reported to contain pars nervosa cells which take up estrogen[4]. As in the armadillo, the functional significance of these findings is unknown.

The number of anterior lobe cells which possess binding sites for estradiol appears to vary considerably from one species to another. A total of 28.8% of the cells in the armadillo bound the tritiated steroid as compared to 17.2% of the pars distalis cells in the adult ovariectomized rhesus monkey[5] and 86% in the mature, castrated rat[3]. Nuclei of a 'relatively small' number of cells in the hypophyses of the intact tree shrew and castrated squirrel monkey were labelled after exposure to the radioactive estrogen[4] while considerably more cells concentrated this hormone in the pituitary glands of the opossum[4] and guinea-pig[9]. The reason for the high degree of interspecies variation in the number of target cells for estradiol is unclear. It may be partially related to the amount of endogenous estrogens which are available to occupy binding sites within the pars distalis at the time the test animal is given the radiolabelled steroid[4]. On the other hand, there may be a phylogenetic relationship to the number of estrogen-sensitive cells; however, considerably more species need to be examined in order to validate this premise.

It has recently been shown that the pituitary cells which concentrate estradiol also secrete luteinizing hormone, follicle-stimulating hormone, growth hormone, prolactin, thyrotrophin and corticotrophin[11]. Studies are currently underway in our laboratory to determine which of these hormones reside in cells that possess estrogen binding sites in the pars distalis of the armadillo.

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PRO EXPERIMENTIS

A simple method of cannulating the portal vein and obtaining multiple blood samples in the rat[1]

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Summary. A simple and rapid technique for cannulating the portal vein in the rat is described. Multiple blood samples can be obtained or alternatively substrate may be infused through the cannula.

We describe a simple means of obtaining sequential blood samples from the portal vein of rats after directly cannulating the vein. Prior descriptions have been more complex[3], or require repeated insertions of a needle[4] or, a cannula has to be inserted through and ligated into a tributory of the portal vein (splenic vein[5], pyloric vein[6]), or a venous shunt has to be constructed[7]. Our method is also readily adaptable to sampling of blood from other (small) blood vessels or to infusion of substrate.

Method. Rats weighing 250–300 g are suitable. After anesthesia a midline abdominal incision is made from the symphysis pubis to the xyphoid process. From the xyphoid process the incision is extended cephalad and laterally on both sides superficially to the bony thoracic cage so that the total incision is Y-shaped. A straight hemostat is clamped to the flap of skin and muscle on each side close to the junction of the thoracic and abdominal tissues. Each flap of tissue is rolled around the hemostat and pulled laterally (this has the effect of raising up the abdominal content and eases further access). The liver is then gently everted over the sternum, the duodenum is freed, reflected to the (rat's) left side and the portal vein is exposed. A 0.5–1-cm segment of portal vein is teased free from the mesentery. Then a curved hemostat (concave dorsally) is slipped under the freed portion of the portal vein and opened just sufficiently to place slight tension on the vein (fig.). With the bevel facing dorsally the point of a 23-gauge needle is partially inserted in a cephalad direction into the wall of the portal vein. The needle is then lifted slightly ventrally to act as a guide for the immediate introduction of the bevelled end of a 13–15 cm long PE 50 polyethylene cannula (Intramedic polyethylene tubing, inside diameter 0.585 mm × outside diameter 0.965 mm, Clay-Adams, Division of Becton Dickinson & Co. Parsippany, N.J. 07054) filled with normal saline and connected to a variable speed infusion pump. The cannula is threaded 3–4 mm into the portal vein by sliding it along the underside of the needle (side nearest to the portal vein). The needle is now quickly removed. The puncture site is immediately sealed and the cannula is simultaneously fixed into place with 1 drop of a cyanoacrylate ester cement (Super Glue 3, Woodhill Permatex, Cleveland, Ohio 44128). Normal saline is continually infused via the cannula at a slow rate (1 ml/h). Duodenum and liver are replaced and the abdominal incision is clamped shut. The cannula is further anchored by applying 1 drop of cyanoacrylate ester cement to the skin at the point where the cannula exits from the abdominal cavity. With practice total operating time, after induction of anesthesia is less than 15 minutes. We allow an equilibration period of 15 min after clamping the abdominal cavity shut before commencing experiments. Pilot studies have shown that this period is adequate for measuring intestinal transport.

Samples of portal vein blood are obtained by temporarily disconnecting the cannula from the pump. Portal blood

pressure easily expells the saline from the cannula. When the cannula is filled with blood, a 23-gauge needle attached to a 1-ml tuberculin syringe is inserted into the cannula and the sample withdrawn. The cannula is then reconnected to the pump and flushed clear of blood by a momentary increase in the rate of saline infusion. The sampling process takes less than 1 min. We have routinely taken blood samples as close together as every 3 min. An example of a validation experiment is given below.

