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Prostaglandin production by rabbit peritoneal polymorphonuclear leukocytes *in vitro*

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It has been suggested (Kaley & Weiner, 1971) that since prostaglandin E_1 (PGE_1) has chemotactic activity *in vitro* on rabbit polymorphonuclear (PMN) leukocytes it may be an important mediator of the leukocyte emigration from blood vessels seen in acute inflammation. If PGE_1 were released by PMN leukocytes during phagocytosis, this could constitute a control system for local leukocyte emigration which would continue as long as phagocytosis was occurring. PMN leukocytes *in vitro* were therefore investigated for their capacity to produce PG.

Rabbit PMN leukocytes were obtained by washing out the peritoneal cavities of animals with Hanks solution 4 h after the intraperitoneal injection of 200 ml 0·1% rabbit liver glycogen in 0·9% sterile saline as described by Hirsch & Church (1960). Each animal was used at intervals of 7–10 days for this procedure. The cell suspensions (over 90% PMN leukocytes) were centrifuged gently and resuspended in Hanks solution enriched with glucose (0·56 mM) and 0·01% bovine serum albumin, to give cell suspensions in the range $3{\cdot}5\text{–}8{\cdot}0\times10^6$/ml. These suspensions (volume 40–50 ml) were incubated at 37°, and aliquots of 4 ml taken at intervals, adjusted to pH 3·0 with HCl and extracted twice with ethyl acetate. The pooled extract was evaporated to dryness in a rotary evaporator, redissolved in 1·0 ml Krebs solution and the resulting solution assayed for PG-like activity on rat stomach strips superfused with Krebs bicarbonate solution containing methysergide 0·2 mg/l, phenoxybenzamine 0·1/mg/l, propranolol 3 mg/l, hyoscine hydrobromide 0·1 mg/l and mepyramine maleate 0·1 mg/litre.

In these circumstances only very small (less than 1 ng/ml original suspension) amounts of prostaglandins were detected, but if killed bacteria (Pertussis vaccine, Burroughs Wellcome, containing 4×10^{10} bacteria/ml) were added in doses of 100–200 bacteria/leukocyte, PG-like activity in amounts up to 10 ng/10^6 PMN leukocytes PGE_2 equivalent were found after incubation for 2 hours. The bacterial suspensions alone had no PG-like activity. Thin layer chromatography of the extract was performed after purification from solution in ethanol by extracting 4 times with petroleum ether, using Dioxan/Benzene/acetic acid (20/20/1) as solvent. Fifty-six per cent of the PG-like activity found moved with PGE_2, 28% with $PGF_{2\alpha}$ and 16% as an unidentified spot between the origin and the $F_{2\alpha}$.

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