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**A REUSABLE ADAPTER FOR COLLECTION OF CEREBROSPINAL
FLUID IN CHRONICALLY CANNULATED GOATS**

**U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts**

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A lightweight, adjustable device has been designed to standardize and advance the technology for chronic cannula implantation in <u>Capra hircus</u> (goats). The adapter facilitates cannula implantation, simplifies surgical procedures, and allows for repeated sampling of -CSF, thereby enhancing the probability of successful cannulation with minimal risk to the animal. This report describes the design, construction and implantation of this device.					
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A REUSABLE ADAPTER FOR COLLECTION OF CEREBROSPINAL FLUID
IN CHRONICALLY CANNULATED GOATS

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This certifies that the cumulative studies described in this report were reviewed and recommended for approval by the USARIEM Laboratory Animal Care and Use Committee and were approved by the Commander, USARIEM before implementation.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of the Institute of Laboratory Animal Resources, National Research Council".

Surgical procedures were performed in accordance with standard veterinary practices and performed or directly supervised by trained and experienced personnel.

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ABSTRACT:

A lightweight, adjustable device has been designed to standardize and advance the technology for chronic cannula implantation in Capra hircus (goats). The adapter facilitates cannula implantation, simplifies surgical procedures, and allows for repeated sampling of CSF, thereby enhancing the probability of successful cannulation with minimal risk to the animal. This report describes the design, construction and implantation of this device.

INTRODUCTION:

A lightweight, adjustable adapter has been designed for chronic cannula implantation in goats (Capra hircus). It provides an accurate, relatively safe means of sampling cerebrospinal fluid (CSF) through a cisternal cannula and has been used for continuous perfusion of synthetic CSF into the fourth ventricle in unanesthetized goats. This technology has also been used for examining the changes in ionic composition of CSF as well as cerebral interstitial fluid (ISF) in the study of adaptations to high altitude (2-5).

Previously, cannulae have been permanently implanted in the lateral ventricles and cisterna magna of goats following the technique described by Pappenheimer et al. (1). While the preparations, surgical procedures, and research applications have been described by Pappenheimer et al., the purpose of this report is to outline significant differences in the design and construction of cannula body and the sampling device and to describe its use in goats. Earlier devices required a number of fixed needles which were inserted through the cisternal and ventricular cannulas one at a time, each sequentially longer until a successful puncture was accomplished. This device allows removal of a blunt needle, and adjustment of the puncture depth without changing the needle.

The new adapter facilitates cannula implantation, and with improvements to the cannula design enhances the probability of successful surgical cannulation with minimal risk to the animal.

MATERIALS AND METHODS:

Pre-surgical Procedures: A total of twenty-four healthy adult female and male goats, weighing 30 to 50 kilograms, were cannulated and used in six studies over a ten year period (2-5). Animals were fasted a few days prior to surgery to allow evacuation of ruminant stomach and reduce the incidence of aspiration. Pre-anesthetics Rompun R (xylazine, 3mg total dose, i.m.) and Pentothal (0.2 to 0.4mg/kg) were administered intravenously in a saphenous vein. A cuffed endotracheal tube was passed to prevent accumulation of salivary fluid in the lungs during the induction of anesthesia. A general gas anesthetic mixture of halothane and oxygen was then given during the remainder of surgery. A stanchion previously described (7) was used for positioning the entire animal during surgical procedures and to align the goat's head to accept a stereotaxic frame. The goat's head was shaved, prepared for surgery, and draped. Sterile latex rubber tubing was used to cover the horns. A midline incision was made in the skin 5-8cm behind and extending to the nuchal ridge for the cisternal cannula. The underlying muscles were incised down to the skull for a distance of 5mm on each side of the midline (exposing occipital bone) and the neck muscles were retracted laterally. With the soft tissues retracted and each surgical site cleaned, the sites were then ready for implantation of the cannulas. Additionally, a 4cm area of each bone surface was cleaned.

One cannula was positioned dorsal to the ependymal lining of the lateral ventricle and the other was placed dorsal to the dura mater of the cisterna magna (Figure 1).

