PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 249–254 (1974)

# Techniques for the *in Vivo* Catheterization of the Portal Vein in the Rat (37787)

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Most studies on the absorption of water-soluble compounds in the rat are carried ou using *in vitro* techniques (1). The identification of a compound (or its derivatives) in the portal blood is of paramount importance to understand the mechanism of intestinal absorption. The methods reported for the cannulation in larger animals such as dogs (2), miniature pigs (3), rabbits (4) or baby pigs (5) cannot be applied to the small and fragile portal vein of the rat. Reported here are three methods for obtaining samples of the portal venous blood with a minimum of trau ma in the unanesthetized rat.

*Materials.* Female albino rats (CD strain, Charles River Breeding Labs.) weighing between 180 and 300 g were maintained on a diet of laboratory Purina chow and water *ad libitum*. Fasting previous to operation was not necessary. Used as an anesthetic, 2 ml of Penthrane (methoxyflurane, Abbott Labs.) were poured into a 100 ml beaker containing a small gauze. Polyethylene tubing (Intrame dic, Medical formulation PHF, Clay Adams) was coated with a 5% Siliclad solution (Clay Adams). Catheters of varying sizes were used as follows: PE 10 (i.d. 0.3 mm, 30 cm long); a T cannula made of PE 10 (30 cm long) glued to PE 160 (i.d. 1.2 mm, 0.8 cm long); PE 50 (i.d. 0.6 mm, 26 cm long), bent in such a way that it forms a 90° angle 1 cm from one end. The small end is cut with a bevel so that it is actually used for the cannulation. The catheters are filled with heparin solution 1000 units/ml and the distal end was occluded with a needle or a thin pin.

Uniformly labeled $^{14}$C-*Chlorella* protein (sp act 100 $\mu$Ci/ml) was a product of the Radiochemical Centre, Amersham. As a carrier, the bulk protein of the strain of the unicellular alga *Chlorella vulgaris*[2] was isolated (6). (Glycyl-1-$^{14}$C) glycine hydrochloride (sp act 12.4 mCi/mmole) was purchased from Amersham/Searle. Glycylglycine carrier was a product from Schwarz/Mann. Radioactivity was measured by the method described recently (7). A Packard Tri-Carb liquid scintillation spectrometer Model 3380 with absolute activity analyzer No. 544 was used.

*Methods and Results. Procedure A.* The abdomen is opened by a midline incision. The mesentery between the spleen and the stomach is gently moved aside and the spleen is withdrawn to the right side of the abdominal cavity. Blood vessels (vein and artery together) related to the spleen are ligated in individual pairs, with silk No. 5-0. The lienal vein is carefully dissected; blunt-tipped, curved, nontraumatic forceps are essential for this maneuver. A loose ligature is placed around the splenic vein 3 mm from its entry into the portal vein. Another loose ligature is placed 5 mm above the entry of the gastric vein into the lienal vein; a third ligature is tied tightly 10 mm from the previous one (see Fig. 1). To prevent blood loss, a nontraumatic clamp (Mayfield aneurysm clip) is placed in position. By means of a Castro-

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[2] We are indebted to the Radiochemical Centre for this gift.

