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A simple technique for the frequent intravenous infusion of fluid without heparin into the rabbit

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A technique is described for the daily intravenous injection over 16 weeks in the rabbit. The system consists of a Teflon cannula passed down the external jugular vein with the tip in the superior vena cava. The cannula is joined at the point of entry to the jugular vein to a length of silicon rubber tubing, which is then passed subcutaneously to the forehead. The silicon tubing is terminated on a Luer needle hub, which is held in a simple Perspex plate secured subcutaneously between the rabbit's ears. The Luer hub is covered with a replaceable rubber cap through which injections may be made. With this system cannulae were maintained patent for 16 weeks by flushing once a day with Hanks' balanced salt solution, pH 7·4.

In our studies on the effect of various substances such as nicotine and phospholipids on atherogenesis, we required a method for the intravenous infusion of solutions into the rabbit, twice daily over about 16 weeks. Repeated direct venesection of the marginal ear vein is unsuitable because the sites of injection become fibrosed and the veins totally occluded by thrombosis, and with frequent daily injections an animal becomes excessively stressed. To overcome these difficulties we have investigated methods using intravascular catheters.

Various techniques for the implantation of venous cannulae have been described in rats (Weeks & Davis, 1964; Greig, 1971; Dow & McQueen, 1972; Hall & Goodyear, 1973), cats (Hall, Gomershall & Heneage, 1967), sheep (Hales, 1972), dogs (Baker & Shields, 1974). Reports of success in maintaining such systems patent for any length of time are, however, few. In general, patency is compromised by thrombosis. This problem can be minimized by the use of heparin as an anticoagulant, either dissolved in the infusion solution or chemically bonded to the catheter (Gott, Mel Ameli & others, 1967; Hall & others, 1967; Grode, Anderson & others, 1969; Dyck, 1972). The presence of heparin is often unacceptable, as in our case, because of interference in lipid metabolism by activation of lipoprotein lipase. We therefore examined the possibility of maintaining patency of intravenous cannulae over periods of months without the use of anticoagulants. The choice of cannula material and the position of the end of the catheter tip in minimizing mechanical damage to the vessel wall and subsequent thrombosis received particular attention. The best composition of the salt solution used for flushing the cannulae was also investigated.

We report the development of a successful method for frequent intravenous infusion of substances into the conscious animal, without stress, over 16 weeks, without the use of an anticoagulant.

MATERIALS AND METHODS

Materials

Of materials investigated for intravascular section of cannulae (silicon rubber, polyethylene, polypropylene and Teflon) only Teflon held promise and thereafter Teflon Medical Grade tubing, ultra thin wall No. 6423, 0·027 inch i.d. × 0·039 inch o.d. (Becton, Dickinson & Co., Rutherford, New Jersey) was used. The connection between the Teflon cannula and the terminating Luer hub was made with Silescol translucent silicone rubber tubing, T.C. 156 0·5 mm i.d. × 1·0 mm o.d. (Esco Rubber Ltd, 14/16 Great Portland Street, London, W1N 5AB U.K.). The exterior end of the Silescol tubing was terminated by a 21 gauge Luer needle (Gillette Industries Ltd, Isleworth, Middlesex, U.K.) with its shaft cut down to a length of 1 cm. The end of the hub was covered with a latex injection cap (Portex Ltd, Hythe, Kent, U.K.).

The base-plate for securing the Luer needle hub was constructed from 2 mm Perspex sheeting (Fig. 1). It was made in two parts. The upright section, which was approximately 1 cm square, contained a slot to allow the silescol tube to be inserted and a circular aperture to receive the Luer hub. The flat base-plate was 1 cm square. The two

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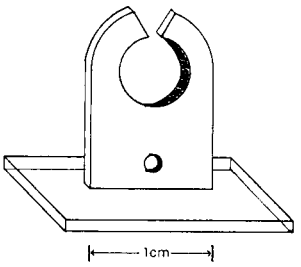

FIG. 1. Perspex base-plate, showing the upright section with slot and aperture for the cannula and Luer hub. The small hole is for the third securing suture.

sections were joined together with Zip Grip 10 glue (Devcon Ltd, Theale, Herts, U.K.) and the plastic needle hub was also fixed into the aperture with Zip Grip 10 glue.

The sterile solutions, which were used for flushing cannulae and infusions were 0·9% saline, pH about 7; Hanks' balanced salt solution (Oxoid Ltd, Southwark Bridge Road, London, S.E.1, U.K.), adjusted to pH 7·4 with 0·1M sodium hydroxide; Plasma-lyte 148 salt solution (Baxter Laboratories Ltd, Thetford, Norfolk, U.K.); 0·1M phosphate buffer, pH 7·4; 0·1M phosphate buffer containing bovine serum albumin (BSA–Cohn fraction V, Armour Pharmaceuticals Ltd, Brighton, U.K.); 10% EP suspension of lecithin (Nättermann, Köln, West Germany).

