land, New Jersey 08360, USA) provided with a 40- or 80-mesh screen (380- or 190-µm opening size) in a dish filled with Ephrussi/Beadle modified Ringer's solution. The homogenate was sieved through 500-, 250-, 140-, 105-, 85-, and 62-µm nylon meshes in different combinations for elimination of contaminants and selection of chosen stages. The final suspension was placed in the cylinder of the glass filtration-apparatus provided with a filter disk (30 mm diameter) of nylon tissue (17- or 27-µm opening size). Whole mounts were obtained with a high yield after different staining methods (Heidenheim's iron-haematoxylin, Mayer's haematoxylin-eosin, Daddi's sudan for lipids, Kunick's methyl green-pyronin, orcein-lactic acid for chromosomes). Paraffin embedding was carried out over cedarwood oil. For electron microscopy (figure 2), after sequential fixation by glutaraldehyde and osmium tetroxide[8], objects were dehydrated in acetone and embedded in epon and araldite.

The above mentioned investigation exemplifies the mode of use of the given method for a specific object. For both light and electron microscopy of these objects, the results were comparable to those previously obtained with much more tedious and less efficient methods[7].

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Intrasplenic infusion. A simple method for intraportal infusion in rats

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Summary. A simple and time-saving method for intraportal acute or chronic infusion in the rat by the insertion of a catheter into the spleen is described. This method has proved to be especially useful for performing chronic infusions in conscious animals.

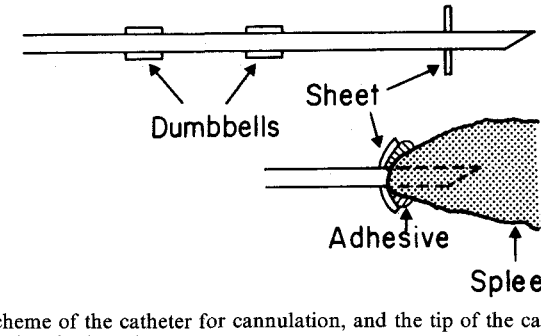
There is a great variety of experiments which require administration of different solutions into the portal system. Cannulation of the portal vein requires extensive surgery, viscera manipulation with loss of fluid and, frequently, great alterations in the portal circulation. Cannulation of a primary mesenteric vein causes great hemodynamic alterations in the splanchnic area, and cannulation of secondary mesenteric veins requires the use of very small catheters, with the problem of small flows and great resistances. The method here presented allows the infusion of any kind of solution even at high infusion rates, without disturbing the portal circulation.

PE 50 tubing (polyethylene tubing, 0.5 mm inner diameter; 1.0 mm outer diameter; Vigon France) was used for this purpose. One end of the catheter was cut with an angle less than 30°, giving a well beveled tip. A small round (1 cm in diameter) piece was cut from flexible polyethylene sheeting, 0.05 mm thick, and a small hole was bored in its center with a sharpened steel tube. The beveled tip of the catheter was then threaded through the sheet and glued to the tube with polyethylene adhesive. 2 small dumbbells made of PE100 tubing were put 3 and 5 cm away from the sheet and used for tying the catheter to the abdominal muscle. All materials were sterilized in a 1:1000 solution of benzalkonium chloride (Armil) for 24 h and rinsed with sterile saline immediately before implantation.

The rats were anesthetized with nembutal (30 mg/kg b.wt i.p.). An abdominal incision about 2 cm in length was made in the linea alba 3 cm below the sternon. The tube was passed under the skin to the back of the neck and filled with heparinized isotonic saline. The spleen was exposed and a loose ligature made in the conjunctive tissue of one of its ends with a double two zero silk. The beveled tip of the catheter was then inserted into the spleen following the direction of its long axis until the sheet touched it, and a drop of cianoacrylate esther adhesive (Eastman 910 adhesive, Eastman Chemical Products, Kingsport, Tenn., USA) was put in the site of puncture (fig.). The ligature was tied to the tube behind the sheet and after 2 min to allow the adhesive to solidify, the spleen was replaced in the abdomen, the catheter tied by the proximal dumbbell to the internal face of the abdominal muscles and the muscular layers closely sutured around the catheter. Then the tube was tied to the external face of the abdominal muscle by the distal dumbbell and the skin was closed.

The external end of the catheter, which during the experiment had been connected to an insulin syringe filled with heparinized saline, was sealed by insertion of a stainless steel rod, and left free in the neck or tied with a suture to the skin.

After the operation the animals were put into individual large cages on top of sterile absorbent pads, and allowed to wake and move freely in the cage. Penicillin was given on the day of surgery and 4 consecutive days thereafter (100,000 U i.m. daily).



