are driven into the skull vault. At the centre of the plate rostrally there are 3 holes. The midline hole serves as a landmark. The other holes, whose centres are 3 mm apart, are the cannulation holes.

The top plate is the same size as the head plate onto which it can be screwed at the midline caudally. The top plate has two wings to protect the cannula assembly which consists of a cannula guide tube (22 SWG stainless steel tubing 15 mm long) mounted in a threaded steel collar which screws into one of two holes which correspond to the cannulation holes of the head plate. The cannula itself is 28 mm long and made of 24 SWG stainless steel hypodermic tubing. It has an adjustable collar so that its depth of projection can be regulated.

Fixation of the head plate. New Zealand white rabbits have been used. The animal is anaesthetised and 2 ml 1% lignocaine solution injected s.c. in the midline half way between the eyes and the ears. A saggital incision about 5 cm long is made down to the bone of the skull. The periosteum is stripped back laterally to expose the skull vault. Using the suture landmarks shown in Figure 1c the head plate is located with the two access and the guide holes straddling the bregma. The plate is then fixed in position using a suitable drill and the 4 screws. The skull under the cannulation holes is also drilled through. The plate sits flat on the skull vault due to its saddle shape. In very large animals it may be necessary to file the nuchal crest somewhat to seat the plate. The skin is now sewn up behind and in front of the plate and the surplus skin is generously excised. It is not necessary to cover the deficit. The animal is left for 5 days before injections are made.

Intracerebroventricular injection. After reperforating the appropriate access hole the top plate is screwed onto the head plate. When an injection is to be made the cannula is attached to a microlitre syringe using polythene tubing and flushed with the solution to be injected. The cannula is then inserted down the cannula guide tube into the ventricle. This insertion is made without any sign of distress to the animal. The usual injection volume is 100 µl.

The ventricle is located at Ba–9 mm (MONNIER and GANGLOFF's[4] coordinates). Thus the cannula and cannula guide tube must be adjusted so that the cannula will project 12 mm below the lower edge of the top plate. These relative positions are shown in Figure 1g.

Results. The device has been used routinely for 3 years and on repeated occasions dissection has confirmed that the ventricle has been accurately injected. Figure 2 shows an Xray photograph of a rabbit after 100 µl of radioopaque dye (Myodil, Glaxo) has been injected into the ventricle using this method. Cardiovascular[5] and temperature regulation[6] experiments have been carried out with the device and it has been found simple and robust in use.

The system has several advantages compared with implanting a cannula directly into the ventricle. Since the brain is untouched before and between injections the infection rate is low. Unlike permanent cannulae the present device has no dead space. Finally, whereas cannulae fixed to the skull with dental cement can often only be used once, the present device can be recovered and reused indefinitely.

Zusammenfassung. Die Konstruktion und der Gebrauch einer Vorrichtung zur Injektion von Substanzen in den lateralen, cerebralen Ventrikel von wachen Kaninchen wird beschrieben.

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[5] P. J. LEWIS, J. L. REID, M. G. MYERS and C. T. DOLLERY, J. Pharmac. exp. Ther. *188*, 394 (1974).

[6] P. J. LEWIS, M. D. RAWLINS and J. L. REID, Br. J. Pharmac. *51*, 207 (1974).

[7] Acknowledgment. I am grateful to Prof. W. I. CRANSTON and Prof. M. D. RAWLINS for introducing me to the original technique and to Mr. L. CHENERY, instrument maker.

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Simple Apparatus for Perfusion Fixation for Electron Microscopy

Accuracy of ultrastructural study of cells and tissues depends largely upon fixation, and, since the start of electron microscopy in biological studies, numbers of fixation techniques have been developed. However, it is generally agreed that perfusion fixation is the method of choice for a majority of tissues[1–3].

Perfusion fixation may be done by any one of different methods, but for the past few years this laboratory has been using an inexpensive, simply constructed perfusion system that is believed to offer several advantages. Most often we have used it for rats of various age groups, immature and mature, but it serves equally well for newborn swine, young dogs, rabits, mice, and Chinese hamsters.

We have developed 2 forms of the system, the first and simpler for morphological studies, the second and more complex for histochemical studies. Both forms consist of pressurized vessels for the solutions, devices for pressurization and for measuring pressure, and vinyl tubing, needles or catheters to deliver solutions into the vascular bed.