Implantation of the Cisternal Cannula: To prepare for the implantation of the cisternal cannula, a 7mm hole was trephined ventrally through the base of the occipital bone to the dorsal surface of the dura mater in the foramen magnum. A channel 1cm in width and 2mm in depth was rongeured from the base of the hole dorsally to the nuchal crest. Two orthopedic (stainless steel) screws were placed in the occipital bone 2-3mm on each side of the channel with a third placed in the parietal bone on the rostral end of the nuchal crest. Gel foam (UpJohn Co., Kalamazoo, Mich.) was used to protect the dura prior to application of dental acrylic ("Perm", Hygenic Dental Mfg. Co., Akron, Ohio). The gel foam was not removed, but left in place to be reabsorbed by the tissues. The dental acrylic was poured around the cannula and over the screws used for anchoring the resin. The stereotaxic equipment was used to center the cannulas into the trephined holes as well as for support while the dental acrylic cured, as described by Pappenheimer, et al. (1).

The cisterna magna cannula was machined from a solid piece of 9.52mm diameter nylon dowel (Micro Group, Inc., Miami, Fl), and was placed into the channel allowing the 45° angle cut of the cannula foot to lie directly on the dura mater (Figure 2). After the acrylic had set (15 minutes), the subcutaneous tissue and skin were brought around the cannula and sutured to complete the surgical procedure. The cannula was then capped with a locking obturator. The original cannula design was modified with the addition of circumferential grooves and a flat surface on one side to prohibit rotation and longitudinal movements.

The obturator was made from a stainless steel rod (1.07mm) that was silver soldered into a closed-end male luer-lok adapter. The opposite end of the obturator was rounded and fit flush with the angular foot of the cannula when fully inserted. The primary use of the obturator was to protect the cannula

from airborne contaminants and to allow for removal of percutaneous matter that could plug the base of the cannula when the cistern puncture needle was extracted.

Implantation of the Ventricular Cannula: The animal's head was placed so the parietal surface was horizontal. The incision for the ventricular cannula was 1-2cm behind the coronal suture and extended 2cm laterally on each side of the midline. The lateral ventricular cannula (Figure 3) was introduced through a 9mm hole trephined in the parietal bone. The stereotaxic apparatus as described in the previous procedure was used for installing the ventricular cannula. The long narrow tip was specially designed to penetrate the brain parenchyma covering the dura with minimal tissue damage. During research procedures, artificial CSF was perfused through the ventricular cannula while native CSF was collected from the cisternal cannula. The cannula was then capped with a locking obturator.

Post Surgical Procedures: As a precautionary practice, all goats received antibiotic (Penicillin, 20,000 units/kg) after surgical procedures as well as four to six weeks of post operative rest before usage. CSF from each animal was cultured shortly after surgery and was found to be negative for bacteria. Post operative measures used to evaluate the goat's health included: daily basal temperature, hematology, food and water consumption, general responsiveness and activity of the animal. Each animal was fitted with a universal aluminum helmet with 16mm nylon straps, similar in design to Pappenheimer et al. (1), to prevent damage to the cannulas while goats were in their cages. The inside of each helmet was lined with a 37mm stack of 10.2cm gauze squares with a small hole at

the center, and this gauze was placed over each cannula.

