tautomerization has occured, a π-excessive ring (ALBERT's nomenclature) tends to *donate* a π charge when fused to a π-deficient ring to form a new heterocyclic skeleton. The reverse is true if prototropy has taken place.

2. No simple prediction can be made regarding the charge distribution of two π-deficient or two π-excessive rings fused together without referring to detailed MO calculations.

Summary. Detailed MO computations reveal that the title definitions do not always represent the actual π-electronic charge distribution: tautomers of purine and pteridine are discussed as an example.

Résumé. Les calcules avec la méthode OM montrent que la terminologie π-exessive ou π-deficient ne représente toujours pas la situation réelle; la purine et la ptéridine sont prises comme exemples.

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PRO EXPERIMENTIS

A Device for Intracerebroventricular Injections in the Conscious Rabbit

Injection of drugs into the lateral cerebral ventricle of conscious animals is a technique used widely in the investigation of the central actions of drugs. Methods for the implantation of chronic cannulae in the cerebral ventricles have been described for the cat[1] and the rat[2]. In the rabbit such injections are usually made with the device described by COOPER, CRANSTON and HONOUR[3], itself a modification of that of MONNIER and GANGLOFF[4]. This apparatus consists of a steel head plate which is permanently fixed to the skull and a second cannula guide plate which is fitted to the head plate when required. However, this method cannot be used in conscious animals unless they are confined in headstocks since the cannula projects from the device and is readily broken off if the animal is allowed freedom of movement.

The device here described is a modification of this system which is suitable for use in the unrestrained rabbit. It can therefore be used for cardiovascular work where the stress of confinement in headstocks can considerably distort responses to drugs. Firstly the cannula itself has been shortened and protected by stout flanges on the cannula holder; secondly the head plate has been simplified to be used exclusively for ventricular injection and thirdly the plates are made not of steel but of transparent plastic. This not only lightens the device but has the important advantage that the suture landmarks on the animal's skull can be seen through the head plate as it is fixed in position.

Materials and methods. Figure 1 shows a scale drawing of the device. It has 3 components, a head plate which is affixed to the skull (Figure 1a + b), a top plate which carries the cannula guide tube (Figure 1d + e) and the cannula itself (1f). Both plates are machined out of perspex. The bottom plate has holes at each corner through which short stainless steel self tapping crews (10 BA 7 mm)

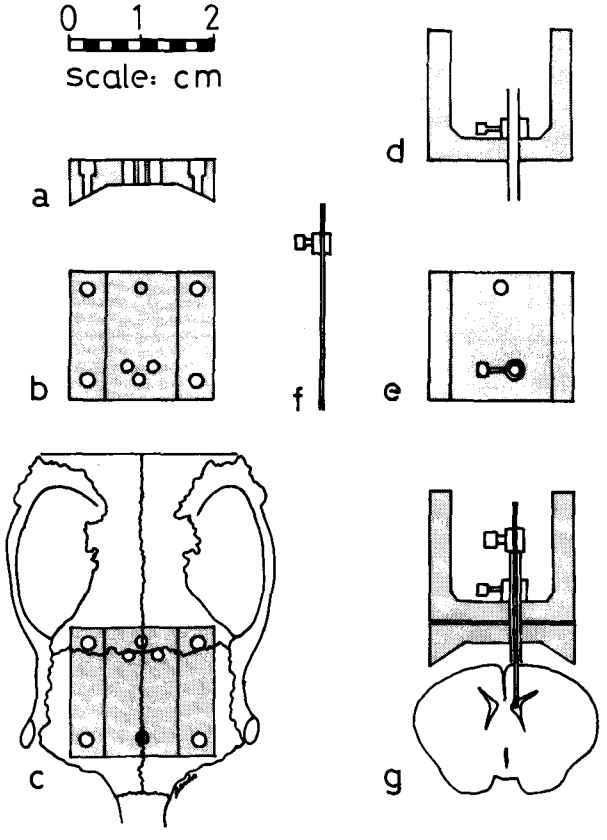

Fig. 1. a + b head plate; c, fixation of the head plate; d + e, top plate; f, cannula and g, whole assembly.

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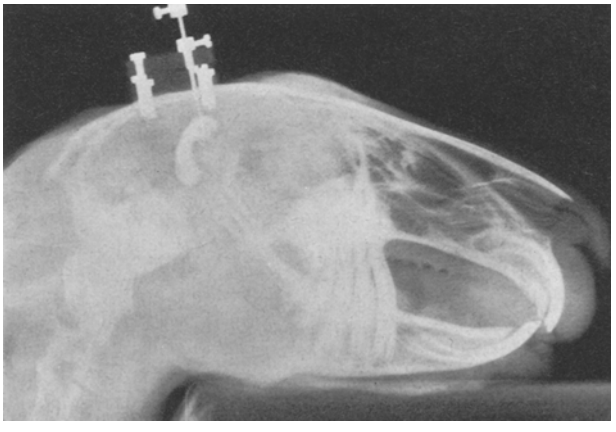

Fig. 2. Myodil ventriculogram with cannula in situ.

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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[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

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