Cannulation of Minute Mesenteric Veins for Continuous Portal Perfusion of Rats

The introduction of a cannula into a very small vein (< 1 mm.) is almost impossible because of collapse and cohesion of the vessel walls. Any looping or clamping of the vein beyond the incision in order to avoid bleeding and to maintain a clear visual field causes spasm which will not permit further passage of the tubing into the vascular lumen. If the cannula is forced, it will perforate the thin vessel. We have, therefore, constructed a cannulating device which permits the introduction of a fine plastic tubing into a small vessel without visual control.

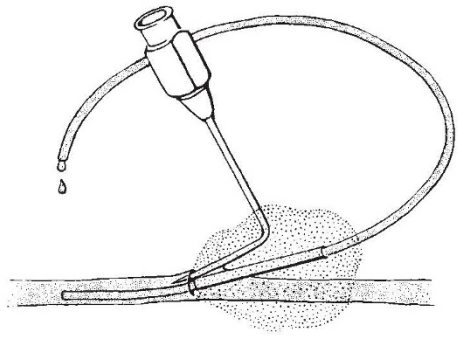

Fig. 1

The device consists of a No. 22 injection needle, 1½ in. long, the distal ½ in. of which is bent to a right angle so as to form a trough terminating at the needle point. To this trough, and just at the base of the needle point, is soldered a straight 1 in. long metal cannula, which forms an angle of about 15° with the base of the needle trough (Fig. 1). A polyethylene tubing (No. 10 Clay-Adams) with a square end is introduced into the metal cannula which will guide the plastic tubing exactly to where the point of the angled needle has punctured the vessel. When the plastic tubing is now pushed forward, it passes close to and beneath the needle point into the vascular lumen (Fig. 1). In spite of lack of visibility due to the bleeding from the punctured vessel, successful cannulation is evident from the flow of blood into the tubing. Threads which have been previously passed under the vessel and looped are tied after the point of the cannulating device has been pulled back about 3 mm. The plastic cannula is now firmly grasped with a thumb forceps in front of the needle point and held in position while the cannulating device is slipped off the tubing. The polyethylene tubing is filled with a 1:10,000 heparin–saline solution and stoppered. Eighty-eight female rats weighing 220–260 gm. were anæsthetized with intraperitoneal 'Nembutal' (6 mgm./100 gm. body-weight) and a laparotomy with exposure of the ileocæcum was carried out. The ileocæcal loop and its mesentery containing the exposed vein were spread on a thin cork plate covered with moist gauze. The mesentery was pinned down to the cork plate in order to permit the smooth sliding of the cannula into the stretched vein. The cannulating device containing the plastic tubing was attached to the barrel of a 2 c.c. syringe, thus providing a steady grip of the instrument. The cannulation of the ileocæcal vein by this technique is done close to the intestinal border of the mesentery. The abdomen is closed in two layers and the cannula brought out through the most cephalad part of the mid-line incision to prevent the rats from catching their hind-feet in the cannula. A small glass bead is slipped over and glued to the cannula at its point of exit from the abdominal wall. The cannula is fixed by putting a suture through the skin and tying it in front of the glass bead. The animals are then restrained[1] and continuously perfused with various solutions.

Of the 88 rats, 26 have been perfused for less than 3 days, 41 have been perfused for 4–10 days, and 21 have been perfused for more than 10 days.

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ANATOMY

Histochemical Demonstration of Aromatic Monoamine in the Locus Cœruleus of the Mammalian Brain

Shimizu *et al.* have reported several interesting histochemical results of the locus cœruleus of the mammalian brain—the strong activity of acid phosphatase[1] and cholinesterase[2] but rather poor action of respiratory enzymes[3,4], and further, characteristically strong monoamine oxidase activity[5]. The last finding led us to suppose that some aromatic monoamines such as catechol amines and serotonin would be concentrated in the locus. Although Falck and Hillarp[6] recently reported that no chromaffin reaction could be observed in the hypothalamus and the caudate nucleus, and concluded that noradrenalin and dopamine are not stored in a small number of nerve cells of these regions, they did not examine the locus cœruleus. In the present investigation several histochemical reactions including argentaffin, Schmorl's, chromaffin, azo-coupling and Gibbs's reaction were carried out in mammalian brain tissues containing the locus cœruleus.

The materials used were taken from the healthy adult rabbit, rat, mouse, guinea pig, dog and cat and also from the scorbutic guinea pig and animals treated with marsilid or catron. All tissue slices were fixed in 10 per cent neutral formalin or 5 per cent potassium bichromate–chromate solution with or without formalin[7]. The specimens were quickly embedded in paraffin, since a yellow compound due to an oxidation of chromaffin substances might be dissolved in hot paraffin[8]. The sections (7–10μ) were subjected to the following histochemical reactions: the argentaffin reaction (Gomori's methenamine silver or Fontana's ammoniacal silver solution, cf. Pearse[9]) for 24 hr. without pretreatment with Gram's iodine solution, Schmorl's ferricyanide reaction (Lillie and Burtner[10]), azo-coupling reaction (diazo blue *B* in alkaline solution, cf. Pearse[9]) and Gibbs's reaction (cf. Gomori[11]). The results are summarized in Table 1.

The silver or Prussian blue granules due to the presence of a reducing substance occurred almost exclusively in the locus cœruleus of all animals hitherto studied except for rats and mice, where the