Use of Spinal Needle Adapter: Prior to puncture of the cisterna, the animal was placed in a stanchion designed to restrict head movement (7). The aluminum helmet with gauze padding was removed. The horns and surrounding areas were swabbed with a Betadine solution, wiped, and then sprayed with 70% isopropyl alcohol. The goat's head was placed on the lap of an animal holder (slightly above the horizontal position with respect to the body of the goat). Using sterile gloves, the obturator was removed, and sterile water was flushed into the deadspace with a syringe and an 18-gauge (or smaller) teflon catheter approximately 50-65mm long to expel any debris from previous cannulations. A blunt stainless steel measuring rod (similar to the obturator) was inserted through the cannula until it touched the dura. The depth was marked by clamping the rod with a sterile hemostat at the top of the luer-lok hub. The rod was removed and the puncture depth was measured using a millimeter scale. The assembled spinal needle adapter (Figure 4) was then fitted to the top of a 19 gauge spinal needle with the split-collar coarse adjustment unit facing caudally. With the fine adjustment luer extension threaded fully inward, the adapter was positioned 6-8mm above the depth indicator line and secured to the needle using the coarse adjustment screw. The luer-slip fine adjustment was then unthreaded down the needle shaft within 1mm of the indicator line to compensate for the dura thickness. The needle was then inserted into the cannula, and the dura was punctured using a quick gentle motion. If the puncture was unsuccessful, the needle depth was increased 0.5mm and the procedure was repeated. To evaluate a successful puncture, the goat's head was lowered, and pressure was applied to each side of the neck to occlude the external jugular veins and increase CSF pressure. This increased pressure

caused oozing of CSF from the punctured dura. The needle adapter allowed use of a single needle and could be adjusted in length by rotating the threaded hub, as opposed to the former practice of using multiple needles of different lengths. The adapter also assured an accurate puncture depth preventing accidental damage to surrounding tissues. The needle could be discarded at any time without discarding the entire apparatus. The adjustable length of the spinal needle adapters was 38mm when the fine adjust luer-slip hub was fully closed and 44mm when completely extended. These spinal needle adapters were used with both cannulae for tapping each respective fluid compartment. The same techniques and procedures were employed for puncturing the dura through either the cisternal or ventricular cannula. At the end of each experiment the adapter was removed and the cannula was flushed with sterile saline using a 40mm teflon catheter. A sterile obturator is then placed into the cannula.

RESULTS AND DISCUSSION:

Six studies, utilizing 24 goats, were performed using this cannulation procedure (2-5). These studies successfully utilized the needle adapter with chronic cannulation. The average time span for a functioning cannula was approximately two years. The volumes of CSF withdrawn were 5 to 8ml during an 8-hour experimental day. During experiments the animals were not retested for at least 3 days. Patency was usually not a problem with above surface cannulae if frequently used, but repeated use of the cannulated goats consistently prevented premature occlusion from occurring in the first 6 months after cannulation.

Loss of the cannula function was eventually due to bone growth which forced

the cannula out of position after a few years. However, the animals did not appear to have any pain or discomfort from this cannula displacement. Finally, tissue replaced the exposed bone area of these animals with no ill effects. During repeated studies all goats remained healthy, developing no systemic disease or infection around implantation areas. In addition, blood cell counts remained at normal levels in all cannulated goats. Moreover, hypertensive hydrocephalus did not occur after any of the cannula implantations. No change in appetite was noticed after loss of the cannula. The goats were not placed on any chronic antibiotic programs during these periods. Proper care post operatively, separate housing, and mechanical protection (helmets with halter straps) provided additional safeguards for the cannulae.

SUMMARY:

The goat's docile nature and resilience make them an excellent experimental model for experiments requiring chronic sampling of CSF through cannulae. Their horns simplify construction of a helmet for cannulae protection, and they became accustomed to wearing the protective helmet. The spinal needle adapter has been successfully used in goats to perfuse and collect CSF for a ten-year period without incident, and the methodologies described in the current report are an apparent improvement over earlier techniques with less discomfort and risk to the experimental animal.

