This investigation was supported by grants from the Italian Research Council, Rome.

Institute of Medical Pharmacology,
University of Rome,
Rome, Italy.
G. ERSPAMER FALCONIERI

Institute for Basic Research,
Farmitalia S.p.A.,
Milan, Italy.
A. ANASTASI

Institute of Biology,
National University of Cuyo,
Mendoza, Argentina.
J. M. CEI

March 6, 1970.

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A simple method for chronic measurement of the electrocardiogram and blood pressure in the conscious rat

The cardiovascular responses to drugs and toxic chemicals may be markedly different in conscious animals and anaesthetized animals. Electrocardiograms (ECG) are difficult to obtain from the conscious rat without training (Farmer & Levy, 1968) or restraint (Fujita & Tedeschi, 1968). The procedure described permits implantation of electrodes which can be used for several months to monitor ECG. With slight modifications, arterial and venous cannulas have also been used for up to 2 weeks.

The mounting device (Fig. 1) consisted of a small curved polyethylene "saddle" approximately 3 × 2 cm which was cut from the side of a standard 16 oz polyethylene reagent bottle. Two electrode leads, about 10 cm in length and consisting of No 30 Teflon coated 7 strand stainless steel wire, were woven through 6 holes in the saddle leaving about 8 cm on one side for attachment to the sternum of the rat and 2 cm on the other side for connection to the electrocardiograph. Approximately 4 mm of the short lead was stripped of Teflon insulation and soldered so as to leave a small solder blob. If required, silicone rubber cannulas were also passed through additional holes in the same "saddle".

Under ether anaesthesia, two 1 cm long skin incisions were made over the anterior and posterior ends of the sternum. A 4 cm dorsal midline incision was made beginning at about the 4th vertebrae and proceeding posteriorly. The skin adjacent to these incisions was dissected free from subcutaneous tissue. The electrode leads were passed under the skin from the dorsal incision to the incisions over the sternum. The end of the electrode wires were then passed through the cartilage at each end of the sternum, using a small curved needle, and secured by twisting around the main electrode lead in the same way as standard electrical connections are made. The saddle was gently manipulated beneath the skin through the dorsal incision and the skin sutured over the saddle leaving the short connecting parts of the electrodes protruding. About 20 min was required to complete this procedure. When required, silicone rubber cannulas filled with heparinized saline, were passed under the skin and implanted in the carotid artery and jugular vein in a similar manner.

After surgery the animals were housed in individual cages to prevent other rats from damaging the electrode leads or cannulas. When an ECG was required the rat was placed in a small open-top stainless steel cage which was grounded to the electrocardiograph. Two wires (made of the same material as the implanted leads) about 60 cm in length and with small aligator clips attached were used to connect the implanted leads to the electrocardiograph. These light-weight leads allowed free movement of the animal within the cage and permitted the measurement of the ECG with only rare artifacts occurring as the result of the aligator clips shorting together.

Where vascular cannulas were used about 2 cm of cannula was permitted to protrude from the "saddle". The cannulas were filled with heparinized saline and ligated about 0·5 cm from the tip. To maintain patency of the cannulas the ligature was untied and about 0·5 ml of heparinized saline (10 units/ml) infused every 12 h. In our experience it was possible to maintain cannula patency for about 2 weeks. To measure blood pressure or give drugs intravenously, extension cannulas about 20 cm in length were attached to the indwelling cannulas by means of appropriate size needle tubing and connected to a pressure transducer or syringe.

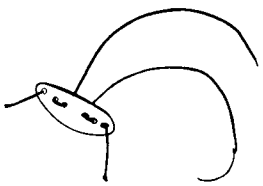

FIG. 1. Device for exteriorizing electrocardiograph leads. If required, cannulas may be exteriorized via the same device.

