petroleum ether (b.p. 60-80° C) and after filtering, this mixture was forced (~20 ml/min) through a small column (0.3-1 g) of silicic acid (Unisil : Clarkson) and the eluate discarded. The endoperoxide is completely eluted in 1-4 ml of cold (−20° C) anhydrous diethyl ether, which may (after rapid evaporation of the solvent) be biologically tested, (or after reduction in volume) be subjected to t.l.c.

The endoperoxide can be rapidly isolated by t.l.c. on microscope slides coated with a slurry of silica gel G (Merck), and developed for ~5 min (2° C) in a solvent system (L1) of analytical grades of toluene : dioxane (1 : 1). Another suitable solvent system (L2) consists of anhydrous diethyl ether : petroleum ether : methanol (80 : 20 : 3.5). Precoated plates (silica gel F_{254} : Merck) can be used and they are developed for ~40 min at −20° C. After sectioning the plates, position of the endoperoxide may be detected by rapidly spraying with a commercially available spray reagent (Nu Peroxy Spra : Supelco). Alternatively radioactivity from ^{14}C arachidonate substrate (Applied Sciences) may be detected or the plates sprayed with phosphomolybdic acid (Gréen & Samuelsson, 1964). For biological tests, the endoperoxide must be eluted immediately (without drying) into 1-10 ml of diethyl ether : methanol (9 : 1). Etherial eluate from the rapid column procedure or t.l.c. can be stored for several days below −60° C without significant loss in biological activity.

For testing biological activity attributable to the endoperoxide, the solvent is evaporated down rapidly under a stream of nitrogen, using latent heat of evaporation to cool the tube. The dried material is then immediately dissolved in 0.05-1 ml of Tyrode's solution (for testing on isolated organs) or in citrated platelet-rich plasma (1 ml) for testing its effects on platelet aggregation.

References

COLLIER, H.O.J., SAEED, S.A., SCHNEIDER, C. & WARREN, B.T. (1973). Arachidonic acid and the analgesic action of aspirin-like drugs. *Advances in the Biosciences*, **9**, 413-418.

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX. Thin layer chromatography of prostaglandins. *J. Lipid. Res.*, **5**, 117-120.

GRYGLEWSKI, R. & VANE, J.R. (1972). The generation from arachidonic acid of rabbit aorta contracting substance (RCS) by a microsomal enzyme preparation which also generates prostaglandins. *Br. J. Pharmac.*, **46**, 449-457.

HAMBERG, M., SVENSSON, J., WAKABAYASHI, T. & SAMUELSSON, B. (1974). Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc. Soc. Nat. Acad. Sci. USA*, **71**, 345-349.

WILLIS, A.L. (1973a). Biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ generates labile material which induces platelet aggregation. *Abstracts, IVth International Congress on Thrombosis and Haemostasis*, p. 79.

WILLIS, A.L. (1973b). Platelet synthesis of pro-aggregating material from arachidonate and its blockade by aspirin. *Circulation, XLVIII (4)*, **Suppl. IV**, 55.

WILLIS, A.L. (1974). Isolation of a chemical trigger for thrombosis. *Prostaglandins*, **5**, 1-25.

WILLIS, A.L. & KUHN, D.C. (1973). A new potential mediator of arterial thrombosis whose biosynthesis is blocked by aspirin. *Prostaglandins*, **4**, 127-129.

WILLIS, A.L., VANE, F.M., SCOTT, C.G., KUHN, D.C. & PETRIN, M. (1974). An endoperoxide aggregator (LASS) formed in platelets in response to thrombotic stimuli: purification, identification and biological actions. *Prostaglandins, submitted for publication.*

An increased reactivity in hypertensive animals after prolonged anti-hypertensive therapy

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An increased reactivity to various vasoconstrictor agents, such as noradrenaline (NA) and 5-hydroxytryptamine (5-HT), in blood vessels seems to be a common feature of several types of experimental hypertension (McGregor & Smirk, 1970; Finch, 1971; Beilin & Ziakas, 1972; Haeusler & Finch, 1972). However, the mechanism for this sustained hyper-responsiveness is unclear. Theoretical and experimental data suggest that structural adaptive changes of the blood vessels may take place (Folkow, 1971) whilst other experimental evidence is compatible with the individual smooth muscle cells becoming supersensitive to vasoconstrictors stimuli (Bohr & Sitrin, 1970).

In the experiments to be reported, rats with blood pressures in excess of 180 mmHg for a period of 8 weeks (made hypertensive by deoxycortisterone acetate 50 mg, unilateral nephrectomy and 0.9% NaCl replacing the drinking water for 4 weeks) were treated with

combinations of antihypertensive agents placed in the drinking water; (a) hydrallazine 0.1 g/litre, hydrochlorothiazide 0.25 g/litre and reserpine 0.005 g/litre; (b) hydrallazine 0.05 g/litre and mecamylamine 0.05 g/litre. Both these drug combinations (after 3-10 weeks of administration) lowered the blood pressures of the hypertensive animals to within normotensive levels. Reactivity to various vasoconstrictor agents was determined in the isolated Krebs-perfused mesentery artery preparation. Using preparations from untreated hypertensive rats the dose-response curves of NA, 5-HT and ATP all showed a marked increased reactivity, a higher maximum, but no alteration in the threshold doses when compared with preparations from age-matched normotensive animals were observed. An increased reactivity to NA, 5-HT and ATP was also still observed in the preparations from 'hypertensive' animals which had received the antihypertensive therapies whilst the responsiveness of preparations from the normotensive treated animals was similar to those obtained using untreated normotensive animals. It was observed that the long-term antihypertensive therapy reduced the incidence of periarteritis nodosa in the mesenteric arteries seen in the hypertensive rats.

The present results support the theory that structural changes may be partially responsible for the increased reactivity observed in blood vessels of experimental hypertensive rats, however, the persistence of the increased reactivity after prolonged lowering of the blood pressure seriously questions the role of the increased reactivity in the maintenance of the elevated blood pressure in experimental hypertension.

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References

BEILIN, L.J. & ZIAKAS, G. (1972). Vascular reactivity in postdeoxycorticosterone hypertension in rats and its relation to irreversible hypertension in man. *Clin. Sci.*, **42**, 579-590.

BOHR, D.F. & SITRIN, M. (1970). Regulation of vascular smooth muscle contraction. Changes in experimental hypertension. *Circulation Res.*, **26** & **27**, (suppl. 11), 83-90.

FINCH, L. (1971). Cardiovascular reactivity in the experimental hypertensive rat. *Br. J. Pharmac.*, **42**, 56-65.

FOLKOW, B. (1971). The haemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. *Clin. Sci.*, **41**, 1-12.

HAEUSLER, G. & FINCH, L. (1972). Vascular reactivity to 5-hydroxytryptamine and hypertension in the rat. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **272**, 101-116.

McGREGOR, D.D. & SMIRK, F.H. (1970). Vascular responses to 5-hydroxytryptamine in genetic and renal hypertensive rats. *Am. J. Physiol.*, **219**, 687-690.