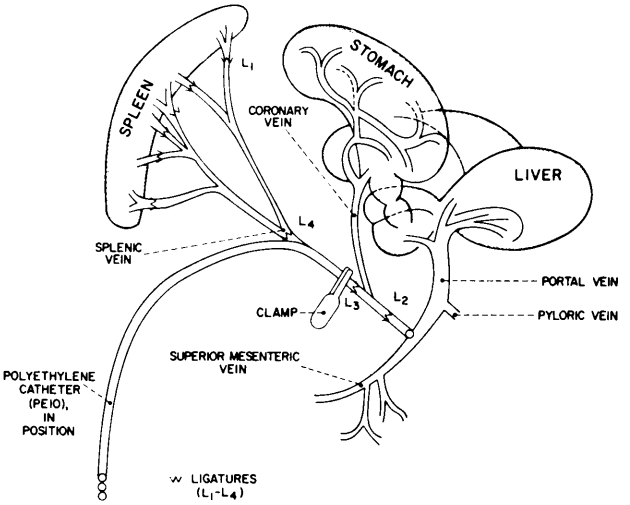


FIG. 1. Catheterization of the portal vein in the rat, Procedure A. The vessels connected to the spleen are carefully dissected and ligated (L1). Splenectomy follows. Loose ligatures ($L_2$ and $L_3$) are placed around the lienal vein. To stop blood flow, a nontraumatic arterial clamp is used. The splenic vein is catheterized just below ligature $L_4$, the clamp is removed and the cannula is inserted in such a way that its tip rests at the opening of the splenic vein into the portal vein. The catheter is secured in position with ligatures $L_2$ and $L_3$ and brought out the abdominal wall. The abdomen is closed and the animal is placed in a restraining cage. During the absorption experiments, continuous sampling of portal blood can be carried out for a period of 60 min.

viejo iris scissors (Miltex E. Miltenberg Inc.), a small opening is made in the splenic vein 2 mm below the distal ligature. The cannula (PE 10) is introduced, the clamp is rapidly removed and the catheter is fastened into the vessel so that its tip rests at the opening of the lienal vein into the portal vein wall (Fig. 1). After splenectomy, the catheter is drawn out the abdominal cavity by procedures described previously (8). Subsequent to closure of the abdomen, animals are put in restraining cages (8). Procedure A was successfully performed 2 to 3 times weekly for a period of 2 yr.

The experiments are carried out 24 hr following surgery. Rats, prepared as described above, received by stomach intubation 3 ml of a saline suspension containing 10 $\mu$C of $^{14}$C-*Chlorella* protein and 250 mg *Chlorella* protein carrier. Samples of blood were taken, continuously, for 60 min in 50 $\mu$l heparinized capillary tubes. Kinetics of appearance of radioactivity in the portal vein (Fig. 2) shows a sharp increase within the first 20 min, a slower increase from 20 to 60 min and minor variations thereafter. Most of this material was identified as free amino acids.

*Procedure B.* Following the opening of the abdomen, the vessels connected to the spleen are individually tied. After splenectomy all viscera are gently drawn to the left side of the abdominal cavity to expose the portal vein. The pyloric and splenic veins are tied, separately, 1–2 mm from their opening into the portal vein. Loose ligatures are then placed around the portal vein; one 3 mm above the pyloric vein opening and the other 5 mm below the splenic vein opening. The next steps require speed and precision and should not take more than 2 min. A nontraumatic clamp is placed around the portal vein above the ligature proximal to the liver; another clamp is placed below the distal ligature. With a Castroviejo iris scissor, an opening is made, transversely, into the portal vein, midway between the two clamps. One end of the PE 160 polyethylene tubing is inserted, the ligature is tied and the portal clamp is immediately removed; some blood appears in the PE 160 catheter. The other end of this catheter is introduced into the distal part of the portal vein and secured by

