mals. This latter material would be both insoluble and free of hydroxyproline and hence would correspond to the insoluble noncollagenous material found by Houck in rat skin(7,9).

Summary and conclusions. Intraperitoneal injections of 100 mg/kg body weight of dilantin sodium to young rats resulted in significant increase in keratinization of the oral epithelium. Connective tissue of oral mucosa showed elevated fibroblastic activity with pronounced collagen fiber formation. Histochemical staining reactions suggested a substitution of glycoproteins for mucopolysaccharides in the epithelial cell layers. Increase in the glycoprotein and mucopolysaccharide staining material in the connective tissue was noted. This investigation suggested a relation between the severity of microscopic changes in the oral mucosa and dose and duration of dilantin therapy.

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Technique of Permanent Cannulation of the Right Ventricle in Rats and Ground Squirrels.* (28437)

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Permanent cannulation of the aorta and vena cava or right atrium(1) permits simultaneous blood sampling and circulatory studies in unanesthetized and undisturbed small animals(2,3). This method is, however, unsuitable for cardiac output determinations based on the Fick principle, because venous blood is not mixed sufficiently in the right atrium(4). The purpose of this paper is to describe a new technique for permanently implanting a cannula in the right ventricle where blood mixing is adequate.

Methods. Preparation of cannulas. For optimal results the ventricular cannulas (polyethylene tubes #PE 10, Clay Adams, diameter .28 × .61 mm) are prepared at least 48 hours prior to implantation. A ventricular cannula 15 cm long and having the shape of a hook (Fig. 1) is manually shaped

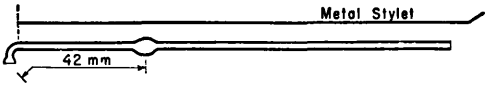

FIG. 1. Schematic presentation of a ventricular cannula made of a polyethylene tube with its stainless steel stylet.

in 90°C water. A small bulge is made 4.2 cm from the internal tip of the cannula by intermittent heating with a nickel wire having a resistance of 2 ohms, through which 3 volts are supplied. A 17 cm long, 0.2 mm wide stainless steel stylet is then inserted into the cannula up to the curvature. The cannula is filled with heparinized saline (4 mg/ml) and its external tip is flame sealed around the stylet. For young rats and ground squirrels the cannulas should be made shorter.

Insertion of ventricular cannula. The anesthetized animals (ether or Nembutal) are placed in supine position. The right internal jugular vein in ground squirrels or external jugular vein in rats is dissected free for one

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FIG. 2. X-ray picture of a rat 45 days after ventricular and aortic cannulation. Both arterial and ventricular cannulas are radioopaque.

centimeter. The cephalad portion of the vein is permanently ligated. The cannula is inserted through a small incision into proximal portion of the jugular vein, loosely tied and advanced toward the heart. One side of the cannula is marked by ink for orientation as to the position of the tip of the cannula. During the first 2 cm insertion, the curved tip of the cannula is directed toward the sternum. The cannula is then turned more to the left for easy passage into the ventricle. The ligature is tied securely once the bulge of the cannula passes just beyond it. The sealed tip of the cannula is then cut off around the stylet which is removed from the cannula. The cannula is flushed with saline in an amount slightly exceeding the volume of the cannula. Then the tip of the cannula is raised 5 cm above the heart level. If blood is still forced out against this hydrostatic pressure, the cannula lies in the ventricle. This can be verified by connecting a pressure transducer to the cannula. If the cannula is misplaced, the stylet is reinserted and the whole procedure repeated. After insertion into the ventricle the cannula is flushed. A hemostat with both tips protected by polyethylene tubing is applied close to the tip of the cannula which is then flame sealed. The ventricular cannula is exteriorized on the top of the neck of the animal after being pushed by forceps or hemostats underneath the skin around the neck. In addition in most of the animals an aortic cannula was implanted as previously described(1). The positions of both cannula are shown in Fig. 2.

