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INCLASSIFIE CURITY CLASSIFICATION OF THIS PAGE READ INSTRUCTIONS REPORT DOCUMENTATION PAGE BEFORE COMPLETING FORM 2. GOVT ACCESSION NO. J. RECIPIENT'S CATALOG NUMBER 4/79 TYPE OF REPORT & PERIOD COVERED Heat Research Division A Method for the Chronic Cannulation of the Technical Report. Superior Vena Cava and the Aortic Arch in the Rat Using Cannulas Made of Silicone Elastomer REPORT N Rather than Polyethlene (8. CONTRACT OR GRANT NUMBER(=) ANTHONY . Ph.D., and Candace B. Kelly, Roger W. Hubbard Murray P. Hamlet 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 6.27.77.A Environ.Stress Phys. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD 21701 Fit. & Med. Factors in Mil. Per. 3E162777A845 94183042 11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research and Development 17 May 179 Command, Ft. Detrick, Frederick, MD 21701 NUMBERO DAG UNCLASSIFIED 15. SECURITY CLASS. (of this report) 14. MONITORING AGENCY NAME & ADDRESS(IL different from Controlling Office) Same 154. DECLASSIFICATION/DOWNGRADING 16. DISTRIBUTION STATEMENT (of this Report) Distribution of this document is unlimited. 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) NA 18. SUPPLEMENTARY NOTES NA 19. KEY WORDS (Continue on reverse side if necessary and identify by block number) chronic cannulation; silicone elastomers; rat; blood sampling age . al identify by block number) A method is presented for chronic cannulation and subsequently obtaining central venous and aortic arch blood samples from an unanesthetized rat. Cannula were put into the right external jugular vein and the left common carotid artery and threaded caudally to the superior vena cava and the aortic arch respectively. Tubing made of medical grade silicone elastomers is used for the cannulas in preference to polyethlene tubing, because the former is more flexible and smoother walled making insertion less traumatic to the vessel walls and reducing problems with blood clots. To anchor the slippery silicone DD , 100 1473 EDITION OF I NOV 65 13 OBSOLETE UNCLASSIFIED

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TECHNICAL REPORT

NO. T 4/79

A METHOD FOR THE CHRONIC CANNULATION OF THE SUPERIOR VENA CAVA AND THE AORTIC ARCH IN THE RAT USING CANNULAS MADE OF SILICONE ELASTOMER RATHER THAN POLYETHYLENE

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by

CANDACE B. KELLY, ROGER W. HUBBARD, Ph.D. and MURRAY P. HAMLET, D.M.V.

Project Reference: 3E152777A845 May 1979

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- 4. The cannula. Contrast and make some as second as showing the

Abstract

A method is presented for chronic cannulation and subsequently obtaining central venous and aortic arch blood samples from an unanesthetized rat. Cannulas made of medical grade silicone elastomers were put into the right external jugular vein and the left common carotid artery and threaded caudally to the superior vena cava and the aortic arch respectively. Tubing made of medical grade silicone elastomers is used for the cannulas in preference to polyethylene tubing, because the former is more flexible and smoother walled making insertion less traumatic to the vessel walls and reducing problems with blood clots. To anchor the slippery silicone cannulas in the vessels a suture is placed through a small amount of silicone elastomer on the cannula and tied around the vessel. The cannulated rat is useful for simultaneous arterial and venous blood sampling for at least a month.

Chronic cannulation; silicone elastomers; rat; blood sampling

A Method for the Chronic Cannulation of the Superior Vena Cava and the Aortic Arch in the Rat Using Cannulas Made of Silicone Elastomer Rather than Polyethylene

This method was developed to permit arterial and venous sampling in an undisturbed unanesthetized rat. A chronic method is desirable, because the animal can recover from the surgery before the experiment. The arterial cannula inserted by way of the common carotid artery and the venous cannula inserted via the external jugular vein have many uses such as: a) the injection of drugs and the withdrawal of samples at precisely timed intervals, or b) the simultaneous sampling of arterial and venous blood for blood gas and other determinations, or c) the measurement of arterial blood pressure and central venous pressure. With practice and the proper equipment, this procedure can be done by one person.

