Research Institute of Pharmaceutical Sciences
School of Pharmacy
University of Mississippi
University, MS 38677

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[x] To whom inquiries should be directed.

# Improved Method for Sampling Hepatic Venous Blood in the Rat

**Keyphrases** □ Blood sampling, hepatic venous—improved method, rats □ Hepatic blood samples, venous—improved method for sampling in the rat □ Elimination—method for sampling mixed hepatic venous blood proposed, rats

*To the Editor:*

In an attempt to study hepatic elimination of drugs *in vivo* in the rat, a method for sampling the mixed hepatic venous blood was devised. For estimation of hepatic extraction of drugs *in vivo*, simultaneous measurements of drug concentrations in hepatic arterial, portal venous, and hepatic venous blood are necessary. Extraction of propranolol by the dog liver was studied by direct measurement of hepatic venous concentrations of the drug *in vivo* (1). To obtain hepatic venous blood of the dog, a cannula was introduced into the right or left hepatic vein of the dog *via* a superficial jugular vein under fluoroscopic visualization. This cannulation applied in the dog is not practical for a hepatic vein of the rat, since hepatic veins of the rat are quite narrow and fragile and the cannulation is liable to cause trauma to the liver.

In this communication, we report a simple method for sampling the mixed blood draining from the hepatic veins of the rat in an *in vivo* study of hepatic drug binding and the subsequent drug metabolism. The method described here consists of introducing a cannula (Cannula A, Fig. 1) into the inferior vena cava *via* the external jugular vein so that the mixed blood from the hepatic veins may flow through an artificial channel in the inferior vena cava. Subsequent insertion of another cannula (Cannula B, Fig. 1) into the inferior vena cava makes it possible to sample the mixed hepatic venous blood.

A rat, 270–330 g, is placed in supine position and is anesthetized lightly with ether at suitable intervals. The abdomen is opened through a midline incision extending from the symphysis pubis to the xiphoid. The right lobe of the liver is reflected to the upper left, and the loops of the intestine are retracted downward to the abdominal cavity to expose the inferior vena cava between just above the right renal vein and the point where the vena cava becomes buried in the liver. The vena cava in this region is freed from its connective tissue, and a ligature is passed under the vena cava.

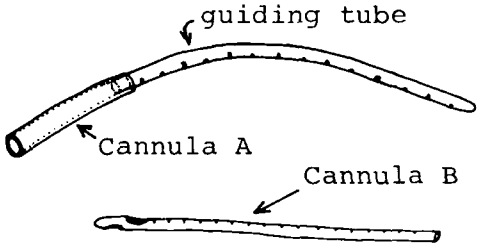


**Figure 1**—*Cannula A with a guiding tube and Cannula B. A guiding tube (polyethylene tubing, 0.2 cm o.d., 15 cm length) with its tip closed is inserted approximately 3 mm into Cannula A (polyethylene tubing, 0.2 cm i.d., 0.25 cm o.d., 3.2 cm length). The slight curve of the guiding tube is helpful in correcting false routes taken by the tip of Cannula A. Cannula A is filled with heparinized blood taken from another rat. Cannula B, with its tip closed, has two lateral holes near its tip. The guiding tube and Cannula B are put with centimeter markers throughout their length.*

The abdominal incision is then temporarily closed with two mosquito forceps. The right external jugular vein is exposed between the cephalic vein and the posterior external jugular vein and is ligated approximately 3 mm proximal to the junction of the posterior external jugular vein and the right external jugular vein. A small cut is made with scissors on the jugular vein, approximately 5 mm proximal to the ligature, while the jugular vein between the cut and the root of the cephalic vein is closed with a clamp to prevent bleeding.

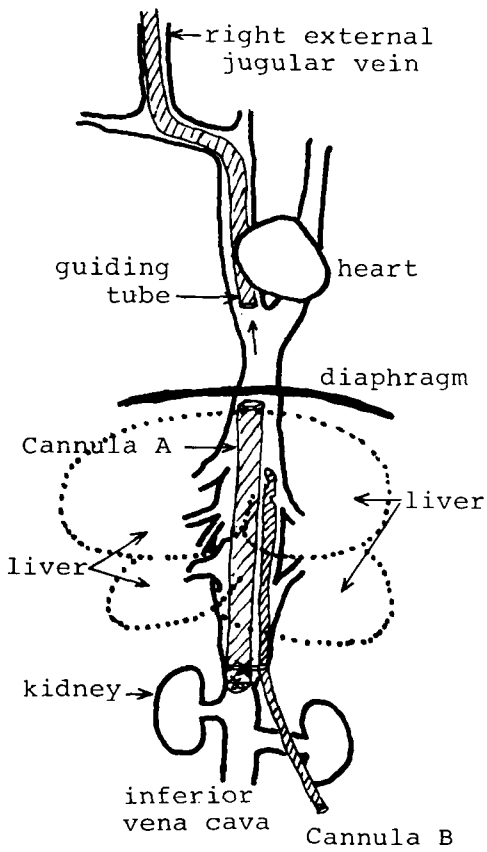


**Figure 2**—*Schematic representation of the inferior vena cava double cannulation for sampling mixed hepatic blood.*

**Table I**—Portal Venous ($C_{pv}$) and Hepatic Venous ($C_{hv}$) Concentrations[a] and Hepatic Extraction Ratios[b] (HER) following Intravenous Administration of Propranolol and Lithium Chloride

