Surgical Catheterization of Hepatic-Portal and Peripheral Circulations and Maintenance in Pharmacokinetic Studies

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Abstract □ A surgical procedure for the chronic catheterization of mongrel dogs was presented with a detailed account of the use and maintenance of these catheters. The methodology allowed for a direct determination of the capacity of the liver to the intact animal to metabolize drugs. The technique permitted the investigator to study the oxidation of drugs by the liver in a specific concentration range and assessment of the first-pass effect of the liver when many drugs are administered *via* the oral route. The dogs were prepared and used in the drug pharmacokinetic studies for periods up to 24 days.

Keyphrases □ Catheterization—hepatic-portal, peripheral circulations, maintenance, pharmacokinetics □ Pharmacokinetics—maintenance of surgical catheterization, hepatic-portal peripheral circulations □ Metabolism—determination of liver capacity in intact dogs, pharmacokinetics, surgical catheterization of hepatic-portal and peripheral circulations and maintenance

Previous investigators (1–17) have reported that following either oral or intravenous administration of ethyl alcohol to humans or animals, there were differences in alcohol distribution in the vascular system. Most authors (1–13) concluded that arterio–venous equilibrium, as defined by zero arterial–venous concentration differences, was attained rapidly in humans. However, previous (14) and more recent studies (15–17) reported distributional trends not seen in the previous work. These results showed, by interpolation, that at only one point in time were equal arterial and venous concentrations attained, and that this time varied with each subject. Increased assay sensitivity and/or longer sampling time perhaps holds the key to the variance in observations.

This methodology permits the direct determination of the capacity of the liver in the intact animal to metabolize drugs. By infusing the liver directly, the blood alcohol concentration entering the liver can be controlled and the oxidation of ethanol in the liver of an intact animal in a specific concentration range under *in vivo* testing conditions can be studied. This technique is useful in assessing the first-pass effect on the liver, observed when many drugs are administered *via* the oral route.

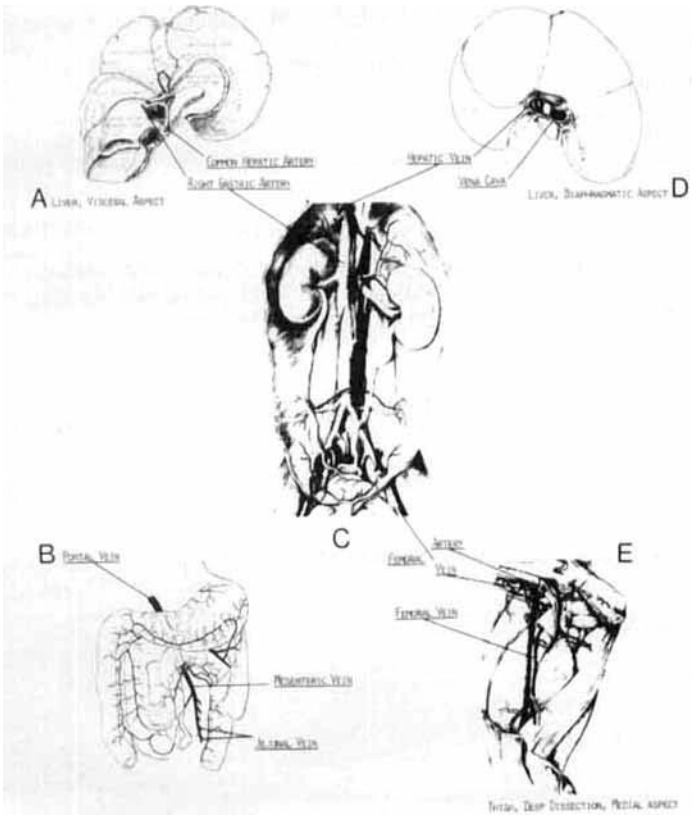

Figure 1—*Diagramatic representation of catheter placements. (Figures from Ref. 18.)*

EXPERIMENTAL

Criteria for Dog Selection—Healthy, full-grown male mongrel dogs (15–31 kg) were selected. The following initial laboratory check-up was performed on each of the dogs: complete blood count (red blood cell count), white blood cell count, differential, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell morphology, sedimentation rate, fecal flotation rate (coccidiosis), creatinine clearance, SGOT, Knott's Test (heartworm), and Brucellosis Screen[1]. Dogs were wormed and vaccinated against rabies[2] and distemper[3].