The animals used were New Zealand White rabbits. They were tranquilized for surgery with the neuroleptanalgesic, Hypnorm (Jannsen, from Crown Chemical Co. Ltd, Lamberhurst, Kent, U.K.).

Surgical procedure

Each rabbit was tranquillized by intramuscular injection of 0·4 ml kg^{-1} Hypnorm, 20 min before surgery, and the right side of the neck and the forehead shaved. A small transverse incision, about 3 cm wide was made in the centre of the forehead between the ears and the surrounding skin freed from the subcutaneous connective tissue. The animal was then turned on its left side and the neck was supported by a cylinder 2 cm in diameter. An incision was then made on the right side of the neck along a line joining the apex of the jaw and the humoral-scapular junction. A subcutaneous passage was then cleared between the two incisions by blunt dissection. A length of sterile Silescol tubing was then pulled through the subcutaneous passage.

The junction of external jugular vein and the facial vein was next located. Two silk ligatures were passed under the external jugular vein and the one further from the heart ligated. A small incision was made in the vein and a Teflon cannula, 5 cm long, connected to a 10 ml syringe and filled with infusion solution, was inserted. The cannula was pushed 2 cm into the vein and tied in place with both ligatures. In some experiments, the cannula was pushed 5 cm into the vein and the end of the cannula was revealed under X rays by placing a thin copper wire in its lumen. The wire was not allowed to protrude from the cannula in order to prevent damage to the vascular wall.

The cannula was then cut to leave about 1 cm protruding from the vein; the Silescol tube, also filled with infusion solution, was then connected to it and the junction secured with Zip Grip 10 glue and a silk ligature. The area was then dusted with penicillin-sulphathiazole powder and the incision in the neck closed with suitable skin clips.

The Luer needle hub was fitted with a short length of Teflon tube, then the Silescol tubing was cut to length and fixed over this using Zip Grip 10 glue and two ligatures. The Teflon covering on the needle ensured a good fit of the silescol tubing and prevented the metal from chafing the Silescol. The skin incision in the forehead was then sprinkled with penicillin-sulphathiazole powder and the base-plate inserted. The Luer hub was then positioned in the aperture and the incision closed with three sutures, one either side of the Perspex upright and the third over the Luer hub and through the hole in the upright. A latex seal was placed over the Luer hub which was then cemented to the base-plate with Zip

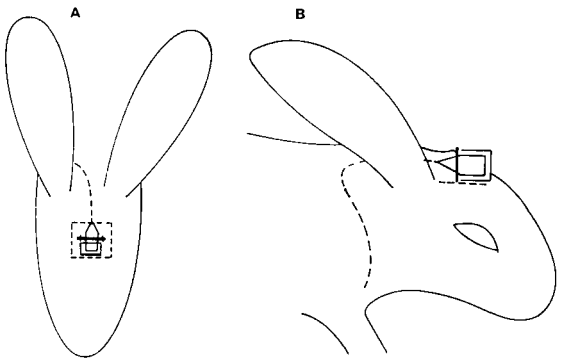

FIG. 2. A. Top view of a rabbit's head with the base-plate, Luer hub and injection cap in place. The dotted line marks the subcutaneous course of the Silescol tubing. B. Side view. The dotted line marks the subcutaneous course of the Silescol tubing up to the point where it joins the Teflon cannula implanted in the external jugular vein.

Grip 10 glue. The entire area was then sprayed with plastic skin (Nobecutane, BDH, Poole, Dorset, U.K.). The position of the capped Luer hub with its base-plate and the course of the subcutaneously implanted silescol tube are illustrated in Fig. 2.

Injections were made through the caps using a 21 gauge needle. A fresh sterile needle was used for each animal to prevent the possibility of infection. Latex caps were replaced once a week.

RESULTS

The method described was evolved after using 127 animals in a series of experiments designed to evaluate flushing solutions, the site of the cannula tip and the time the cannulae remained patent. Four separate experiments were made.

1. To determine the best solution for maintaining patency of catheters six pairs of rabbits were cannulated with the tips approximately level with the diaphragm. In one pair the cannulae had the tips sealed off to act as controls for direct damage. In each pair with open cannula, the cannulae were flushed twice daily with 0·5 ml of one of the solutions as shown in Table 1.