| | Propranolol | | | | | | Lithium Chloride | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 mg/kg | | | 12.5 mg/kg | | | 40 mg/kg | | |
| Minutes | $C_{pv}$, ng/ml | $C_{hv}$, ng/ml | HER | $C_{pv}$, ng/ml | $C_{hv}$, ng/ml | HER | $C_{pv}$, mEq/liter | $C_{hv}$, mEq/liter | HER |
| 5 | 1915 ± 600 | 7 ± 7 | 0.996 | 5224 ± 491 | 414 ± 162 | 0.921 | 1.58 ± 0.28 | 1.51 ± 0.27 | 0.04 |
| 30 | 1151 ± 476 | 1.7 ± 1.7 | 0.999 | 2061 ± 478 | 306 ± 93 | 0.852 | 0.96 ± 0.10 | 0.97 ± 0.08 | 0 |
| 60 | 639 ± 217 | 12 ± 12 | 0.981 | 1611 ± 404 | 104 ± 50 | 0.935 | 0.91 ± 0.07 | 0.91 ± 0.10 | 0 |
| Mean | | | 0.992 | | | 0.903 | | | 0.01 |

[a] Drug concentrations are expressed as mean ± *SE* for three rats. [b] Hepatic extraction ratios are expressed as the difference between the mean drug concentrations in the portal venous and hepatic venous blood divided by the concentration in the mean portal venous blood, since the drug concentrations in the portal venous blood and the hepatic arterial blood (estimated from the drug concentrations in the aortic blood) were not different significantly.

The operator starts to introduce Cannula A, provided with a guiding tube (Fig. 1), into the jugular vein from the cut toward the heart; at the same time, an assistant widens the cut carefully with dissection forceps and subsequently removes the clamp. The assistant ties softly a loose ligature, which is placed in advance around the jugular vein between the cut and the root of the cephalic vein to prevent loss of blood from the cut when the rear end of Cannula A passes the cut of the jugular vein. The cannula is pushed gently over a length of approximately 50 mm into the jugular vein until its tip is located near the diaphragm. Then the abdomen is again opened.

The assistant exposes the vena cava between the diaphragm and the upper margin of the liver so that one can observe the tip of Cannula A passing under the liver. The operator must guide Cannula A carefully near the right auricle, 30–35 mm from the cut, so as not to force it into the right auricle or the left superior vena cava. The operator must also be careful during the passage in the region of the junctions of the hepatic veins and the inferior vena cava. If Cannula A does not advance smoothly, one should not force it. This difficulty can usually be overcome by drawing Cannula A back slightly or rotating it slightly. The tip of Cannula A is then held approximately 5 mm proximal to the junction of the right renal vein and the inferior vena cava by tying the previously placed loose ligature (Fig. 2).

Immediately after ligation, the operator pulls the guiding tube out of Cannula A and extracts it from the vena cava and external jugular vein; the assistant holds Cannula A tightly over the vena cava with dissection forceps. It is important at this stage that the guiding tube be pulled out as quickly as possible after the ligation to relieve the obstruction of blood flow in the vena cava. Then the operator ligates the jugular vein just proximal to the cut to close the cut of the jugular vein.

The assistant reflects the right lobe of the liver to the left again and retracts the central and left lobes gently to expose the inferior vena cava between the lower margin of the liver and the right renal vein. Then the operator makes a venous puncture by a needle point, approximately 3 mm proximal to the ligature of the vena cava, and introduces Cannula B (Fig. 1) over a distance of approximately 15 mm toward the heart, picking up the vena cava near the cut by dissection forceps. Cannula B should be inserted up to 20–25 mm from the caudal tip of Cannula A to avoid reflux of the blood of the inferior vena cava into Cannula B. Usually no bleeding is encountered during this maneuver.

A syringe is attached to the other end of Cannula B, and it is then confirmed that the hepatic venous blood flows out in Cannula B on slight suction. Cannula B is secured by ligation, and the abdominal incision is closed. At the end of experiment the animals are sacrificed to make sure that the lateral holes of Cannula B are located in such a way as to draw the mixed hepatic venous blood. The duration of operation for this double-cannulation method is approximately 15 min.