Intraventricular electrocardiogram electrodes. The implanted ventricular cannula can serve as an intraventricular electrode for electrocardiography. A T-tube is made from 2 short PE 90 polyethylene tubes by heat sealing. A 0.25 mm silver wire is inserted and heat sealed in one of the openings of the T-tube. For ECG monitoring, the second opening of the T-tube is connected to a PE 10 ventricular cannula through a needle adapter. The saline in the cannula serves in this case as one ECG electrode (Fig. 3). A permanently implanted skin electrode or a constantan wire of an implanted copper-constantan thermocouple is employed as the second electrode. The third lead of the T-tube is connected through a needle adapter to a PE 10 coiled tube, exteriorized from the chamber in which the animal is placed, thereby allowing undisturbed simultaneous blood sampling and cardiac output determinations. When the blood pressure is measured, a polyethylene tube #90 (diameter .86 $\times$ 1.27 mm) is used.

The animals were kept in individual cages to prevent them from biting each other's cannula. Two hundred forty-one adult male Sprague-Dawley rats and 61 adult ground squirrels (*Citellus tridecemlineatus*) of both sexes were cannulated. Postoperative survival was 100%. Body weight of experimental rats was 361 $\pm$ 43 g ranging from 285 to 444 g. The average postoperative body weight loss in rats was 4.7%, never exceeding 7.2%. The animals regained preoperative body weight after 3.9 days and continued afterwards to grow at the normal rate (Fig. 4).

Except for a few animals in which the cannula accidentally slipped from the ventricle into the atrium, the patency of the cannulas seemed to be limited only by the life span of the animal. The same applied to PE 10 aortic cannulas. In the first group of 6 rats both cannulas are still patent 124 days after implantation. Periodical flushing of the cannulas with heparinized saline assured the patency although some cannulas remained open for 40 to 50 days without flushing.

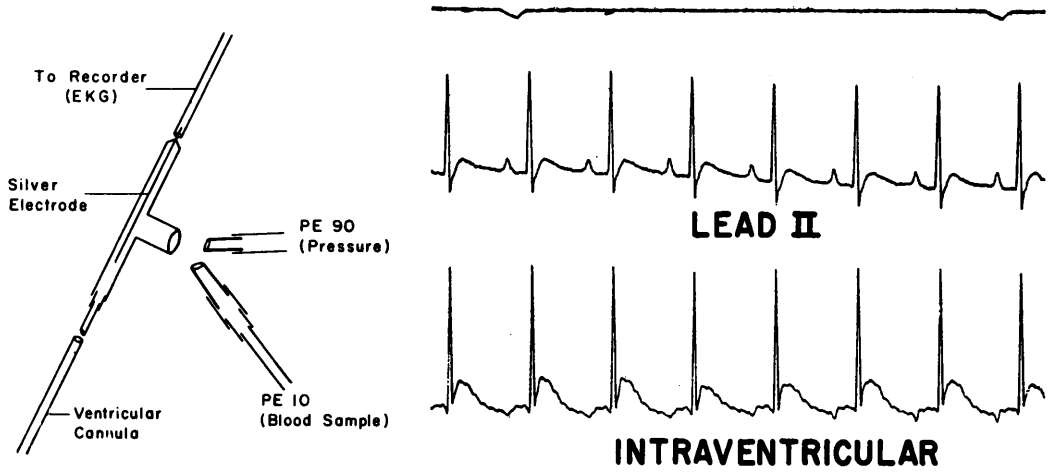

FIG. 3. Adapter serving as an intraventricular electrode (left) and an intraventricular ECG taken 27 days after cannulation in a rat.

To determine whether any cannulated blood vessels had undergone pathological changes, 67 rats were sacrificed between 5 and 100 days after implantation of the aortic and ventricular cannulas and their blood vessels examined. No phlebitis or any abnormal tissue reactions were found. Occasionally, a one millimeter wide white spot was seen on the apex of the ventricle, which developed probably as the consequence of mechanical irritation of the right ventricular wall by implanted cannula.

The competence of the right atrio-ventricular valves after ventricular cannulation was tested in 3 rats after they were cannulated for 5, 25, and 50 days. A second PE 10 cannula was implanted into their superior vena cava *via* the subclavian vein. The venous pressure curve remained unaffected by the right ventricular pressure changes, indicating that no measurable regurgitation occurred.