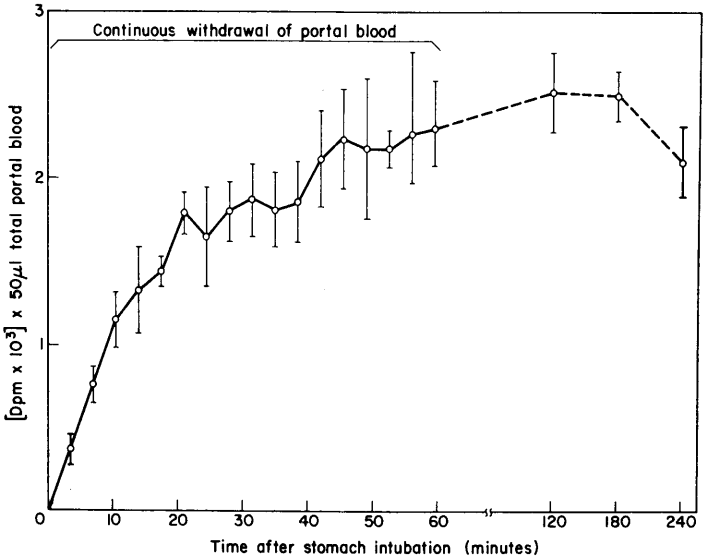


FIG. 2. Appearance of radioactivity in the portal vein of unanesthetized rats administered $^{14}C$-*Chlorella* protein. Female albino rats were fasted for 15 hr and prepared, surgically, by Procedure A. On the day following the operation, the animals were administered, by stomach intubation, 3 ml of a saline suspension containing 10 $\mu$C of $^{14}C$-*Chlorella* protein and 250 mg *Chlorella* protein carrier. Samples of portal blood were collected, continuously, for 60 min in heparinized tubes of 50 $\mu$l capacity. Radioactivity was measured as reported previously (7). Every point is the mean (± SE) from 3 experiments.

the ligature (see Fig. 3). The second clamp is then rapidly released; mesenteric blood flows immediately. The PE 10 portion of the T-cannula is brought out to the right side of the animal through the abdominal wall. The postoperative care of the animals is the same as described above. Although we have had only limited experience with this procedure,

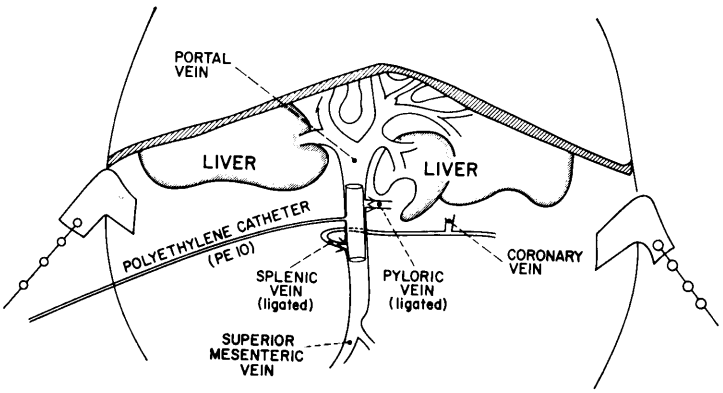


FIG. 3. Cannulation of the portal vein in the rat, Procedure B (final position). Previous splenectomy, tied ligatures are placed around the pyloric and splenic veins to stop their blood flow into the portal vein. Loose ligatures are placed around the portal vein. To block the passage of mesenteric blood, arterial clamps are placed around the portal vein, above the two previous ligatures. A transversal opening is made on the anterior wall of the vein. One end of a special T-cannula is inserted, secured, and the clamp proximal to the liver is removed. The other end of the catheter is introduced, secured, and the distal clamp is removed. The thin portion (PE 10) of the T-cannula is then brought out the abdominal cavity. The treatment thereafter is as in Procedure A.

it has been successful when performed.

When animals prepared by Procedure B were administered by stomach intubation, the same $^{14}C$-containing *Chlorella* protein suspension as described above, the kinetics of appearance of radioactivity in the portal blood did not differ significantly from that shown in Fig. 2.

*Procedure C.* This technique implies direct puncture of the portal vein. In the biological preparation here employed, in order to exclude variable gastric emptying-rate, duodenal catheterization preceded that of the portal vein. PE 50 is introduced 6 mm into the duodenum at a point 2 mm distal to the pyloric junction and secured by silk No. 5-0, and a small piece of polyvinyl plastic is glued with Eastman 910 adhesive. This cannula is brought out the abdominal wall. The portal vein is now carefully exposed. With the beveled end of the heparin-filled cannula (PE 50) an opening is made into the anterior wall of the portal vein and the catheter is introduced so that its tip rests about 5 mm below the entry of the splenic into the portal. The tubing is then fixed in position with Eastman 910 adhesive and a round piece of polyvinyl plastic (from disposable gloves), 2.5 mm in diameter, placed around the portal opening (Fig. 4). Care must be exercised not to apply too much glue to avoid coalescence of the abdominal organs. The cannula is then drawn out the abdominal cavity; traction on the catheter must be minimum to avoid rupture of the portal vein. The postoperative care is also as described above. We have had success with this procedure for over 1 yr; the number of animals used each week varied from 3 to 6. Occasionally, the use of this technique has enabled us to experiment on 10 to 12 animals at one time.