The simpler system (Figures 1 and 2) consists of 2 glass containers (coffee jars), about 1 l capacity, with screw-on lids made airtight by suitable O-rings. Lids are pierced by two 3–4 mm holes, through which lengths of PVC-tubing are inserted and cemented into place with araldite. One tube (Figure 1) provides connection between the air spaces in the 2 jars through vinyl tubing in which is inserted a 4-way joint for connection with a manometer (Figure 1, b) and with a rubber-bulb syringe (Figure 1, a), fitted with a 1-way valve for pressurization. The second PVC-tube through each jar lid connects lengths of vinyl tubing that extend to the bottoms of the jars with lengths of this tubing (about 150 cm) joined at their outer ends by a 3-way plastic valve (Figure 1, c). The third arm of his valve carries the needle or catheter for entering abdominal aorta.

In using this system, one jar is filled with Ringer, the second with the fixative, e.g. glutaraldehyde solution[1]. Lids are screwed on tightly, the system pressurized, and freed of air bubbles by manipulating the 3-way valve. Then the valve is turned to an intermediate position to prevent further loss of solutions and pressure raised to

[1] W. FORSSMANN, G. SIEGRIST, L. ORCI, L. GIRARDIER, R. PICTET and C. ROUILLER, J. Microsc. *6*, 279 (1967).

[2] G. C. NOUET and M. KUJAS, Z. Zellforsch. *143*, 535 (1973).

[3] J. A. G. RHODIN, Microvasc. Res. *5*, 285 (1973).

physiological levels for the animal (rats, 120–130 mm Hg). If cold perfusion is desired, jars should be immersed in an ice-water bath.

Under i.p. Nembutal anesthesia, the abdomen is opened, viscera displaced until aorta and vena cava are visible from renal arteries distally. Then the aortic bifurcation is freed with rounded tipped forceps, the aorta clamped with rounded edged surgical forceps just distal to the renal arteries, the aorta opened with fine scissors at the bifurcation and entered with a catheter or needle to the level of the surgical clamp which is removed after catheter or needle has been locked in place with a grooved clamp (Figure 4, a and b). Then Ringer is admitted into the aorta through the 3-way valve at a pressure slightly above physiological level to ensure flow against aortic pressure. Immediately with the start of the Ringer's flow, the vena cava is cut and within about 2 min blood has been removed by Ringer and the valve turned to admit glutaraldehyde solution, which gives adequate fixation within 10–15 min. Pressure within the system is maintained until fixation ends. Thus all organs, except lungs and those caudal to the catheter or needle, are perfused.

The more complex system (Figure 3) differs from the first in 2 features: 1. a third jar connecting to the others in the system and 2. a third 3-way valve for attaching a syringe (Figure 3, d) for a blood sample or for injecting solutions, e.g. horseradish-peroxydase for vascular permeability studies[4] before fixation begins. As before, the system is pressurized (Figure 3, a) with a rubber-ball syringe, which connects to a manometer (Figure 3, b) and to the jars, the vascular system rinsed with Ringer through a 3-way valve (Figure 3, *c* 2) followed by perfusion fixation for the desired interval (e.g. 5 min) then by adequately positioning valves C 1 and C 2 washed by buffer solution. At this point partially fixed tissue may be removed for cryostate sectioning.

[4] I. Hüttner, M. Boutet and R. H. More, Lab. Invest. *28*, 678 (1973).

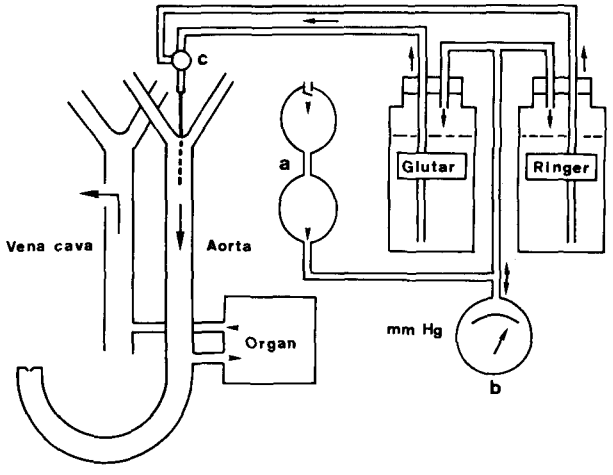

Fig. 1. Diagram of the simpler perfusion system with 2 bottles. a) rubber-ball syringe; b) manometer; c) 3-way valve.