Dogs selected for the study were quarantined for 1 month and maintained on an adequate diet of regular canine laboratory chow[4]. Prior to their release from quarantine, a similar laboratory check-up, as just described, was performed on each dog to detect any clinical changes and to determine the fitness of each dog for surgery.

Surgical Procedure—Indwelling venous catheters were secured surgically in the hepatic artery, portal vein, hepatic vein, and the femoral artery. An additional catheter was implanted within the saphenous vein.

Prior to surgery, the dogs were fasted (including water) for 6 hr. An enema[5] was administered to each dog 60 min prior to surgery. Acepromazine maleate[6] (0.55–1.10 mg/kg of body weight) was administered intramuscularly as a preanesthetic and/or tranquilizer, and an intravenous injection of sodium pentothal[7] was administered (22 mg/kg of body weight) for induction of anesthesia prior to intubation. With sedation, a 10-mm endotracheal tube[8] was fitted in the dog's throat to facilitate the administration of the anesthetic, methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether[9]) during surgery. A catheter[10] (20 gauge,

[1] Pitman-Moore, Inc., Washington Crossing, N.J.
[2] Trimune Rabies Vaccine, Rolynn Laboratories, Lenexa, Kan.
[3] Delcine HL, Dellen Laboratories, Omaha, Neb.
[4] Ralston Purina Co., St. Louis, Mo.
[5] C. B. Fleet Co., Murray Hill, N.J.
[6] Ayerst Laboratories, Rouses Point, N.Y.
[7] Abbott Laboratories, North Chicago, Ill.
[8] Portex, Hythe, Kent, England.
[9] Metofane, Pitman-Moore, Washington Crossing, N.J.
[10] Jelco Laboratories, Raritan, N.J.

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15 mm) was inserted in the cephalic vein so that 5% dextrose in Ringer's Irrigation Solution[11] could be infused by intravenous drip during the entire surgical procedure (3–4 hr) and for 1 hr postoperation to prevent fluid loss through dehydration.

Aseptic technique was observed at all times within the surgical bay. Anesthetic intake, temperature, pulse, heart rate, respiratory rate, fluid intake, as well as urine and blood loss were monitored throughout the surgical procedure.

The dog was placed on its back and a catheter[12] was introduced into the urinary bladder. A 12-cm vertical abdominal midline incision was made. Skin, fascia, and muscle were spread laterally until the liver and the greater omentum were exposed. Capillary bleeding was arrested by electrocautery. The liver was deep red in color, firm in consistency, and friable.

Implantation of the first catheter into the hepatic artery required location of the right gastric artery. The right gastric artery leaves the common hepatic artery at nearly a right angle, runs into the lesser omentum at the pylorus, and continues to the lesser curvature of the stomach. The point at which the right gastric artery leaves the common hepatic artery was exposed, and the connective tissue surrounding it and on its surface was removed (Fig. 1A). A 1-mm longitudinal incision was made, and a 60-cm venous catheter[13] was fitted in the artery and advanced into the common hepatic artery. It was secured in the artery by suturing with nonabsorbable silk (000)[14] using a square knot stitch. The catheter was flushed using a 10-U heparin sodium/ml[15] solution *via* a 3-ml plastic syringe[16] attached to the sampling barrel of the catheter, and testing for patency by drawing back until bright red arterial blood appeared in the syringe. The catheter was kept patent by the maintenance of the 10-U heparin sodium/ml solution within the catheter throughout the surgical period. A similar procedure was followed for maintaining patency with the implantation of the three other catheters.

Following the insertion of the hepatic artery catheter, an indwelling catheter was placed in the portal vein. A 90-cm catheter[17] was advanced cranially into the portal vein through one of the mesenteric veins.

A mesenteric vein was isolated by the separation of connective tissue from its surface. With isolation, a 1-mm longitudinal cut was made and the catheter inserted and secured with nonabsorbable surgical silk (000), flushed, and kept patent (Fig. 1B).

With the hepatic artery and portal vein secured and patent, the peritoneum, fascia, and muscle were sutured by a horizontal mattress stitch using surgical chromic gut[18] leaving the skin to be sutured later. The two catheters were set aside for further placement.