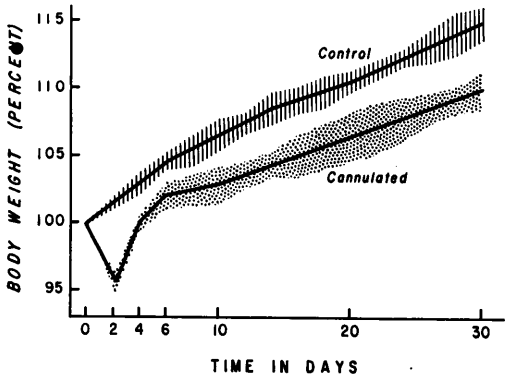

FIG. 4. Body wt of rats after right ventricular and aortic cannulation.

Discussion. The permanent cannulation of the right ventricle as well as the aortic arch offers new possibilities for physiological and pharmacological studies on small laboratory animals during their normal activities. Presently long-term cardiac output studies are made in our laboratory on unrestrained and unanesthetized rats exposed to extremely low external temperatures or to forced exercise. In ground squirrels the cardiac output and intraventricular ECG are measured without interrupting hibernation(5).

Although none of the rats died during first 100 days after cannulation from causes that can be attributed directly to the operation, in several cases the body weights of cannulated rats continued to decrease even after the initial 3 days. These rats were sacrificed and autopsy showed that they all had markedly changed, sometimes hemorrhagic, lungs indicating a pneumonia or a virus lung infection. It is therefore extremely important to check the rat colony for possible pulmonary infection before beginning long term studies on permanently cannulated rats.

Summary. Recently a technique for permanent cannulation of the aorta and vena cava was described which permits simultaneous blood sampling and circulatory studies in unanesthetized and undisturbed

small animals. To make this technique usable for cardiac output determinations (Fick principle), the venous cannula is now permanently implanted in the right ventricle where mixing of the venous blood is adequate. Two hundred forty-one adult male rats and 61 adult ground squirrels of both sexes were cannulated in this way. Postoperative survival was 100%. The animals regained preoperative body weight after 3.9 days and continued afterwards to grow normally. Except for a few animals in which the cannula slipped from the ventricle into the atrium, the patency of the cannulas seemed to be limited only by the life span of the animal.

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Infection of Human Volunteers with Pett Virus.* (28438)

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Although the Pett virus had been recovered from children during an outbreak of respiratory disease(1,2), it could not be etiologically associated with this illness. Moreover, the relationship of this virus to disease in adults also remains undetermined. To gain data on clinical, virologic and serologic responses of Pett virus infection, adult volunteers were inoculated with virus strains immunologically related to the known serotype. This report describes the results of these experiments.

Materials and methods. Clinical procedures. The participants in this study were adult male inmates between the ages of 21 and 35 from various Federal Correctional Institutions. Selection was based on willingness to participate, apparent good health, and lack of detectable neutralizing antibody. Prior to inoculation each volunteer received a complete history and physical examination. Laboratory tests included urine analysis, complete blood count, chemical tests of liver and kidney function, X-ray of chest and bacteriologic examination of nose, throat and rectum. These laboratory studies were repeated at weekly intervals for 3 weeks.

The volunteers were isolated 3 per room. Oral temperature, pulse and respiratory rates were determined 4 times daily. Once daily physical examination was performed by a team of 2 physicians and this, with a record of any complaints of illness, was recorded before leaving the room. The examining physicians were unaware of the type of inoculum administered the volunteers until termination of the experiment.

Virus inocula. The virus used to prepare inoculum No. 1 was recovered from an anal specimen of a young child residing in an institution in Washington, D. C.† The virus used to prepare inoculum No. 2 was recovered from a throat specimen of a volunteer in this study who had been given inoculum No. 1. Inocula were identified in neutralization tests with prototype hyperimmune rabbit serum. The material used was the second passage in primary human embryonic kidney. The maintenance medium, which consisted of 98 parts Eagle's basal medium(3) and 2 parts chicken serum (inactivated 56°C 30 minutes), also contained 250 units of penicillin and 250 μg of streptomycin per ml. The inocula were prepared and safety-tested as previously described(4).

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