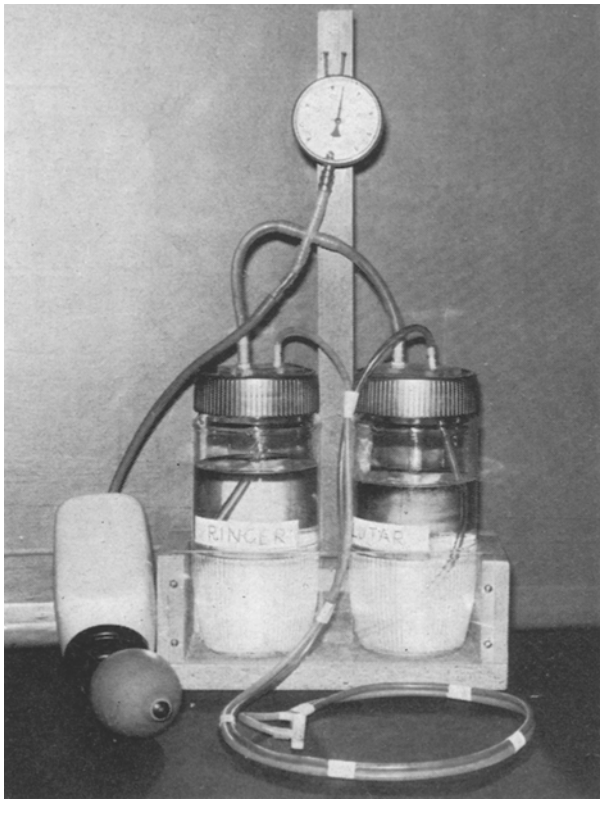

Fig. 2. Photograph of the perfusion system diagrammed in Figure 1.

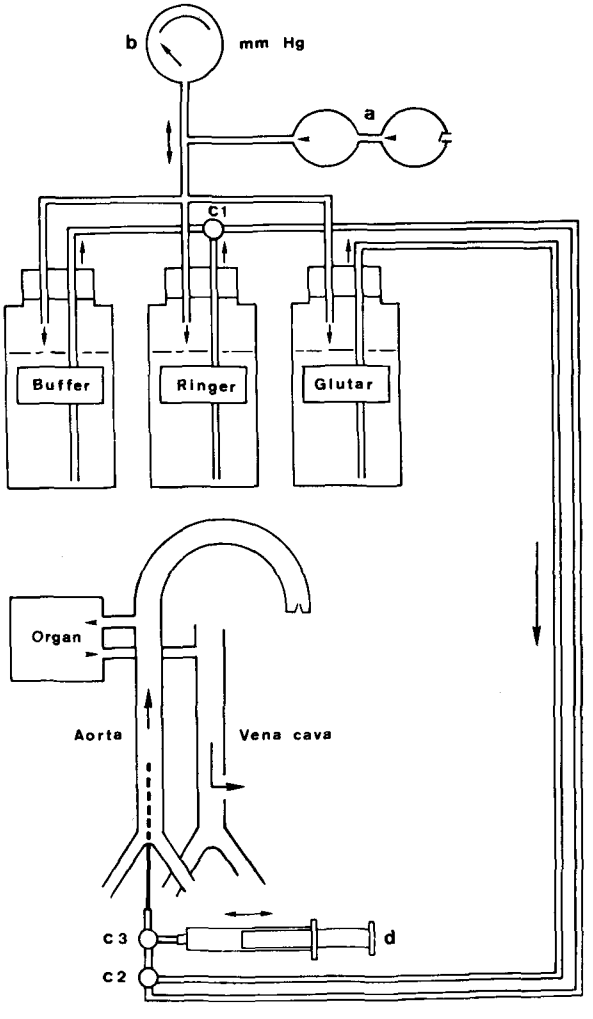

Fig. 3. Diagram of the more complicated perfusion system. a) rubber-ball syringe; b) manometer; c) 1, 2, 3 = 3-way valves; d) syringe.