Rats, in which the portal vein had been catheterized by Procedure C were infused, intraduodenally, with 0.5 ml of a solution containing 7.14 $\mu$C of $^{14}C$-glycylglycine · HCl and 50 mg of glycylglycine carrier. Figure 5 shows the kinetics of appearance of radioactivity into the portal vein plasma, with a "peak absorption" at about 10 min and relatively small changes thereafter.[3]

*Discussion.* The methods reported here permit the monitoring of changes in the appearance of a compound, in its original or derivative form, in the portal vein of the conscious rat. If, in addition, portal blood

[3] Preliminary results indicate that 8 to 20% of this radioactivity is in the form of dipeptide.

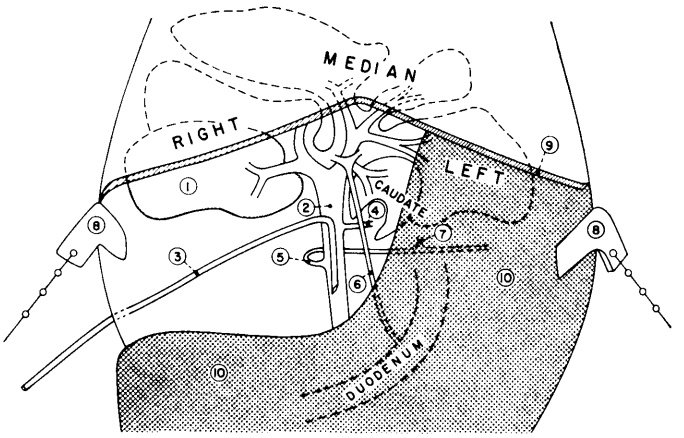


FIG. 4. Direct catheterization of the portal vein in the rat, Procedure C. The beveled end of a heparin-filled cannula (PE 50) is used to perforate the anterior wall of the portal vein. The catheter is introduced so that its tip rests 5 mm below the entry of the splenic vein into the portal. The tubing is then fixed in position with a round piece of polyvinyl plastic placed around the portal opening and secured with Eastman 910 adhesive. The steps that come after are the same as in Procedure A. (1) liver and its lobes; (2) portal vein; (3) PE 50 polyethylene cannula, in position; (4) pyloric vein; (5) splenic vein; (6) common bile duct; (7) coronary vein; (8) abdominal retractors; (9) diaphragm; (10) gauze.

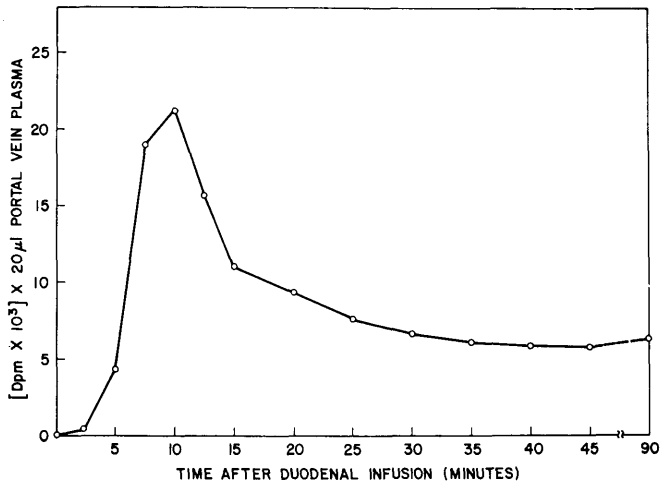


FIG. 5. The appearance of radioactivity in the portal plasma of rats administered $^{14}$C-glycylglycine·HCl. Rats underwent catheterization of the duodenum and of the portal vein, by Procedure C. On the day following operation, they were administered 0.5 ml of a saline solution containing 7.14 $\mu$C $^{14}$C-glycylglycine·HCl in addition to 50 mg of glycylglycine carrier. Blood samples were collected in heparinized tubes at the specified times and 10 $\mu$l of plasma counted, in duplicate. The figure is representative from a group of 4 experiments.

