DEMONSTRATIONS

Time lapse photomicrography of cell migration inhibition

M.A. BRAY (introduced by J. MORLEY)

Laboratory of Applied Physiology, Division of Immunology, Kennedy Institute, Bute Gardens, London W6 7DW

The migration inhibition test is widely used as an *in vitro* indicator of Type IV allergy (delayed hypersensitivity, cellular immunity). In such a test, guinea-pig peritoneal exudate cells or human peripheral blood leucocytes are packed into capillary tubes and allowed to migrate into tissue culture fluid (Eagle's minimum essential medium + 15% normal guinea-pig serum) so that they form a fan of cells on the floor of the chamber. The basis of the test is that an antigen, evoking delayed skin reactions in the donor *in vivo*, will inhibit the migration of sensitized cells *in vitro* (George & Vaughan, 1962; Søborg & Bendixen, 1967). It has been shown that such inhibition is due to non-antibody material released into the medium by sensitized lymphoid cells in response to antigen, and that such material will similarly inhibit the migration of either sensitized or non-sensitized macrophages (Bloom & Bennett, 1966; David, 1966).

The time course of migration from the capillary tube and its inhibition by various active materials has been recorded on 16 mm film by time lapse photomicrography of chambers using dark field illumination. Recordings have been made of migration of peritoneal exudate cells in the presence and absence of the following materials: specific antigen (Bovine gamma globulin, purified protein derivative of tuberculin (PPD)), phytomitogens, lymphokines, Kurloff cell material and continuous cell line culture products. Measurement of the time course of migration inhibition assists distinction between these agents and allows determination of an optimal time for measurement of inhibition (in bioassay of guinea-pig lymphokine preparations or in clinical tests utilizing leucocyte migration inhibition.)

References

BLOOM, B.R. & BENNETT, B. (1966). Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science*, **153**, 80-82.

DAVID, J.R. (1966). Delayed hypersensitivity *in vitro*: its mediation by cell free substances formed by lymphoid cell-antigen reaction. *Proc. natn. Acad. Sci.*, **56**, 72-77.

GEORGE, M. & VAUGHAN, J.H. (1962). *In vitro* cell migration as a model for delayed hypersensitivity. *Proc. Soc. exp. Biol. Med.*, **111**, 514-521.

SØBORG, M. & BENDIXEN, G. (1967). Human lymphocyte migration as a parameter of hypersensitivity. *Acta med. scand.*, **181**, 247-256.

Some easy and rapid endoperoxide isolation procedures

A.L. WILLIS[1]

Department of Pharmacology, Hoffmann–La Roche Inc., Nutley, New Jersey 07110, U.S.A.

Direct biological testing of incubates of arachidonate with enzymes which synthesize prostaglandins (PGs) E_2 and $F_{2\alpha}$ has demonstrated the presence of additional factors which produce contractions of the isolated rabbit aorta (Gryglewski & Vane, 1972), induce nociception in mice (Collier, Saeed, Schneider & Warren, 1973), or aggregation of human platelets (Willis & Kuhn, 1973; Willis, 1973a, b). In the latter case, 'labile aggregation-stimulating substance' (LASS) has been isolated and identified as an endoperoxide intermediate (Willis, 1974; Hamberg, Svensson, Wakabayashi & Samuelsson, 1974; Willis, Vane, Scott, Kuhn & Petrin, 1974). This endoperoxide (15-hydroxy-9_α, 11_α-peroxidoprosta-5, 13-dienoic acid) can be isolated from enzyme incubates or platelet suspension by the procedures below.

LASS is rapidly extracted at pH 3 (adjusted with 1 M citric acid) into 4 volumes of an ice cold mixture of anhydrous diethyl ether and methanol (7.5 : 1) or else 8 volumes of ethyl acetate. After evaporation of the solvent, these extracts can be biologically tested or further purified by liquid chromatography or thin layer chromatography (t.l.c.).

A rapid column procedure was developed which removes >95% arachidonate and PGs E_2 and $F_{2\alpha}$, but with no loss of LASS activity. The ethyl acetate was diluted (1 : 30) with cold (−70° C)

[1] Present address: Department of Pharmacology, Roche Products Ltd, Welwyn Garden City, Hertfordshire, England.

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

L. FINCH

School of Studies in Pharmacology, University of Bradford, Bradford 7, Yorks.

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