A 6-cm cutdown was then made on the left pelvic limb, exposing the femoral artery and femoral vein. A 90-cm indwelling venous catheter was implanted into one of the numerous branches of the femoral vein and fed into the postcava with eventual placement in the hepatic sinus. This served as a site for sampling hepatic vein blood alcohol concentrations. The catheter was flushed and kept patent with heparin.

Nonabsorbable surgical silk (000) was again used for securing the catheter. To ensure proper placement, the distance from the femoral vein to the hepatic sinus was measured and marked on the catheter. When the catheter was secured with the silk, its position within the hepatic sinus was verified by feeling for its tip within the sinus through the wall of the vena cava (Figs. 1C and D). In addition, when the study was complete, a necropsy was performed on each dog revealing the proper position of all catheters.

A fourth catheter was then placed in one of the many branches of the femoral artery. A 1-mm longitudinal cut was made and the indwelling catheter fitted within the femoral artery and secured using nonabsorbable surgical silk (000) (Fig. 1E) and maintained with heparin.

The femoral fascia was sutured with surgical chromic gut and the catheters passed between the medial femoral fascia and the skin to the location of two abdominal catheters. This was done by using a sterile 10-mm endotracheal tube. The tube was inserted between the fascia and the skin after they had been separated by blunt forceps. The syringes were removed from the barrel of each catheter and the catheters advanced through the tube. Once the passage was complete and the endotracheal tube removed over the exposed ends of the catheters, heparin solution was again flushed through each of the catheters.

[11] Travenol Laboratories, Deerfield, Ill.
[12] Model No. 1730, C.R. Bard, Inc., Murray Hill, N.J.
[13] Style No. 1914, C. R. Bard, Inc., Murray Hill, N.J.
[14] Ethicon, Summerville, N.J.
[15] Organon, Inc., North Orange, N.J.
[16] Becton, Dickenson and Co., Rutherford, N.J.
[17] Style No. 1936, C. R. Bard, Inc., Murray Hill, N.J.
[18] Deknatel, Queens Village, N.Y.

