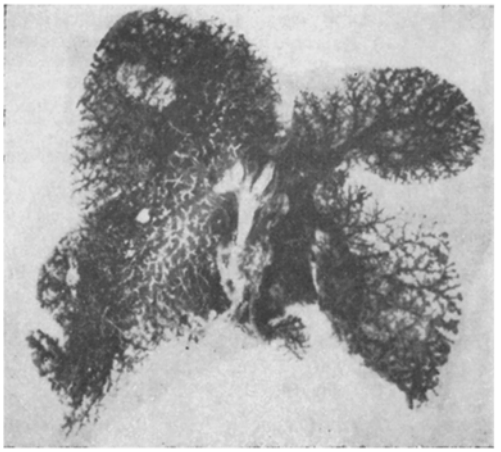

Fig. 1

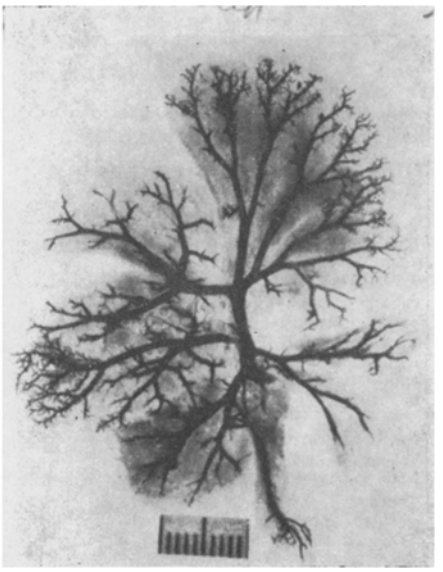

Fig. 2

Fig. 1. Three-dimensional preparation of the intrahepatic system of the portal vein in the liver of an albino rat after resection of the central and left lobes; the main trunks and their branches in the interior of the lobes are clearly seen. Combined illumination (transmitted and incident light). Magnification 1.7:1.

Fig. 2. Two-dimensional preparation of intrahepatic architectonics of the portal vein in the liver of an albino rat (normal). Lobes of the liver and relations of vessels to them are clearly visible. Magnification 1.5:1.

A distinguishing feature of the suggested method is the use of preliminary putrefaction of the liver tissue and of ammonia solution during the bleaching process with hydrogen peroxide.

Photographs of a three-dimensional (Fig. 1) and a two-dimensional (Fig. 2) preparation of the albino rat liver, with injection of the portal vein, are given by way of illustration.

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