The influence of this improved technique for sampling hepatic venous blood on hepatic blood flow was examined by the method usually employed in humans and dogs (2, 3). This method for calculation of hepatic blood flow is based on the Fick principle, using constant infusion of sulfobromophthalein, which is removed exclusively by the liver. The procedure used here was the same as that used by Chauvaud *et al.* (4) in the rat except for the sampling of hepatic venous blood. The result obtained with 30 rats after this operation showed a mean flow rate of 4.06 ± 0.22 *(SE)* ml/min/100 g of body weight. This value is comparable to reported values (5, 6). This finding indicates that the basal hepatic blood flow is not altered by the double cannulation into the inferior vena cava.

An extremely high extraction ratio during intravenous administration of propranolol was obtained in the dog by measurement of hepatic venous drug concentrations (1). Our previous result also suggests that the hepatic extraction of propranolol might be almost complete in the rat in a dose range of 2.5–12.5 mg/kg (7). The described double vena cava cannulation was used for estimation of the hepatic extraction ratio, using propranolol and lithium chloride as test compounds. Propranolol was administered to rats in doses of 5 and 12.5 mg/kg in 30 sec into the femoral

vein, and lithium chloride was administered in a dose of 40 mg/kg in the same manner. Hepatic extraction ratios of propranolol and lithium chloride were measured by obtaining blood samples simultaneously from the portal vein and the hepatic veins at various intervals after administration.

The portal venous blood of the rat can be taken through a cannula introduced *via* the pyloric vein into the hepatic portal vein (8, 9). Propranolol and lithium concentrations in whole blood were determined by the spectrophotofluorometric method of Shand *et al.* (10) and by the flame-photometric method of Amdisen (11), respectively. The hepatic extraction ratio was calculated from drug concentrations of the portal venous and hepatic venous blood samples taken simultaneously. The drug concentrations in the portal venous and hepatic venous blood and the mean hepatic extraction ratios are shown in Table I.

Propranolol concentrations in the mixed hepatic venous blood were always much lower than those in the portal venous blood, while lithium concentrations in the two venous blood samples were almost equal. Hepatic extraction of propranolol was essentially complete in the dose of 5 mg/kg, and it was more than 85% even in the dose of 12.5 mg/kg. On the other hand, lithium was essentially not extracted in the dose of 40 mg/kg.

A method for sampling hepatic venous blood may also be used for measurement of hepatic blood flow. In humans and in dogs, blood samples from a hepatic vein may be easily taken under direct fluoroscopic visualization by catheterization of a hepatic vein *via* the median basilic vein and the superficial jugular vein, respectively. However, in the rat, several technical problems arise on account of the small size of the vessels and liver. A major problem is to place a cannula in such a manner as to avoid trauma to the liver and to prevent contamination by the blood of the inferior vena cava.

For sampling hepatic venous blood, Dhumeaux and Berthelot (12) applied a catheter to one of the small hepatic veins through an incision made in the central lobe of the liver. The main disadvantage of the hepatic vein catheterization as well as this transhepatic vein catheterization is that one does not sample the mixed blood draining from all of the hepatic veins, since the hepatic veins empty themselves separately into the inferior vena cava. Drug concentrations in blood samples obtained by these methods are not always representative of the level in the entire hepatic venous outflow (13). Therefore, data obtained from such localized samplings may not be satisfactory for estimation of true hepatic extraction of a drug by the whole liver.

The technique of the double cannulation of the inferior vena cava presented here overcomes the disadvantage inherent in the previous methods, since the mixed hepatic venous blood, instead of blood from only one hepatic vein, can be obtained. This improved method for sampling the mixed hepatic venous blood is simple and practical. When applied in the rat, this method has proved useful for the determination of the drug hepatic extraction ratio in the study of *in vivo* drug metabolism as well as for measurement of hepatic blood flow in the rat. Details of these studies will be reported.

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*Tokuji Suzuki* [x]
*Toshiaki Ohkuma*
*Tadaaki Rikihisa*
Faculty of Pharmaceutical Sciences
University of Chiba
Yayoi-cho, Chiba, Japan

*Sadao Isozaki*
Research Laboratory
Department of Pharmaceutical Services
University of Tokyo Hospital
Hongo, Bunkyo-ku, Tokyo, Japan

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[x] To whom inquiries should be directed.

## Concentration Dependence of Ethanol Effect on Intestinal Absorption of Theophylline in Rats

**Keyphrases** □ Ethanol—effect on intestinal absorption of theophylline, concentration dependence, rats, □ Theophylline—intestinal absorption, concentration dependence of ethanol, rats □ Absorption, intestinal—theophylline, concentration dependence of ethanol, rats

*To the Editor:*

Koysooko and Levy (1) recently reported that the rate of absorption of theophylline from the perfused small intestine of anesthetized rats is increased significantly in the presence of a constant 2% concentration of ethanol and that there is a positive rank-order correlation between theophylline absorption from the ligated small intestine of rats and water net flux from