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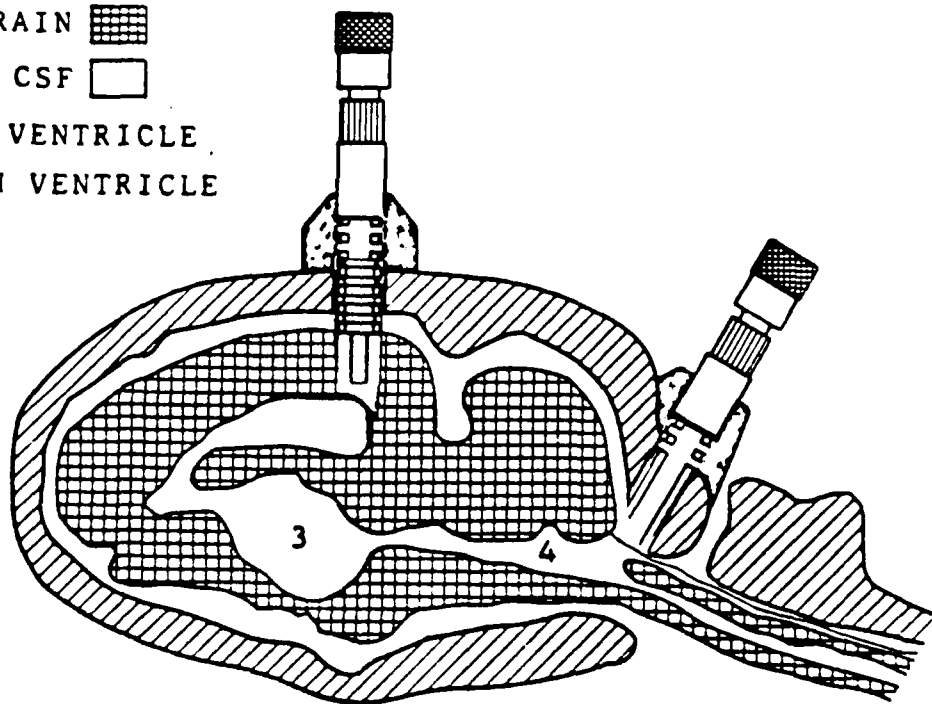
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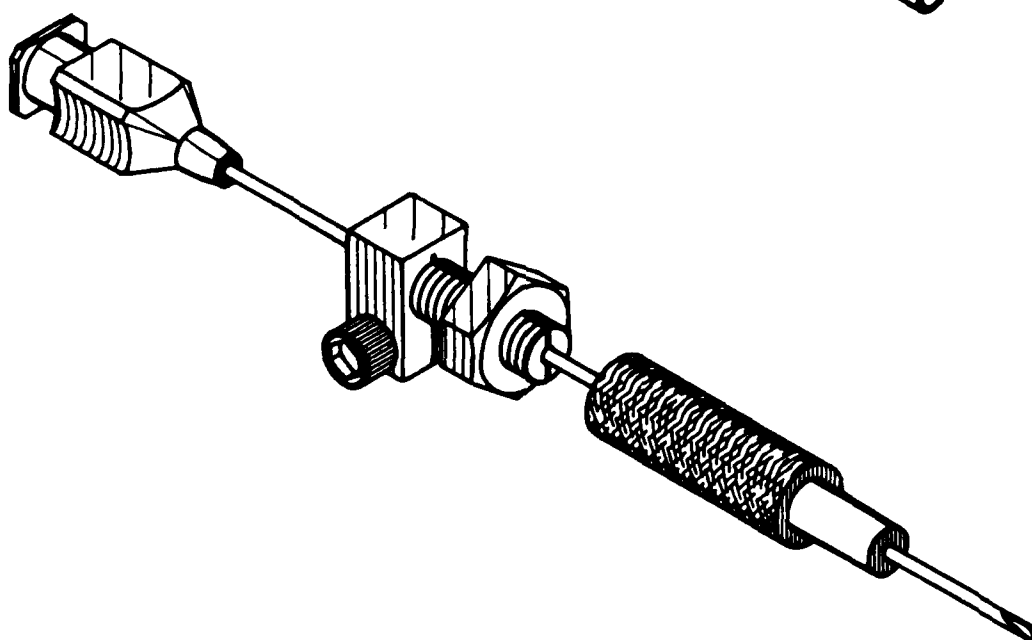