Isotonic Ringers solution with 1 mM galactose and tracer ^{14}C galactose was instilled, via a plastic catheter, into the lumen of a segment of either duodenum or ileum approximately 12 cm long, the segment lumen being isolated by ligatures from continuity with the rest of the small bowel lumen. Samples of portal vein blood were withdrawn just prior to and every 3 min after instillation of the luminal solution. We chose galactose because it is not metabolized by rat small bowel mucosa in vivo[8] yet shares the same transport pathway as glucose[9]. The table confirms very obvious differences in hexose transport in duodenum and ileum as manifested by the differences in radioactivities of sera from samples of portal vein blood removed at 3-min intervals.

Galactose concentration in portal blood[a]

		Duodenum (n = 8)	Ileum (n = 8)	p-value
Segment length (cm)		12.1 ± 0.5	11.9 ± 0.4	NS
Small bowel luminal solution ^{14}C galactose[b]				
Before instillation		19273 ± 232	19722 ± 182	NS
At conclusion		5095 ± 427	9352 ± 348	< 0.001
Portal vein ^{14}C galactose (serum)[b]				
Time (min)	0[c]	8 ± 3	4 ± 2	NS
	3	399 ± 51	28 ± 4	< 0.001
	6	401 ± 41	35 ± 6	< 0.001
	9	397 ± 43	35 ± 5	< 0.001
	12	392 ± 46	36 ± 5	< 0.001
	15	386 ± 43	41 ± 6	< 0.001
	18	380 ± 43	50 ± 7	< 0.001
	21	365 ± 38	48 ± 9	< 0.001

[a]Data are mean ± 1 SEM; [b]cpm per 0.1-ml sample; [c]sample obtained before instilling solution into the segment lumen; n, Number of animals studied; NS, no significant difference ($p > 0.05$).

Discussion. The method for cannulating the portal vein described here is simple and fast. Technical failures are low (< 10%). With practice we are now able to cannulate the portal vein of rats weighing as little as 75–80 g. No special attributes other than a steady hand are necessary. There is virtually no loss of portal vein blood during the insertion of the cannula. No engorgement of the intestinal veins or acute reduction in blood flow to the liver occurs during or after cannulation. The cyanoacrylate ester adhesive seals the junction of cannula and portal vein almost instantaneously, hardens extremely rapidly and holds the cannula securely. Care is required in using the cement, 1 drop is adequate. Its great adhesiveness and rapid hardening make it difficult to separate surfaces (including fingers!) once the glue is applied. The low volume infusion of saline through the cannula avoids the need for heparinization of the animal's blood. The infusion rate can be adjusted to replace the volume of blood removed by sampling. There is also no dead space loss of blood from the cannula after each sample – a loss that could become significant with multiple samples.

The technique is applicable to in vivo studies of intestinal transport (table) or to infusions of biochemical, nutritional or pharmacologic substrates into the portal vein. In conjunction with cannulations of the common bile duct and/or a systemic blood vessel, the technique is also applicable to a variety of metabolic and pharmacokinetic investigations. Studies in conscious rats are also feasible. The sampling/infusion catheter is then brought out dorsally between the scapulae by means of a s.c. tunnel and connected to an infusion apparatus via a harness and swivel assembly in a manner analogous to that described for i.v. feeding in unrestrained rats[10].

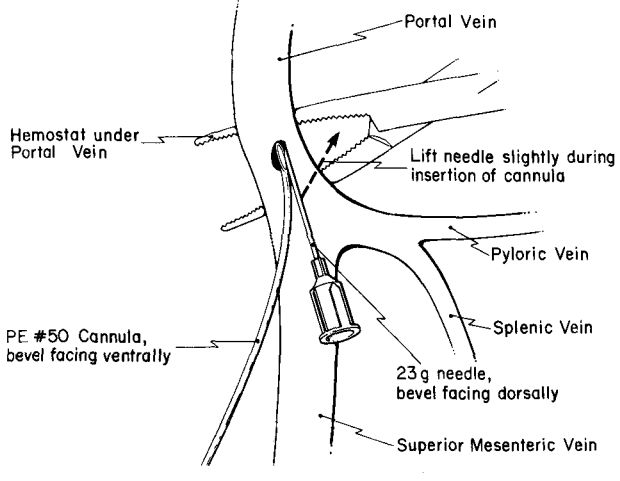

Diagram of the anatomy of the portal vein and technique for insertion of the polyethylene cannula.

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