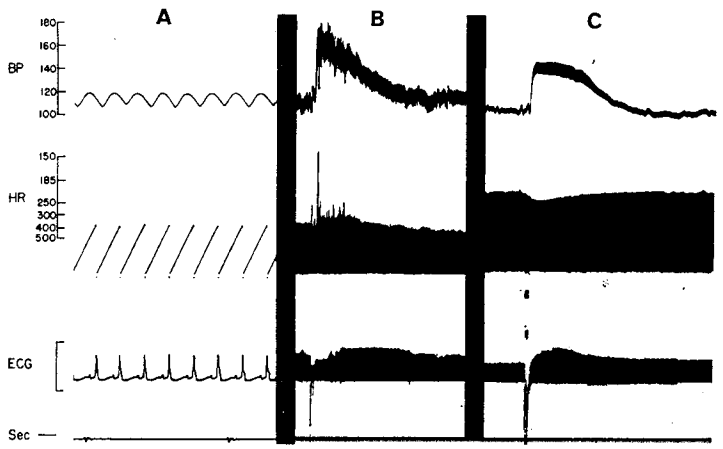

FIG. 2. Blood pressure (BP in mm Hg), heart rate (HR beats/min) and electrocardiogram (ECG) in conscious unrestrained rat. A-control tracing; B-response to 2 μg/kg adrenaline; C-response to 2 μg/kg adrenaline 30 min after administration of 1 mg/kg atropine; H.R. in C must be multiplied by 2 because of the change in attenuation of the cardiotachometer.

The epidermis overlying the plastic plate showed a moderate degree of acanthasis and papillomatosis. The plastic saddle was embedded in a moderately dense connective tissue sheath the inside of which was lined by a smooth shining mesothelial like membrane. The electrodes were enveloped by a thin connective tissue sheath the inside of which was lined by a smooth shining membrane similar to that seen around the plastic plate. The smooth mesothelial like membrane and the connective tissue sheath enveloped the electrodes throughout their entire length in the subcutaneous tissue so that the electrodes were permitted to move freely with the movement of the animal, but were not displaced from their general position in the subcutis.

The distal ends of some of the electrodes were surrounded by a small 3 × 3 mm whitish-grey tissue nodule. On sectioning, these nodules were found to be composed of granulation tissue with a small central cavity surrounded by a variable degree of fibrous tissue. The reaction was characterized by central polymorphonuclear infiltrates, epithelial cells, fibroblasts and histiocytes.

Figure 2 shows a blood pressure and ECG tracing obtained in this way and the response to 2 μg/kg adrenaline before and after administration of atropine. The reflex bradycardia produced by adrenaline before atropine and the tachycardia after atropine emphasizes the differences in cardiovascular response in the conscious animal compared with the anaesthetized animal where tachycardia predominates. This type of phenomenon has been described previously in other species (Whitty & Shepard, 1967; van Miert, 1969).

In a series of 6 animals prepared to determine how long satisfactory ECG recordings could be obtained, excellent recordings showing all components of the ECG were obtained 5 months after surgery in 4 animals. Electrode failure occurred in 2 animals after 3 weeks. Thus, this method has been found useful for the long-term assessment of cardiotoxic effects of chemicals (Grice, Heggtveit & others, 1970) and for the study of the cardiac effects of drugs in the conscious rat.

Division of Pharmacology,
Research Laboratories,
Food and Drug Directorate,
Ottawa 3, Canada.

G. R. VAN PETTEN
J. A. EVANS
F. A. SALEM

February 12, 1970

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The isolation and identification of quininone from *Cinchona ledgeriana*

We have isolated and identified an alkaloid from the bark of a variation of *C. ledgeriana* collected in Guatamala. To the mother liquors remaining after the industrial isolation of quinine (I, R=OMe, R′=H, R″=OH) from this bark (these were supplied, as the residual bases in the form of their thiocyanate salts in aqueous solution, by Lake & Cruickshank Ltd.) (1 litre) was added excess sodium carbonate. The liberated bases were extracted with ether, the total ethereal extract was reduced to small volume under reduced pressure and the residue was subjected to column chromatography on alumina. Elution with ether–chloroform (2:1 v/v) afforded quinamine (II) (0·21 g) (Henry, 1949; Turner & Woodward, 1953), and subsequently with ether–chloroform (1:1 v/v) a white crystalline solid (0·04 g) (initial eluate) and cinchonine (I, R=H, R′=H, R″=OH) (1·24 g) (Henry, 1949; Turner & Woodward, 1953) (latter eluate). Both quinamine and cinchonine, along with quinine, have been isolated previously (Henry, Kirby & Shaw, 1945) from *C. ledgeriana*. The above white crystalline solid was recrystallized from ether–light petroleum (b.p. 40°–60°) to afford prisms, m.p. 98–101°. Elemental analysis gave an empirical formula $C_{20}H_{22}N_2O_2$ which was confirmed and shown to be the molecular formula by mass spectrometry. The ultraviolet spectrum in absolute ethanol showed λ_{max} 361–364 nm