Scheme of the catheter for cannulation, and the tip of the catheter in place in the spleen.

The catheter was rinsed daily with heparinized saline and the wounds kept clean with cotton swabs soaked in betadine. Usually in the same surgical operation some other, arterial, venous and urinary bladder catheters were also implanted and exteriorized in the same site as previously described[2,3].

The surgical procedures did not last more than 10 min. Intra- and postoperative mortality was very low when only the splenic catheter was placed. When this method was used in a group of 39 normal rats, 2 had to be discarded because the tip of the catheter appeared in the spleen's surface, 2 died at days 3 and 5 after surgery and an other revealed a splenic hematoma in the postmorten examination. From the 34 rats studied only 1 showed signs of catheter obstruction as verified in the postmorten examination. More difficulties were found when this technique was applied on animals made cirrhotic by a method previously described[4,5]. From a group of 26 cirrhotic rats, 6 were discarded because of intraoperative problems and 3 died in the first 4 days after surgery. Splenic bleeding, hypotension and infection were the most frequent complications. No catheters were found to be obstructed during the 7-day period after surgery.

Experiments were performed from 4 h to 7 days after the surgical preparation. We prefer to use large cages in which the rat can walk freely rather than restraining cages, because more stable arterial pressure and pulse rates have been found. Experiments of more than 6 h duration can be performed. To carry out the portal infusions, the external end of the catheter is connected to a syringe infusion pump (Unita I, Braun Melsungen, Germany) and a pressure transducer (Statham) and recorder (Poligraph, Grass Inst Co. Quincy, Mass., USA) using a 3-way stopcock. Basal intrasplenic pressure was measured by injecting 0.1 ml of heparinized saline in the catheter as a bolus. This procedure causes an abrupt rise in the pressure which slowly decreases and stabilizes at basal intrasplenic pressure. Intrasplenic pressures recorded for different experiments averaged 13.3 ± 0.6 cm H_2O for 26 control Wistar male rats and 29 ± 3.7 cm H_2O for 18 cirrhotic rats of the same strain, sex and weight. These values are very similar to the ones previously reported by our laboratory using direct splenic puncture[3] and do not increase with the time of cannulation.

The possibility of using high infusion rates without altering the splenic pressure has also been studied. Actual pressures in the tip of the catheter have been obtained by subtracting from the pressures recorded with the catheter in place, the pressures obtained at the same infusion rates with the tip of the catheter open at air. Isotonic saline infusion at rates as high as 9 ml/h can be performed without detectable changes in intrasplenic pressure. The infusion of isotonic saline into the spleen for 6 h at a rate of 15 ml/h did not cause any increment in organ weight (0.25 ± 0.06% of b.wt in 8 animals, compared with 0.24 ± 0.04% in 8 not infused animals). Also, 3 h after infusion of isotonic saline containing 0.1 μCi of $^{22}NaCl$, less than 0.05% of the activity injected remained in the spleen, an amount similar to the activity measured in the spleen of rats which had received the ^{22}Na infusion i.v. This fact demonstrates that no significant amounts of the substance injected are retained by the spleen.

Some pieces of spleen have been histologically studied after 7 days of cannulation, showing a slight fibrous reaction around the catheter and in the surface in contact with the adhesive.

Aziz et al.[6] have reported a method for cannulation of the vena porta and vena cava in conscious rats using a 4 mm long steel cannula attached to a polyethylene catheter. This method has been successfully used by the same authors[7,8], but it requires considerably more time and greater handling of the viscera that the procedure here described. Furthermore the possibility of vein damage by the steel cannula in more chronic studies can be important.

We think the present method allows repeated intraportal infusions, pressure measurement and angiographic studies of the portal area in conscious or anesthetized rats without a requirement for extensive surgery or alteration of the normal pattern of vascular flow in the splanchnic area.

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A simple filtration device to study the interaction of RNA-polymerase with DNA

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Summary. An accessory for a commercial 10-place filtration apparatus is described, which allows the easy recovery of both filter-bound material and flow-through in the study of DNA-protein interactions by assaying binding of protein to cellulose nitrate filters.

The specific interaction of DNA with some proteins can be monitored by making use of the binding of proteins to cellulose nitrate filters. In this case, if the protein has a high affinity in binding to DNA, the DNA will also be retained by the filter. Using DNA fragments produced by restriction endonucleases these fragments can be characterized by their differing ability to bind a certain protein and the sites where the protein binds specifically can be determined. This type of study is especially useful when promotor sites of RNA polymerase are studied[2-6]. As these assays are normally carried out, only the retained DNA fragment is recovered. We found, however, that the relation of bound