DEMONSTRATIONS

Time lapse photomicrography of cell migration inhibition

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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

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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)

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