dissection and a polythene cannula inserted. The ear is then perfused at a flow rate of 1·0 ml/min with the blood collected previously. The resting pressure, usually between 60 and 70 mmHg is measured by a pressure transducer and recorded on a potentiometric recorder. A peristaltic pump draws the blood from a film oxygenator consisting of a polypropylene cylinder 8·5 cm in diameter, 19 cm long, inclined at an angle of 30° from the horizontal and rotated at 30 r.p.m. Moistened 95% oxygen 5% CO_2 is blown down into the base of the cylinder. The blood is pumped through the filter removed from an Avon A10 blood administration set and then runs through a heat exchange coil at 37° C prior to entering the ear through the cannula. The ear is suspended by the tip inside a glass heating jacket which excludes draughts and warms the ear. The venous blood flowing out of the ear is collected by a polythene funnel and either returns to the reservoir or is collected for assay, Injections are made through thick-walled rubber tubing into the blood as it enters the heating-coil.

The advantage of blood perfusion over saline perfusion of the ears is that there is little tissue deterioration. Saline perfusion for 90 min results in a severe oedema ; an 80% weight gain being found. A weight gain of only 20% resulted after blood perfusion for over 3 hours.

With this system records can be made of the perfusion pressure and, after the injection of ^{3}H-noradrenaline, the radioactivity of the venous effluent can be assayed by standard liquid scintillation techniques.

REFERENCES

BLAKELEY, A. G. H., BROWN, G. L., DEARNALEY, D. P. & WOODS, R. I. (1969). Perfusion of the spleen with blood containing prostaglandin E_1: transmitter liberation and uptake. *Proc. Roy. Soc. Lond.* B. **174**, 281–292.

The isolated perfused rat liver in the study of the metabolism of foreign compounds

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The use of the isolated perfused rat liver in studies upon the metabolism of foreign compounds is not new (Bahr, Alexanderson, Azarnoff, Sjoqvist & Orrenius, 1970). The present method of perfusion differs from those described previously in certain important respects. The liver was perfused through the portal vein with a semi-synthetic perfusate consisting of aged human cells (packed cell volume 20%) suspended in Krebs bicarbonate buffered saline pH 7·4, containing 3% bovine serum albumin and 0·2% glucose, at a constant flow rate of 1 (ml/g)/min, corresponding to a portal venous pressure of 10 mmHg. The perfusate was gassed with air containing 5% CO_2 to avoid the degenerative changes associated with the use of higher oxygen tensions reported by Abraham, Dawson, Grasso & Goldberg, 1968. The liver was suspended in liquid paraffin maintained at 37° C, this served to support the lobes and allowed an even perfusion.

The preparation remained both macroscopically and histologically normal for up to 6 h. The metabolic criteria of viability adopted, the values of which proved similar to those reported *in vivo* were glucose production, K^+ efflux and the ratio of lactate to pyruvate in the perfusate. Bile production fell from an initial rate of 1 ml/h to 0·1 ml/h at the end of 6 h. Hexobarbitone or aniline was added to the vascular perfusate to give an initial concentration of 1 mM. The disappearance of substrate was followed by extracting the unmetabolized substrate from aliquots of perfusate taken at 15 min intervals, into an organic phase. Hexobarbitone was measured by the method of Remmer (1959) and aniline by the method of Bratton & Marshall (1939).

The half life for the removal of hexobarbitone was 33 min and for the removal of aniline 82 min. Attempts to measure the formation of metabolites from aniline were only partially successful. At the end of 3 h perfusion 22·8% of the aniline remained unmetabolized, whilst aniline conjugates accounted for a further 20%, and conjugates of p-aminophenol for 10%. Free p-aminophenol could not be detected in the perfusate. Thus 61% of the aniline disappearing could not be accounted for in terms of the major

metabolites which are known to occur in the urine after the administration of aniline to rats *in vivo* (Parke, 1960).

A.B. is an M.R.C. Scholar.

REFERENCES

ABRAHAM, R. DAWSON, W., GRASSO, P. & GOLDBERG, L. (1968). Lysosomal changes associated with hyperoxia in the isolated perfused rat liver. *Expl. Molec. Path.*, **8**, 370–387.

BAHR, C. VON, ALEXANDERSON, B., AZARNOFF, D. L., SJOQVIST, F. & ORRENIUS, S. (1970). A comparative study of drug metabolism in the isolated perfused liver and *in vivo* in rats. *Eur. J. Pharmac.*, **9**, 99–105.

BRATTON, A. C. & MARSHALL, E. K. JR. (1939). A new component for sulphanilamide determination. *J. biol. Chem.*, **128**, 537–550.

PARKE, D. V. (1960). Studies in detoxication: **84.** The metabolism of [^{14}C] aniline in the rabbit and other animals. *Biochem. J.*, **77**, 493–503.

REMMER, H. (1959). Der beschleunigte Abbau von Pharmakain den Lebermikrosomen unter dem Einfluss von Luminal. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **235**, 279–290.

The effects of electrical stimulation of the sympathetic nerves on the size and mitotic index of rat salivary glands

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Catecholamines can influence cell proliferation in rat salivary glands. The sympathomimetic compound isoprenaline causes both hyperplasia and hypertrophy of the parotid and submaxillary acinar cells (Selye, Veilleux & Cantin, 1961). Evidence that electrical stimulation of the sympathetic nerves itself can also cause both hyperplasia and hypertrophy has recently been obtained (Muir, Pollock & Turner, unpublished observations) and the present demonstration illustrates the methods used.

Rats were anaesthetized and one superior cervical nerve tract stimulated supramaximally for 1 h (20 Hz, 1 ms, for 30 s min^{-1}). Glands on the contralateral side acted as controls. Hypertrophy was estimated by:

(a) comparing the lengths of two acinar axes from the stimulated glands with the corresponding axes from the controls (the longest axis and that at right angles to and at the mid-point of this axis).

(b) comparing the wet and dry weights of the stimulated glands with the controls. Hyperplasia was estimated by comparing the mitotic indices of the stimulated glands with the controls.

In one group of animals the wet and dry weights of the salivary glands were determined 33 h after the commencement of stimulation. In another group, to determine the mitotic index of each salivary gland, colchicine (1 mg/kg I.P.) was injected 28 h after the beginning of stimulation to arrest dividing cells in metaphase. These animals were then killed a further 8 h later. The lengths of the acinar axes were measured in the glands taken from this group of animals.

Electrical stimulation increased the weight and acinar size of the parotid and submaxillary glands and the mitotic index of the former. The major sublingual gland which receives no sympathetic innervation was unaffected.

C.J.T. is an M.R.C. Scholar.

REFERENCE

SELYE, H., VEILLEUX, R. & CANTIN, M. (1961). Excessive stimulation of salivary gland growth by isoproterenol. *Science*, **133**, 44.

The response of the rat anococcygeus muscle to electrical stimulation of the inhibitory nerves and to drugs, using, simultaneously, mechanical and intracellular electrical recording techniques

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The rat anococcygeus muscle (Gillespie, 1972) has a high ($\approx$60 mv) stable resting membrane potential and shows no spontaneous activity (Gillespie, Creed & Muir, 1973).