A Simple Device to Facilitate Rapid Blood Sampling in Small Animals (37321)

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The rat is widely used as an experimental animal. However, the extremely rapid plasma clearance of such materials as drugs, dyes and endogenously generated organic anions, coupled with technical difficulties in obtaining frequent and accurately timed blood samples, has largely precluded kinetic studies which depend on accurately defining the plasma disappearance curves of injected materials. The purpose of this paper is to describe a simple device, easily constructed from inexpensive and readily available materials, which permits the rapid and frequent sampling of arterial blood. Use of this device has permitted accurate definition of multicomponent plasma disappearance curves of such substances as radiobilirubin and sulfobromophthalein in the normal rat.

Materials and Methods. The device consists of a specially modified 3-way teflon stopcock with one end connected to a PE-50 polyethylene arterial cannula, the opposite end connected to a 6-ml syringe containing saline or heparinized saline and the third outlet modified to accept standard 75-mm microhematocrit tubes in which the samples are collected.

The device is illustrated in Fig. 1. Parts necessary for its construction are listed in Table I, and have been chosen to insure snug leakfree connections. Steps for modification of individual components are illustrated in Figs. 2A, B, and C. When the apparatus is assembled as in Fig. 1, the stop-

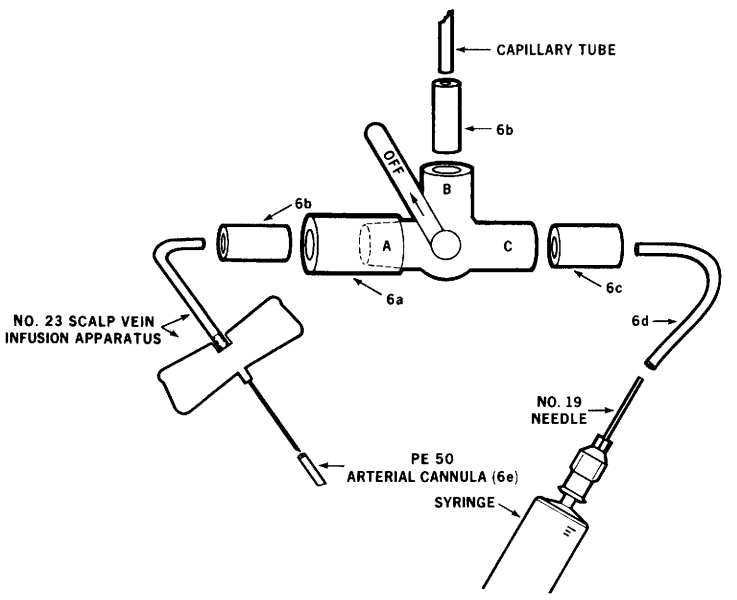

FIG. 1.

cock handle is turned to position B and the system flushed with saline. The handle is now turned to position A and the apparatus is ready for arterial cannulation with the free end of the PE 50 tubing. Note that the length of the PE 50 tubing should not be longer than 5–8 cm, the minimum distance required to stretch from the cannulation site to the sampling apparatus next to the animal. Under these circumstances, the volume of the arterial line from the distal end, in the blood vessel, to the base of the sample tube is approximately 100 μl. After arterial cannulation is completed, the plastic butterfly handle is taped to the animal board to prevent the arterial cannula from accidentally being withdrawn.

When a sample is to be taken, the stopcock handle is turned from position A to position B and the syringe plunger withdrawn until arterial blood appears undiluted in the tubing distal to stub C. A glass capillary tube is then inserted into site B and the stopcock handle turned toward C until the capillary tube is filled. Filling should require no more than 2-3 seconds with proper operation. When the capillary tube is filled, the stopcock handle is turned to position B and the system flushed until the PE 50 tubing

TABLE I. Components of Sampling Apparatus.

