REFERENCES

BALLANTYNE, B. & SWANSTON, D. W. (1972). Ocular irritation tests. *Br. J. Pharmac.*, **46**, 577–578P.

CALLAWAY, S., GAZZARD, M. F., PRICE THOMAS, D. & SWANSTON, D. W. (1973). The calibration and evaluation of a hand held tonometer as a means of measuring intraocular pressure in the conscious rabbit. *Exp. Eye Res.*, **15**, 383–393.

ESPLIN, D. W. (1966). Antiseptics and disinfectants. In *The Pharmacological Basis of Therapeutics*. Goodman, L. S. & Gilman, A. (eds.). The Macmillan Company, New York, 1021–1117.

JOHN, R. W. (1968). Necrosis of oral mucosa after local application of crystal violet. *Br. med. J.*, **1**, 157–158.

MEYLER, L. & HERXHEIMER, A. (1968). *Side Effects of Drugs*, **6**, 336.

SLOTKOWSKI, E. L. & REDONDO, D. (1966). Mucosal irritation following use of gentian violet. *Amer. J. Dis. Child.*, **112**, 40–42.

Chemical sympathectomy of the rabbit with 6-hydroxydopamine

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Despite the now considerable literature on 6-hydroxydopamine (Thoenen, 1972), we could find no recommended dose régime for producing chemical sympathectomy in the rabbit. The present study describes a schedule of pretreatment in this species which produces a marked reduction in sympathetic nervous function in tissues of the cardiovascular system and gut.

6-Hydroxydopamine was dissolved in 0·7 ml ice-cold, nitrogen-saturated, 1% ascorbic acid and injected immediately into a marginal ear vein. Rabbits were given 30 mg/kg at 17.00 h on day 1, followed by 20 mg/kg at 13.00 h and again at 17.00 h on day 2. The animals were killed for removal of tissues about 10.00 h on day 3. The preparations used were the perfused heart (Langendorff, 1895), spirally-cut aorta (Furchgott & Bhadrakom, 1953), spirally-cut renal artery (Kelly, 1971), perfused ear artery (de la Lande & Rand, 1965), spirally-cut portal vein (Kelly, 1971) and duodenum (Finkleman, 1930). Whenever practical, the sympathetic nerves were stimulated electrically. Those of the heart were stimulated chemically with dimethylphenylpiperazinium. The responses of each tissue to noradrenaline and tyramine were also routinely tested. In most cases, full log concentration/frequency-response curves were established in order that accurate comparisons of potency could be made. Tissue noradrenaline levels were assayed by the method of Welch & Welch (1969), and demonstrated histochemically by the method of Spriggs, Lever, Rees & Graham (1966). The results are summarised in Table 1.

TABLE 1. *Changes in a number of parameters recorded from tissues taken from rabbits pretreated with 6-hydroxydopamine.* (*20 mg/kg, day 1; 2 × 20 mg/kg, day 2; killed, day 3*).

Tissue parameter	Heart	Aorta	Renal artery	Ear artery	Portal vein	Duodenum
Sympathetic nerve stimulation	—		—	——	——	—
Noradrenaline sensitivity	O	+	+	+	+	O
% Reduction in noradrenaline content	97	74	65		86	86
Fluorescence characteristics	——		—	——	——	——
Tyramine sensitivity	——	—	——	——	——	

——=Absent
—=Much reduced
O=No apparent change
+=Increased
A gap indicates, where, for practical reasons, the test proved inconclusive.

If the effects produced by stimulation of the sympathetic nerves are taken as the most reliable indication of their functional integrity, then, in all the tissues investigated (except aorta), a very considerable degree of sympathectomy is apparent. This inter-

pretation is further supported by the observation that all the tissues had greatly reduced noradrenaline levels after treatment with 6-hydroxydopamine whether assayed biochemically, or assessed by histochemical fluorescence microscopy. Furthermore, responses to the sympathomimetic amine, tyramine, were greatly reduced or abolished in those tissues (heart, ear artery, renal artery and portal vein) where its mode of action is known to involve the release of noradrenaline from sympathetic nerves.

It is concluded that pretreatment of rabbits with 6-hydroxydopamine according to the dose schedule described above, produces marked impairment of sympathetic neuronal function in the tissues examined.

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REFERENCES

FINKLEMAN, B. (1930). On the nature of inhibition in the intestine. *J. Physiol., Lond.*, **70**, 145–157.

FURCHGOTT, R. F. & BHADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

KELLY, M. J. (1971). Receptors for dopamine in some isolated vascular preparations. *M.Sc. Thesis, University of Manchester.*

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol./med. Sci.*, **43**, 639–656.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Säugetierherzen. *Pflügers Arch. ges. Physiol.*, **61**, 291–332.

SPRIGGS, T. L. B., LEVER, J. D., REES, P. M. & GRAHAM, J. D. P. (1966). Controlled formaldehyde-catecholamine condensation in cryostat sections to show adrenergic nerves by fluorescence. *Stain Technol.*, **41**, 323–327.

THOENEN, H. (1972). Surgical, immunological and chemical sympathectomy. Their application in the investigation of the physiology and pharmacology of the sympathetic nervous system. In: *Catecholamines*, Ed. Blaschko, H. & Muscholl, E. Springer-Verlag, Berlin. pp. 813–844.

WELCH, A. S. & WELCH, B. L. (1969). Solvent extraction method for simultaneous determination of norepinephrine, dopamine, serotonin, and 5-hydroxyindoleacetic acid in a single mouse brain. *Analytical Biochem.*, **30**, 161–179.

Prostaglandin E_2, inflammation and pain threshold in rat paws

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We have examined relationships between disappearance of injected prostaglandin (PG) E_2 from the paws of rats and the duration of the PG-induced oedema and hyperalgaesia.

Prostaglandin E_2 (1 μg, 0·1 ml), containing ^{3}H-PGE_2 (0·47 ng, 0·12 μCi) was injected into the subplantar surface of hind paws in rats. At various times after injection, 'pain threshold' (Randall & Sellito, 1957) was measured and the paws excised, weighed and frozen in liquid nitrogen. The prostaglandins were extracted (at pH 2·8) from crushed paw material into ethyl acetate. Radioactivity in the extracts was determined by liquid scintillation counting.

Extractable radioactivity, disappeared very rapidly from the paws, and when peak swelling was attained (20 min), the equivalent of only 25 ng of PGE_2 could be recovered. The oedema produced by the PGE_2 was short lived, decaying at a rate which was superficially similar to, but slower than disappearance of the injected PGE_2. In contrast, the hyperalgaesia developed more slowly and lasted for at least 6 h, even though the amounts of originally injected PG were apparently very low (equivalent to about 0·7 ng of PGE_2).

The occurrence of hyperalgaesia has been reported following or during injection or infusion (respectively) of E-type PGs into human skin (Solomon, Juhlin & Kirschenbaum, 1968 ; Juhlin & Michaelsson, 1969 ; Ferreira, 1972). However, chronic hyperalgaesia is produced after prolonged subdermal infusion of low concentrations of PGE (Ferreira, 1972) or within four days of repeated daily injections of PGE_2 (1–2 μg) in rat paws (Willis & Cornelsen, 1973). A little (equivalent to about 0·4 ng PGE_2) radioactivity could still be extracted from the paws at 24 h, and it is possible that some of the PGE_2 could have been converted to poorly extractable material. Thus the chronic hyperalgaesia in rat paws might be partly due to accumulation of PG metabolites which produce hyperalgaesia and this is being investigated.