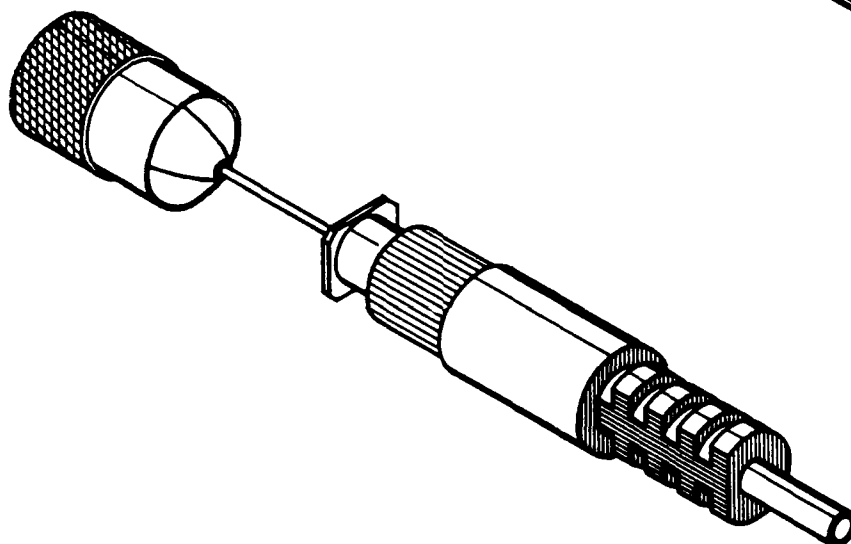
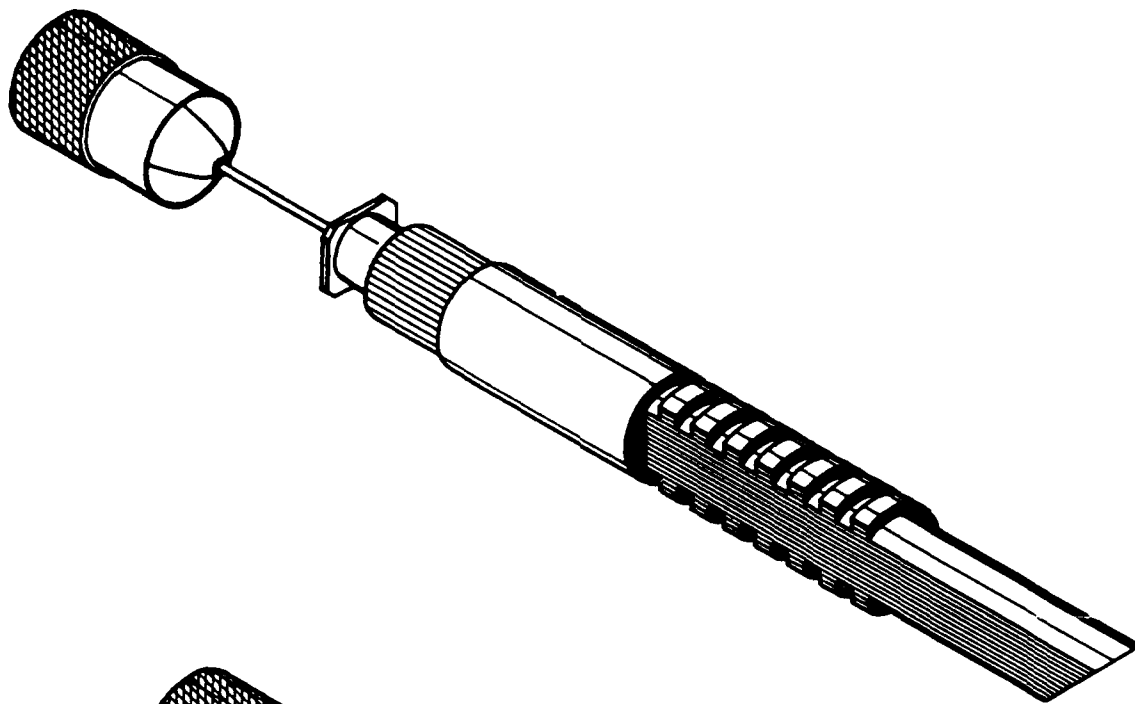
BRAIN 

CSF 

3 - THIRD VENTRICLE

4 - FOURTH VENTRICLE





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