1. Standard 3-way stopcock (Pharmaseal K-75)
2. #23 scalp vein infusion set (Abbott # 4565)
3. #19 needle
4. 6 cc syringe
5. Heparinized capillary tubes (Arthur H. Thomas RED TIP[a] Tubes) with internal diameter 1.1–1.2 mm, wall thickness 0.2 mm, and length 75 mm.
6. Tubing with the following lengths and internal and external diameters:

	Internal diameter (inches)	External diameter (inches)	Length (inches)
(a)	1/8	1/4	1/2 (Tygon)[a]
(b)	1/16	1/8	3/16 (Tygon, two pieces)
(c)	3/32	5/32	3/16 (Tygon)
(d)	1/32	3/32	2 (Tygon)
(e)	0.023	0.038	2–4 (Intramedic PE-50)

[a] Registered trademark.

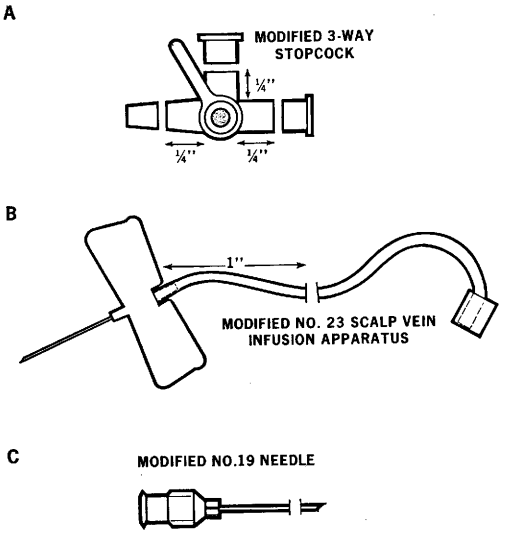

FIG. 2.

is cleared with saline. The stopcock handle is then repositioned at A where it remains until the next sample. With practice, samples can readily be obtained at intervals of 30 sec or less.

For sample processing, it is convenient to seal one end of the capillary tube with putty (Critoseal[1]) and to centrifuge the tube in a standard microhematocrit centrifuge. With proper use of the device, the hematocrit will remain constant from sample to sample, although a gradual downward trend may result from hemodilution if excessive volumes of saline are used to flush the arterial line. Significant variation in hematocrit from sample to sample indicates poor technique, with a variable degree of sample dilution with saline.

To obtain the plasma, the capillary tube is fractured at the meniscus and the plasma withdrawn from the upper fragment with a microliter pipette. The capacity of the standard microhematocrit tubes is approximately 75 μl, yielding plasma samples in excess of 30 μl. Longer capillary tubes, with correspondingly greater sample sizes, are also available.

Results. This apparatus has proved useful both in obtaining disappearance curves of

[1] Registered trademark.

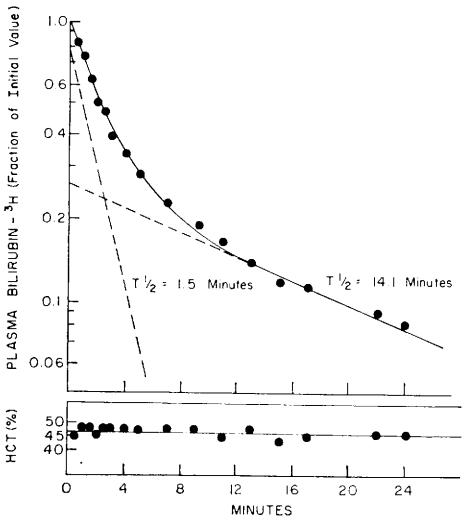

FIG. 3

various materials and in repeated sampling at infrequent intervals over a period of hours. Samples can comfortably be obtained every 30 sec, and 25 samples can be taken in rapid succession without introducing more than 1 ml of saline into the animal. A typical plasma disappearance curve for bilirubin-^{3}H in a normal Sprague–Dawley rat is illustrated in Fig. 3. In this study, samples were obtained from the right internal carotid artery after injection of radiobilirubin into the right external jugular vein. Note that this device permits accurate definition of the initial disappearance component, with a $T_{1/2}$ of 1½ min, and that sample hematocrits remained constant throughout the study.

Summary. A device has been described which facilitates rapid, timed sampling of arterial blood in the rat. This device is especially useful for definition of plasma disappearance curves of various injected compounds.

Received Jan. 25, 1973. P.S.E.B.M., 1973, Vol. 143.