After 3 days and 10 days one rabbit from each pair was killed and the vessel adjacent to the cannula tip examined. The extent of thrombosis was assessed

Table 1. *Estimates of the size of the thrombus found in the inferior vena cava at the cannula tip at 3 and 10 days.*

Solution, all sterile 37 °C	After 3 days	After 10 days
Blank	+	+
Hanks'	+	2+
Plasma-lyte	2+	3+
Saline	2+	4+
Phosphate buffer	+	3+
Hanks' + BSA 2 g per 100 ml	2+	3+
Hanks' + BSA 5 g per 100 ml	2+	3+

\+ Small thrombus.
5+ Vessel occluded.

on an arbitrary scale from 1+ to 5+; 1+ indicated a small thrombus, which barely narrowed the vascular lumen, whilst 5+ indicated that the vessel was occluded.

With the sealed cannula, there was a small white thrombus adhering to the vein at 3 and 10 days. Only with Hanks' solution alone was the thrombus a similar size to that found in the control.

2. In the second experiment, 55 rabbits were cannulated with the tips level with the diaphragm. The catheters were flushed twice daily with 0·5 ml Hanks' solution at 37° delivered slowly from a 1 ml syringe to avoid damage to the vein.

38 animals died within 8 weeks, either because of cardiac or liver failure brought about by a massive thrombus occluding the inferior vena cava or right atrium. In all of these the cannula tip was level with the diaphragm. In the remaining 17 rabbits killed after 16 weeks, the cannulae were patent. In 13 the tip was in the inferior vena cava, level with the diaphragm and thrombi originating from the tip had developed. Congestion of the liver was present and 10–50 ml of blood-stained fluid was frequently found in the abdominal cavity. In 4 there were, however, none of these abnormalities and the cannula tip was in the superior vena cava opposite the right atrium or in the external jugular vein. The findings are summarized in Table 2.

Table 2. *Position of cannulae and thrombi and survival time for 55 rabbits.*

Number of animals	Survival weeks	Location of thrombus	Cannula patency	Liver
38	<8	Inferior vena cava	Patent	Damage
13	16	Inferior vena cava and heart	Patent	Damage
4	16	Superior vena cava and jugular vein	Patent	Normal

3. In the third experiment 40 animals had the cannula tip placed in the external jugular vein at the junction with the superior vena cava. Other conditions were as in the previous experiment.

Three animals died soon after cannulation, one from an infection which spread along the cannula from the head incision, one from a large thrombus in the right atrium and one from unknown causes. The remaining 37 were killed 16 weeks after cannulation when all cannulae were patent. In 4 animals, the cannula tip was adjacent to the right atrium and white thrombi were observed on the walls of the heart and in the inferior vena cava. The liver was congested, suggesting a restriction of the venous return. In the other 33 animals the cannula tip was in the external jugular vein, which although severely narrowed around the cannula, was not occluded. There were no thrombi in the inferior vena cava or right atrium and the liver was normal.

4. In the fourth experiment 20 rabbits were cannulated with the tip in the external jugular vein.

In 10 rabbits the cannulae were flushed once a day, 5 days a week with 3 ml of sterile Hanks' solution at 37°. In the other 10 animals 3 ml of a sterile solution of lecithin (10% EPL suspension) was similarly used.

Two animals died soon after cannulation; one with a thrombus in the right atrium, and the other with severe scouring. The remainder were killed 10–16 weeks after cannulation. All cannulae were patent, despite the fact that they were not flushed 2 days in each week and that in the second group a viscous solution of lecithin was infused. In 1 animal the cannula tip was in the inferior vena cava, there was a thrombus in the atrium and the liver was congested. In the remaining 17 the cannula tip was in the external jugular vein and there was no congestion of the liver.

DISCUSSION

We have shown that it is possible to maintain the patency of indwelling intravenous cannulae in rabbits for up to 16 weeks, with minimal thrombosis using a Teflon cannula. Trials of various materials in our laboratory suggested that Teflon gave less thrombosis than polyethylene or Silescol silicone rubber. Teflon has also been shown to be better than polyethylene for prolonged radial artery cathererization in man (Downs, Chapman & Hawkins, 1974) even when the polyethylene cannulae were loaded with heparin.

In addition to the material of the cannula we also considered the effects of the solution for flushing the cannula. Saline, Plasma-lyte and phosphate buffer all enhanced the formation of thrombus, which originated at the end of the cannula. This effect may have occurred because, although isotonic, these solutions did not contain the full complement of plasma ions or were not buffered to pH 7·4. Hanks' solution, on the other hand, contained the balanced ionic constituents of plasma at pH 7·4 and caused a minimal amount of thrombosis. Adjustment of the colloid osmotic pressure of the Hanks' solution by addition of BSA increased the thrombosis compared with Hanks' solution alone. The foreign protein must have been responsible for this.

The device for terminating the cannulas proved both simple to insert and reliable. It is cheaper than commercially made devices (Hall & others, 1967; Day & Owen, 1970; Day & Whiting, 1972) and the seal can be replaced easily. It is also highly convenient, because repeated injections can be made without stress to the animal, whilst it is still in its cage.

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