flow is measured by relatively simple methods (9), then it is possible to study the rate of appearance (kinetics of absorption) of a substance in the portal vein blood. The operative techniques are difficult to carry out and offer both advantages and disadvantages. The catheterization of the portal vein through the splenic vein (Procedure A) is the most difficult, delicate and laborious; it takes approximately 30 min and involves splenectomy. The tip of the catheter must be placed exactly at the opening of the splenic vein; when left a few millimeters distal to the portal vein, then this portion of the lienal vein could collapse and stop blood flow. If, on the other hand, the catheter is inserted inside the lumen of the portal vein, then the portal flow is disturbed. On the other hand, this method allows the *continuous* withdrawal of portal blood for 60 min and with the infusion of heparin solution, this preparation remains functional on the average for 1 wk. This technique has permitted a comparison of the portal absorption of $^{14}$C-*Chlorella* protein in fed and fasted rats (10). Procedure B requires speed, precision, and adequate assistance. The most common complication when the operation is prolonged is the pronounced engorgement and congestion of the mesenteric circulation, with the subsequent liver hypoxia and the clotting of blood within the portal vein. Other disadvantages of this technique are splenectomy and the closure of the pyloric and splenic veins. However, this preparation remains functional for several months[4] and, during experiments, it also permits the continuous withdrawal of portal blood. The similarity of the kinetics of absorption curves using this and the previous procedure indicates that these techniques give a reliable picture of the absorption process. The most advantageous technique here described is the direct catheterization of the portal vein (Procedure C). It is the easiest, takes only a few minutes and the experimental conditions closely approximate normal physiology. Using this method, there is no blood loss, the veins related to the portal vein are undisturbed and no splenectomy is necessary. The major disadvantage of this procedure is securing the cannula over the

[4] Six months after the operation, rats which underwent catheterization of the portal vein by this procedure are in apparent good health. Opening of the abdomen demonstrated pronounced peritoneal reaction, characteristic of the rat, but no mesenteric congestion or gross signs of portal hypertension.

wall of the portal vein but this is obviated by the use of adhesive. This preparation remains functional for 3–4 days. This method has been used by us to evaluate the appearance of radioactivity after administration of $^{14}$C-glycylglycine to overnight fasted and fed rats (10). This technique, in association with the catheterization of the aorta, has been used to study[5] simultaneous changes in glucose, insulin and free fatty acids associated with the initiation of lipogenesis and of gluconeogenesis elicited by an acute glucose load. Other possible complications with any of the 3 procedures described are rupture of the vein(s), formation of abundant fibrous tissue in the vicinity of the catheter, and, in the case of Procedure C, compression of the tip of the catheter against the wall of the portal vein and minor phlebitis. In these series, no pyemia, liver necrosis or pulmonary embolism have been observed.

The reported procedures can be used in conjunction with the catheterization of the stomach (11), the common bile duct (8), the duodenum or the small intestine to evaluate the absorption of water-soluble compounds under several conditions. When the chemical composition of a compound is such that during its absorption there is a partition between the portal vein and the lymph, then these techniques could be associated with the catheterization of the thoracic duct (8) in the same animal. An example of such a study has been reported (12). Procedures A and B could also be used for the infusion of fluids, at a constant rate, into the portal blood stream. Procedure C is not recommended for the infusion of fluids into the portal vein.

*Summary.* Techniques are reported for the catheterization of the rat portal vein. These methods permit drawing of blood samples without pain in unanesthetized animals under near physiological conditions. These procedures can be used for the assessment of the rate of intestinal absorption via the portal vein and/or the infusion of materials into the portal bloodstream.

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Received July 2, 1973. P.S.E.B.M., 1974, Vol. 145.