tion of pentagastrin-induced gastric acid secretion by terbutaline and salbutamol, like the inhibition by isoprenaline, is not secondary to a fall in mucosal blood flow.

The development of β-adrenoceptor stimulants which, in man, have no direct effect on the heart, raises the possibility of using these drugs for their anti-secretory action. A substance which inhibits acid secretion but causes a relative increase in mucosal blood flow might be expected to hasten the healing of some types of gastric lesions.

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REFERENCES

CURWAIN, B. P. (1972). Comparison of the plasma clearances of ^{14}C-aniline and amidopyrine for the measurement of gastric mucosal blood flow. *J. Physiol., Lond.*, **222**, 1–3*P*.

CURWAIN, B. P., ENDERSBY, K. & HOLTON, PAMELA (1971). Effect of isoprenaline on histamine induced gastric acid secretion in dogs. *Br. J. Pharmac.*, **41**, 384–5*P*.

CURWAIN, B. P. & HOLTON, PAMELA (1971). Radioactive aniline clearance from canine gastric pouches for the measurement of gastric mucosal blood flow. *Br. J. Pharmac.*, **41**, 384*P*.

CURWAIN, B. P. & HOLTON, PAMELA (1972). Effects of isoprenaline on gastric acid secretion and mucosal blood flow during stimulation by pentagastrin or feeding. *Br. J. Pharmac.*, **44**, 332*P*.

Development of acetylcholine, choline acetyltransferase and acetylcholinesterase in rabbit corneal epithelium

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The mammalian corneal epithelium contains a very high concentration of acetylcholine (ACh) and high activities of choline acetyltransferase (ChAc) and acetylcholinesterase (AChE), but the function of the cholinergic system in this tissue is unknown. Hemicholinium-induced depletion of epithelial ACh is accompanied by loss of the corneal reflex, suggesting involvement of ACh in sensory mediation (Fitzgerald & Cooper, 1971). However, significant amounts of ACh remain in the cornea after denervation (Brücke, Hellauer & Umrath, 1949).

Corneal epithelia from female rabbits and their offspring were scraped off under pentobarbitone anaesthesia. Material from one eye of each animal was bioassayed for ACh while ChAc activity was estimated in the extract from the contralateral eye using the method of Schrier & Shuster (1967). AChE was assayed by the technique of Ellman, Courtney, Andres & Featherstone (1961) on any remaining material, as well as on that from several other litters of rabbits. In the case of very young animals, it was necessary to pool tissue from several littermates.

Great variation in ACh content was found, although this was less noticeable within each family. Thus, after expressing ACh levels in terms of %ACh content of the mother's corneal epithelium, a logarithmic increase with age could be observed. At 12 days after birth the corneal ACh was about 3% of that of the mother, reaching 100% at about 50 days. ChAc activities were also subject to considerable variation, but showed a linear increase with age. The enzyme was

first detectable at approximately 12 days and reached adult levels at about 56 days. The relationship between ACh content and ChAc activity within each corneal extract was also logarithmic. Compared with ACh and ChAc, AChE activity showed less variation among different families and an entirely different relationship to age. A sharp peak in AChE was observed in the region of 3–10 days, and activity levelled off at adult values at values at about 25 days.

The coincidence of the opening of the immature rabbit's eyelids (approximately 10 days after birth) with the first appearance of ACh and ChAc in the epithelium, together with the long time taken to achieve maturity of the cholinergic system, do not support a neural role for the bulk of ACh in the cornea.

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REFERENCES

BRÜCKE, H. VON, HELLAUER, H. F. & UMRATH, F. (1949). Azetylcholin- und Aneuringehalt der Hornhaut und seine Beziehungen zur Nervenversorgung. *Ophthalmologica, Basel*, **117**, 19–35.

ELLMAN, G. L., COURTNEY, K. D., ANDRES, V., JR. & FEATHERSTONE, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

FITZGERALD, G. G. & COOPER, J. R. (1971). Acetylcholine as a possible sensory mediator in rabbit corneal epithelium. *Biochem. Pharmac.*, **20**, 2741–2748.

SCHRIER, B. K. & SHUSTER, L. (1967). A simplified radio-chemical assay for choline acetyltransferase. *J. Neurochem.*, **14**, 977–985.

Some pharmacological properties of RX 67668—a new anticholinesterase

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Cis-2-phenyl-1-(N-pyrrolidinyl) cyclohexane hydrochloride (RX 67668) has been shown to be an anticholinesterase whose chemical structure is unrelated to the chemical structures of established anticholinesterases. In a comparative study with a number of other anticholinesterases only RX 67668 was able to reverse the neuromuscular blockade induced by D-tubocurarine at doses which did not also produce symptoms of muscarinic stimulation.

The inhibitory effect of RX 67668 on acetylcholinesterase (acetylcholine acetylhydrolase, 3.1.1.7) and butyrylcholinesterase (acetylcholine acetyl-hydrolase, 3.1.1.8) was initially measured *in vitro* using the method of Michel (1949). Concentrations of anticholinesterases necessary to produce 50% inhibition of the enzymes were determined. It was found that approximately 5×10^{-6}M RX 67668 was necessary to produce 50% inhibition of both acetylcholinesterase and butyrylcholinesterase.

Among the pharmacological tests used to assess anticholinesterase activity *in vivo* are the rat chromodacryorrhoea test (Burgen, 1949) and mouse miosis test (Schneider, 1970). Using these tests a dose of 1·4 mg/kg i.p. RX 67668 was needed to reduce by half the dose of methacholine necessary to produce red tears, whilst a dose of 7·2 mg/kg s.c. RX 67668 was necessary to reduce the pupil diameter of the mouse to 50% of the control value. Comparable doses for neostigmine are 0·036 mg/kg i.p. and 0·06 mg/kg s.c. respectively.

Anticholinesterases find their principal clinical use in the reversal of muscle relaxation at the termination of surgical procedures. Experiments using either the