Cannula Construction

Silastic (a registered trademark of Dow Corning Corporation, Medland, Michigan) was chosen for the cannula material over the previously used polyethylene (p.e.) tubing. Smoother more flexible Silastic eliminates accidently puncturing the vessel wall with the cannula. Also, blood clots, which do not form as readily in smoother Silastic tubing, adhere more tenaciously to p.e. This makes it easier to dissolve and withdraw a clot from Silustic than from p.e. tubing. The smoothness of Silastic makes cannula slippage a problem. This problem is solved by running a ligature through a small amount of silicone adhesive on the cannula and tying the ligature around the vessel and cannula.

A small diameter (0.020 in. ID, 0.037 in. OD) tubing was chosen to minimize interference with blood flow in the aortic arch and the vena cava, and a 20 cm length proved to be convenient. The ends of the cannulas should be only slightly beveled to prevent the cannula tip from folding back on itself. To determine the proper insertion distance (see Fig. 1), a cannula must be inserted and the animal sacrificed to determine its position in the aorta or vena cava. For 500 g male Sprague-Dawley rats (Charles Rived CD strain) 3.5 and 4.0 cm lengths (arterial and venous respectively) work well. The insertion distance is marked on the tubing, which has been cleaned of grease with acetone, and a small amount of Medical Adhesive Silicone Type A (Dow Corning) is placed on the mark and allowed to cure for 24 hrs. A 6-0 silk suture is then run through the cured adhesive (bump) but not the cannula (see Fig. 4). The prepared tubing is soaked in 50% ethanol for 5 min. and rinsed in saline prior to insertion.

Cannulation

A blunted 22 G needle and syringe filled with heparinized saline (10 U per ml) is attached to the distal end of the cannula. The rat is anesthetized (sodium pentobarbital, 50 mg/kg, i.p.), shaved ventrally from the angle of the jaw caudally to halfway down the chest and dorsally between the shoulder blades, and the shaved areas cleaned with Betadine. A single midline incision is made from the angle of the jaw to the top of the sternum. From this incision both the left common carotid and the right external jugular can be cannulated.

We have selected the left carotid because it is dissectable for more of its length than the right carotid. Bluntly dissect between the sternomastoideus and sternohyoideus and under the levator scapulae (see Fig. 2) to find the left common carotid. Carefully separate the carotid from the vagus nerve as far

cephalad and caudad as possible. Place a small buildog clamp on the artery at the caudal extreme (see Fig. 3A). Place 2 ligatures (3-0 silk) under the artery; tie off the artery with one as far cephalad as possible leaving the ends long to be retracted with a hemostat. Tie a surgeon's knot in the other ligature, but leave it loose enough to pass the cannula through it. Make a small hole in the artery with small dissecting scissors between the ligatures. Hold the incision open with a horizontally held blade of the scissors to insert the cannula to the clamp. Holding the cannula in the vessel with fine tipped forcepts, release the clamp. Feed the cannula down to the adhesive bump being careful to keep a hold of the cannula in the vessel or the cannula near the insertion point at all times. Finish tying the surgeon's knot tightly enough to hold the cannula but to not occlude the vessel. Remove the hemostat from the cephalad ligature and tie the ends around the cannula above the Silicone bump (see Fig. 3B). Take the ends of the 6-0 suture in the bump and tie them around the vessel and cannula. This third ligature will insure that the cannula will not slip. Test the cannula for patency by injecting a small amount of saline, and then withdrawing blood. Flush the blood back in leaving saline (plus optional heparin) in the cannula.

The right jugular vein was chosen because it has a more direct route into the vena cava than the left jugular. The external jugular can be found superficial to the muscles (see Fig. 2) and should be bluntly dissected for a distance of about 1 cm. from the clavical cephalad. The cannulation proceeds as for the carotid except that a bulldog clamp is not necessary.

Once both cannulas are tied in place they must be exteriorized from between the shoulder blades. The skin is bluntly dissected away from the underlying tissue on each side of the neck to allow for the passage of a 16 G

needle inserted from the back to act as a trocar for the passage of the cannula. The midventral incision is then closed. Plugs made from small lengths of paper clip wire bent into a right angle with sharp edges filed are put into the open ends of the cannulas.

The animal should be allowed to recover for 3-4 days before use. The cannulas should be flushed to withdraw clots with heparinized saline to maintain patency, but this need be done only about once a week. The properly cared for cannula should function indefinitely, but we routinely use them for less than a month.

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Fig. 3 Arterial Cannulation (A) Cannula Inserted To The Clamp (B) Cannula Tied In Place.



The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official department of the Army position, policy, or decision, unless so designated by other official documentation.

In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences -National Research Council.

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