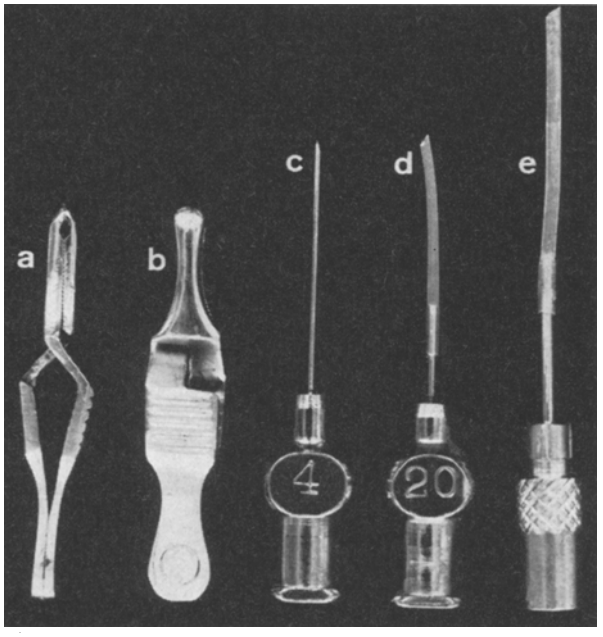

Fig. 4. Clamp with groove for securing the needle or catheter in position in the aorta. a) lateral view; b) frontal view, and needles or catheters for the perfusion of Chinese hamsters (c), mice (d), and rats (e).

This more complex system provides accurately controlled fixation where this is required for histochemical reactions, e.g. nucleoside phosphorylase determinations in endothelium and fibroblasts[5]. The short fixation period does not drastically inhibit enzymatic activity and permits cryostate sectioning without artefacts which could impair subsequent electron microscopic study of the tissue.

Catheters or needles used for entering the abdominal aorta differ in outside diameters depending on the size of the animal. For larger animals, such as adult rats, a short length of polyethylene arterial catheter may be mounted on a cut hypodermic needle (Figure 4, e). With smaller animals, Chinese hamsters for example, a hypodermic needle (Figure 4, c) outer diameter 0.3 mm, with tip shortened and slanted to 45°, in which case the aorta can be entered directly without need for clamp. A grooved clamp to hold catheters or needles in aortas is shown in lateral (Figure 4, a) and frontal views (Figure 4, b).

These systems avoid the use of peristaltic pumps or of gravity to ensure perfusion. Pressures provided by peristaltic pumps are not easily reproducible, and the pumps costly. Pressure by gravity, for example for rats with hypertension of 250 mm Hg or higher would be difficult to achieve in the laboratory[6].

Summary. Control on duration of application and pressure of fixative is achieved with an apparatus of simple construction. This allows optimal fixation of laboratory animals for morphological and cytochemical studies.

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17 February 1975.

[5] M. Borgers, I. Schaper and W. Schaper, J. Histochem. Cytochem. *19*, 526 (1971).

[6] Thanks are due to Dr. J. W. Buchanan, University of Pennsylvania, for advice during the development of the system.

CONGRESSUS

Canada

International Symposium on Flammability and Fire Retardants

in Toronto, 6–7 May 1976

Papers should deal with flammability and fire retardancy of polyurethanes, plastics, textiles and fabrics, paints and coatings, testing procedures and marketing. Papers are now being solicited and tentative titles should be sent by October 15, 1975 to: Vijay Mohan Bhatnagar, Editor, Advances in Fire Retardands, 209 Dover Road, Cornwall, Ontario, Canada K6J 1T7.

Italy

International Symposium on Thrombosis and Urokinase

in Roma, 30 October–1 November 1975

The Symposium is organized by the Istituto Superiore di Sanità and the chairmen are: Prof. Sol Sherry of Philadelphia, USA, and Prof. R. Paoletti of Milano, Italy. Main topics: Physiopathology of thrombosis. Chemical, biochemical and pharmacological aspects of urokinase. Effects of urokinase on thrombosis. Clinical applications of urokinase.

Registration fee will be US Dollars 30.00. Information and registration by Prof. Rodolfo Paoletti, Via A. Del Sarto 21, I–20129 Milano, Italy.

Corrigendum

G. Tortolani and E. Ramagnoli: Estimation of Molecular Weight of Acid Mucopolysaccharides by Thin-Layer Electrophoresis on Sephadex-Cellulose, Experientia 31, 389 (1975). The explanation of the symbols in the caption of Figure 3 should correctly read as follows: ●, CSC; ○, CSB; ◐, FB; ◑, CSA; ◓, 3GS; ◒, HP.