of plasma levels, the above calculations being based on available data for debrisoquin plasma levels in man (Roche Products, Ltd., 1967).

Since the experiments described were carried out under conditions which may be considered to approximate to those occurring *in vivo* in man, there seems to be a *prima facie* case for considering that uptake of the above compounds may occur in patients undergoing anti-hypertensive therapy, and that any circumstance that interferes with the uptake of debrisoquin or guanethidine into platelets, or causes the discharge of these drugs from the platelets, may alter the magnitude and duration of their clinical effects.

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Absence of inhibitory effects of catecholamines on lower vertebrate arterial strip preparations

SIR,—Many pharmacological studies on spiral strips of large arteries from mammals have been reported and both α- and β-adrenotrophic receptors have been demonstrated (Furchgott, 1952, 1954; Furchgott & Bhadrakom, 1953; Bevan, 1960; Maxwell, 1965; Paterson, 1965). In the present work, the pharmacological responses of spiral strips of arteries from lower vertebrates have been examined as part of an investigation of the evolution of the autonomic innervation of the vasculature.

Spiral strips were cut from both right and left systemic arteries of the sleepy lizard (*Tiliqua rugosa*), the toad (*Bufo marinus*) and from the ventral aorta of the trout (*Salmo trutta*) and the eel (*Anguilla occidentalis australis*). The arterial strips from the sleepy lizard and toad were suspended in McKenzie solution as used by Campbell, Burnstock & Wood (1964). The teleost arterial strips were suspended in a modified Krebs solution as used by Bülbring (1953). Recordings were made at 25° either with an isotonic frontal writing lever or isometrically with a tension transducer.

In the lizard, toad, trout and eel, adrenaline tartrate and noradrenaline bitartrate monohydrate caused contraction of the arterial strips. The threshold concentration (salt) for contraction of the systemic artery of the sleepy lizard

was 10^{-9} g/ml and 10^{-8} to 10^{-7} g/ml for the toad. In teleosts the threshold concentration was higher and more variable (10^{-4} to 10^{-6} g/ml).

Isoprenaline hydrochloride contracted the lizard systemic artery preparation, although it was the least potent of the three catecholamines; the minimal sensitivity was 10^{-5} g/ml. However, isoprenaline hydrochloride (10^{-9} to 10^{-4} g/ml) had no effect on either the toad systemic artery or the teleost ventral aorta. Application of isoprenaline never produced a relaxation in any of the preparations, even when the strips had been brought initially to a state of moderate contraction by another agent, for example, acetylcholine 10^{-7} g/ml or noradrenaline 10^{-7} g/ml (Table 1). In contrast, experiments on mammalian aortic muscle have shown that the β-effects of isoprenaline can be revealed in this way (Furchgott, 1952; Furchgott & Bhadrakom, 1953).

The contractile effects of the catecholamines in the lizard and toad preparations were blocked by the α-blockers, phentolamine methanesulphonate 10^{-7} to 10^{-6} g/ml, phenoxybenzamine hydrochloride 5×10^{-7} to 10^{-6} g/ml and Dibenamine 5×10^{-7} to 10^{-6} g/ml. Reversal of the contractile response of the catecholamines to relaxation was never observed, whereas reversal of the action of adrenaline on the rabbit aorta after α-blockade by Dibenamine has been reported (Furchgott, 1952; Furchgott & Bhadrakom, 1953). The β-blocker, pronethalol, 5×10^{-7} to 10^{-6} g/ml blocked the contractile actions of catecholamines on the lizard and toad preparations. The effects of α- and β-blockers on the response of the teleost ventral aorta to adrenaline and noradrenaline appeared to be similar to their effects on the toad, but were difficult to assess because of the variability in the control response.

In conclusion, it appears that there are no catecholamine receptors which mediate inhibition in the systemic artery muscle of the lizard or toad, or in the ventral aorta of the eel and trout. However, in the lizard, the potency ratio for noradrenaline, adrenaline and isoprenaline for contractile effects is consistent with that observed for α-stimulation in mammals. Thus, the catecholamine receptors in this preparation appear to be of the α-type, and the blockade of the responses to catecholamines by the β-blocker, pronethalol, must be unspecific. Arterial strips from lower vertebrates could prove to be useful vascular smooth muscle preparations for studies of the excitatory action of sympathomimetic agents uncomplicated by their effects on receptors which mediate relaxation.

TABLE 1. COMPARISON OF THE RESPONSES OF SPIRAL STRIPS OF LARGE ARTERIES TO CATECHOLAMINES (CONCENTRATIONS IN G/ML) IN DIFFERENT VERTEBRATE CLASSES, AND THE ACTIONS OF α- AND β-BLOCKERS ON THESE RESPONSES

	Mammalia*	Reptilia	Amphibia	Teleosteii
Noradrenaline	+ (10^{-10}–2×10^{-9})	+ (10^{-9})	+ (10^{-9}–10^{-7})	+ (10^{-6}–10^{-4})
Adrenaline	+ (10^{-10}–2×10^{-9})	+ (10^{-9})	+ (10^{-8}–10^{-7})	+ (10^{-6}–10^{-4})
Isoprenaline	+ (10^{-6}) − (10^{-9}–10^{-8})	+ (10^{-8})	no effect	no effect
α-Blockers	Block +	Block +	Block +	Block +†
β-Blockers	Block −	Block +	Block +	Block +†

+ = *contraction.* − = *relaxation.* Block + = blockade of contractile response. Block − = blockade of inhibitory response.
*Figures from Furchgott & Bhadrakom (1953).
† See text.

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The anticholinesterase activity of physostigmine

SIR,—When physostigmine in solution undergoes decomposition, its urethane grouping is first lost and a colourless compound termed eseroline is formed; subsequent oxidation leads to rubreserine, a red quinone, which is later converted to eserine blue or eserine brown (Ellis, 1943). We have now investigated the anticholinesterase activities of these degradation products.

Samples of eseroline, rubreserine, eserine blue and eserine brown were kindly supplied by Mr. G. Smith, Department of Pharmacy, Heriot-Watt University. They were dissolved in freshly distilled water and stored at 4° until required. The anticholinesterase activities of the different solutions were compared with that of physostigmine.

In the first experiments, comparisons were made using horse serum as the source of pseudocholinesterase and acetylcholine as the substrate. Both the Warburg manometric technique and the biological method which involves measuring the residual acetylcholine on a piece of isolated tissue (rat colon, rat uterus, guinea-pig ileum) were used. All the degradation products of physostigmine were less active than the parent compound; eserine blue, the most potent, was 100–500 times less active whilst eseroline and rubreserine were 10 times less active than eserine blue. When the comparisons were made using both serum and red blood cells of rabbit, horse and man as the sources of enzyme and acetylcholine as the substrate, all of the degradation products were more active against the pseudocholinesterases than against the true enzymes; on the other hand, physostigmine at a very much lower concentration was equally active against both enzymes.

Finally, tests using the chromodacryorrhoea response in rats (Burgen, 1949) showed that eserine blue, rubreserine and eseroline were about 1,000 times less active than physostigmine in potentiating the *in vivo* action of acetylcholine.

The results are of relevance in that ophthalmic solutions of the British Pharmaceutical Codex are now required to be sterile, and physostigmine eye-drops B.P.C. (Supplement, 1966) may be sterilized by heat. Hydrolysis may occur during the heating process, resulting in the formation of an inactive colourless compound, eseroline, before the appearance of the pink oxidation product, rubreserine. Thus solutions of physostigmine may be colourless but relatively inactive.