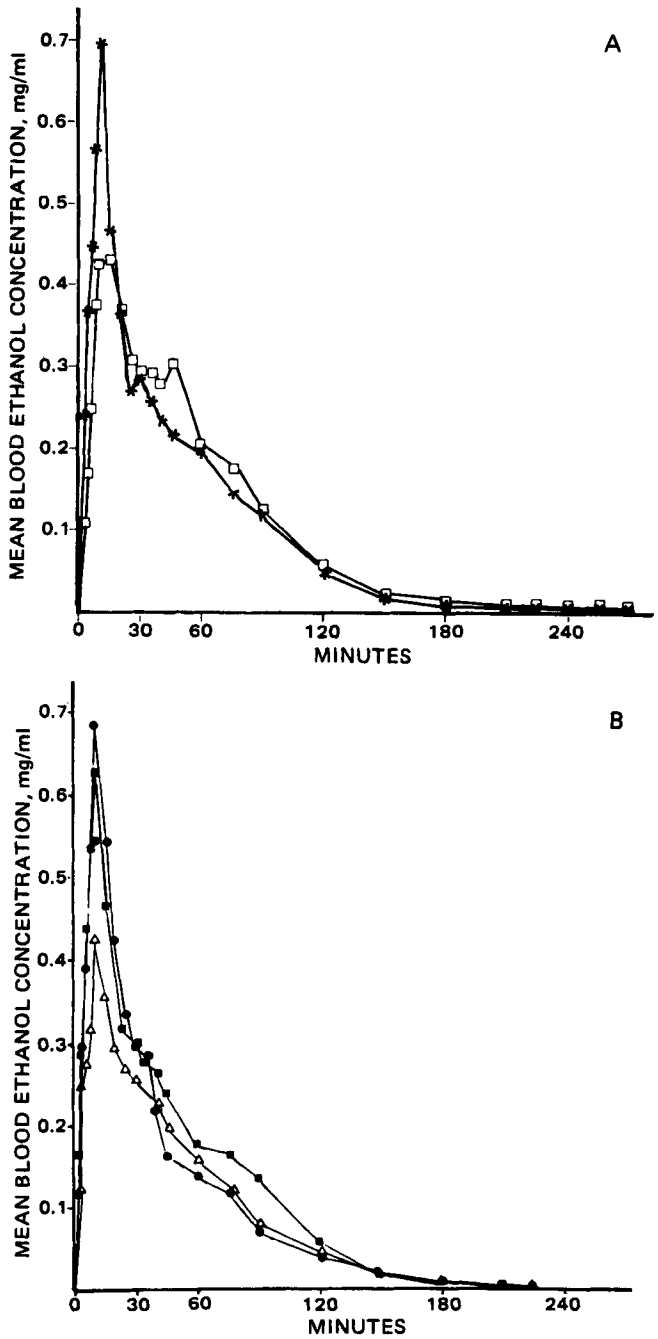

Figure 2—*Typical results of study conducted following surgery. Mean blood–ethyl alcohol concentrations following the 10-min intravenous infusion of 0.26 g/kg of ethyl alcohol* via *the cephalic vein to three dogs. Key: A: (✱) femoral artery; (□) femoral vein; B: (■) hepatic artery, (△) hepatic vein, (●) portal vein.*

The four catheters were placed under the skin to a location behind the dog's neck. This was accomplished by laterally separating (by blunt forceps) the skin and fascia so that the endotracheal tube could be placed between the tissues. A 2-cm incision was made in the dog's skin for the exit of the catheters from the tube. After the tube was positioned, the syringes were removed from the previously labeled catheter barrels and the catheters advanced through the tube. The tube was then removed and a polytef 3-way stopcock[19] was attached to the barrel of each catheter. Each catheter was checked for patency, flushed with 3-ml of the 10-U heparin sodium/ml solution, and the attached stopcock closed off.

The skin at the midline incision and the femoral triangle was then closed using surgical silk (000)[20]. Penicillin G benzathine and penicillin

[19] Model No. K-75, Pharmaseal, Inc., Toa Alta, P.R.
[20] Davis & Geck, Division of American Cyanamid Co., Stamford, Conn.

G procaine[21] (20,000 U/kg, respectively) was administered intravenously to prevent infection.

Following the surgical implantation, there was a need to protect the externalized vascular catheters from the trauma of biting and rubbing. A dog jacket[22], made of durable nylon net, afforded maximal protection and allowed the wound to heal. The jacket was adjustable, so as to fit a range of sizes, and contained two pockets, one on either side of the dog, enabling the housing and protection of the indwelling venous catheters. Clove oil was used as a chewing deterrent. Clove oil was found to be effective and long lasting without ill-effects. In addition, routing the catheters under the skin and through the dermis into an area covered by the jacket's pockets failed to attract the dog's attention and a deterrent from biting was accomplished and proved effective.

Postsurgical Procedure—The dogs were allowed to stabilize postsurgically for a period of 2–3 days. It was found that the heavier dogs required a shorter recovery time. They were permitted their normal diet *ad libitum*. Prior to each animal study the dogs were fasted and water withheld overnight. The four catheters were checked for patency and flushed daily with a 100-U heparin sodium/ml solution in physiological saline.

RESULTS AND DISCUSSION

The experimental design (19, 20), for which the above surgical procedure was developed, entailed the administration of several different doses of ethyl alcohol (8–10% v/v solutions in saline) *via* either the hepatic artery indwelling catheter or a 20-cm catheter[23] inserted at the time of each study into a cephalic vein. Studies were conducted at 2- or 3-day intervals to allow a sufficient wash-out period for the ethyl alcohol. Figures 2A and B show typical results from one such study in which 0.26 g of ethyl alcohol/kg of body weight was administered over 10 min *via* the cephalic vein. Blood samples from all four surgically placed catheters and a fifth catheter (20 cm) inserted at the time of study in the saphenous vein (a branch of the femoral vein) could be collected within a 15–20-sec time period. Thus, simultaneous blood collection at several locations in the vascular system is possible. Simultaneous sampling is essential in order to obtain accurate concentration gradients with time across the liver and within the peripheral circulation. Using the above surgical techniques, mongrel dogs were maintained with patent catheters up to 24 days (postsurgery). The catheters were patent at this time and could have been maintained longer. In this length of time, numerous studies could be conducted.

While the surgical procedure described was for a specific study with ethyl alcohol, it has utility for the study of any drug suspected of liver first-pass metabolism. With appropriate maintenance the catheters could remain patent for extended periods with minimal discomfort to the dog.

[21] Longocil, Fort Dodge Laboratories, Fort Dodge, Ia.
[22] Alice King Chatham Medical Arts, Los Angeles, Calif.
[23] Style No. 1617 C. R. Bard, Inc., Murray Hill, N.J.

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