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Simplified thin-layer chromatography of prostaglandins in biological extracts

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Commercially prepared plates for thin-layer chromatography are disposable and can be sectioned, allowing phosphomolybdic acid visualization of prostaglandin (PG) markers run on the same plate as a biological extract. The AI and AII systems (Gréen & Samuelsson, 1964) were used with glass plates (Merck) precoated with 0·25 mm of silica gel, sometimes containing fluorescent indicator (plates backed with aluminium foil were less satisfactory). As recommended by Ramwell & Daniels (1969), tanks were not equilibrated before use.

For the AII system, the plates were saturated with ethanolic AgNO) (Fig. 1). The edges were wiped and the plates thoroughly dried and sealed in a black polyethylene envelope for up to 10 days before use.

Extract and markers were applied to the plate as in Fig. 1. After development, the plate was dried, cut and the marker PGs visualized (Gréen & Samuelsson, 1964). Silica gel containing separated extract was scraped off and eluted. Zones from AII plates were eluted into acidified Krebs solution or into 3 ml of acidified 2% NaCl solution (pH 2·5–3·0 after elution). PG in the eluate was extracted twice into an equal volume of ethyl acetate and the dried extracts dissolved in 1 ml of Krebs solution for bioassay. Zones from AI plates were eluted into 1–4 ml Krebs solution and, after centrifugation, activity in the supernatant determined directly. Eluted activity was assayed in parallel (Willis, 1969) on isolated tissues superfused in cascade with Krebs solution.

Good separation was achieved: in the AII system, mean ($\pm$S.E.M.) R_fs on eighteen plates were $0{\cdot}79 \pm 0{\cdot}02$ for PGE_1; $0{\cdot}54 \pm 0{\cdot}02$ for PGE_2 and $0{\cdot}26 \pm 0{\cdot}01$ for $PGF_{2\alpha}$, with no significant difference across the plates. Recovery was 60–80% in the AI and 30–50% in the AII system. $PGF_{1\alpha}$ was only used on four occasions, when it ran just in front of PGE_2. Detection of $PGF_{1\alpha}$ in extracts thus relied upon parallel assay and rechromatographing in the AI system.

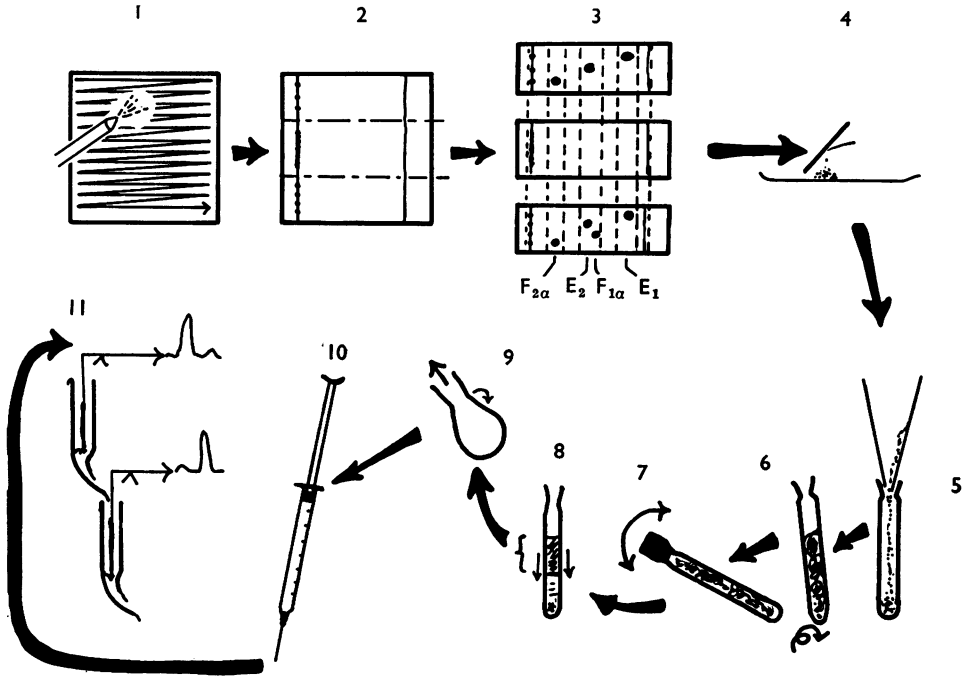

FIG. 1. Separation in the AII system. Using a glass spray (Aimer), the plate was sprayed to saturation with a solution of 10 g of $AgNO_3$ in 100 ml of 86% aqueous ethanol (1). This solution must be used fresh, otherwise substances are formed (probably ethanol oxidation products) which interfere with the separation. The plate was thoroughly dried with a hair drier, followed by 3 h in an oven at 60° C. Marker PGs (5 μg) were applied as single spots of 1 mg/ml solution in ethanol or ethanolic Na_2CO_3 solution. Biological extract was dissolved in 0·5 ml of ethanol and a measured volume applied to the plate as a 5 cm band using a stream of *cool* air to evaporate the ethanol (2). After development, the plate was dried, cut and marker PGs visualized with phosphomolybdic acid (3). Zones of silica gel (-------) were scraped from the extract portion of the plate onto glossy paper (4), transferred into glass-stoppered tubes (5) and eluted into aqueous saline solution (6) which precipitated silver ions as AgCl. After adjusting to pH 3 with HCl, PGs in the eluent were extracted into ethyl acetate mixing the two phases by inversion of the tubes (7). After centrifugation (8), the ethyl acetate extracts were dried on a rotary evaporator at 50° C (9) and dissolved in 1 ml of Krebs solution (10) for bioassay (11).

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The Vidian nerve and the sphenopalatine ganglion in relation to lacrimation in the cat

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The secretory fibres to the lacrimal gland are conveyed by the lacrimal branch of the fifth cranial nerve (Botelho, Hisada & Fuenmayor, 1966; Elsby & Wilson, 1967) but they are believed to leave the brain stem with the seventh nerve (Landolt, 1903). Transfer of these secretory fibres from the Vidian branch of the seventh to the fifth