PROSTAGLANDIN PRODUCTION BY A MILD INFLAMMATORY LESION IN SHEEP

B. GREENWOOD & P.J. KERRY
A.R.C. Institute of Animal Physiology, Babraham, Cambridge

By implanting a Teflon chamber subcutaneously into a sheep a mild aseptic inflammatory lesion was produced from which wound fluid could be sampled daily. The prostaglandin content of this wound fluid was examined. Prostaglandin E_2 activity was found in all samples, and a peak was apparent on the fourth or fifth day after producing the lesion. The amount of prostaglandin activity appeared to be unrelated to the total number of polymorphs in the fluid or to the volume of this exudate.

Introduction Prostaglandins have been detected in fluid from several types of lesion, but in few species. The presence of prostaglandin E_2 was demonstrated in the exudate from a carrageenin-air bleb, in rat subcutaneous tissue (Willis, 1970) and in lymph from scalded dogs' paws (Anggard & Jonsson, 1971). Prostaglandin E_1 and smaller amounts of E_2 were found in the aqueous humour of rabbit eyes in which uveitis had been induced (Eakins, Whitelocke, Perkins, Bennett & Unger, 1972), and prostaglandins E and F were recovered from inflamed human skin (Greaves, Sondergaard & McDonald-Gibson, 1971). Rat pleural and peritoneal exudates induced by carrageenin have contained prostaglandins E and F, as has synovial fluid in rheumatoid arthritis patients undergoing anti-inflammatory therapy (Velo, Dunn, Giroud, Timsit & Willoughby, 1973).

There must inevitably be some discussion about species differences in the prostaglandin types produced, and, within those species, about the cells which produce them. In the experiments described here, relatively large volumes of sterile wound fluid have been obtained from the subcutaneous tissues of the sheep. As well as prostaglandins, the cell content of this fluid has been studied.

Methods Wound fluid was collected from sheep with a perforated Teflon or stainless steel cylinder (Greenwood, 1970) of 29 mm external length and 19 mm external diameter. One end of the chamber was closed, while a catheter passed through a lid at the other end so that its tip lay centrally in the chamber. The cylinder was placed subcutaneously in the shoulder region beneath the *M. cutaneus colli*. The muscle layer and skin were closed over the chamber, the catheter passed out through the top of the incision and was held in place by Michel clips and sutures. The catheter remained plugged except when wound fluid samples were being taken.

The wound fluid was removed every 24 hours. The catheter was kept clear between sampling by injecting a small quantity of heparinized 0.9% w/v NaCl solution (saline). Each collection continued until no further fluid was obtained. Blood samples were taken from the jugular vein at the same time. Total and differential white blood cell counts were carried out on both wound fluid and blood. Evidence of bacterial contamination was sought, but not found, by culturing each wound fluid sample on blood agar.

The prostaglandins were extracted by acidifying the wound fluid to pH 3 with HCl before extracting the sample twice with equal volumes of ethyl acetate. The ethyl acetate was evaporated to dryness and the residue dissolved in 1.0 ml of Krebs bicarbonate solution. The prostaglandin content of each extract was assayed in terms of prostaglandin E_1 equivalents on the rat fundic strip preparation (Vane, 1957). The tissue was superfused at 5-10 ml/min with Krebs bicarbonate solution, containing mepyramine maleate (0.1 mg/l), hyoscine hydrobromide (0.1 mg/l), propranolol (3 mg/l), phenoxybenzamine (0.1 mg/l), methysergide (0.2 mg/l) and indomethacin (1 mg/l). Evidence for the identification of individual prostaglandins in the extracts was obtained by thin layer chromatography, with the AI and AII systems of Gréen & Samuelsson (1964).

Results Daily determinations in two individual sheep (Figure 1) showed that the wound fluid volumes and total leucocyte counts progressively rose and then declined, whereas total prostaglandins remained comparatively steady save for one outstanding peak. In sheep (a) the peak occurred on day 5, and in sheep (b), on day 4. In neither animal did the peak coincide with the peak total polymorph count. In one sheep the prostaglandins peaked one day later than the total polymorphs, while in the other they peaked two days earlier. The peak polymorph count in both

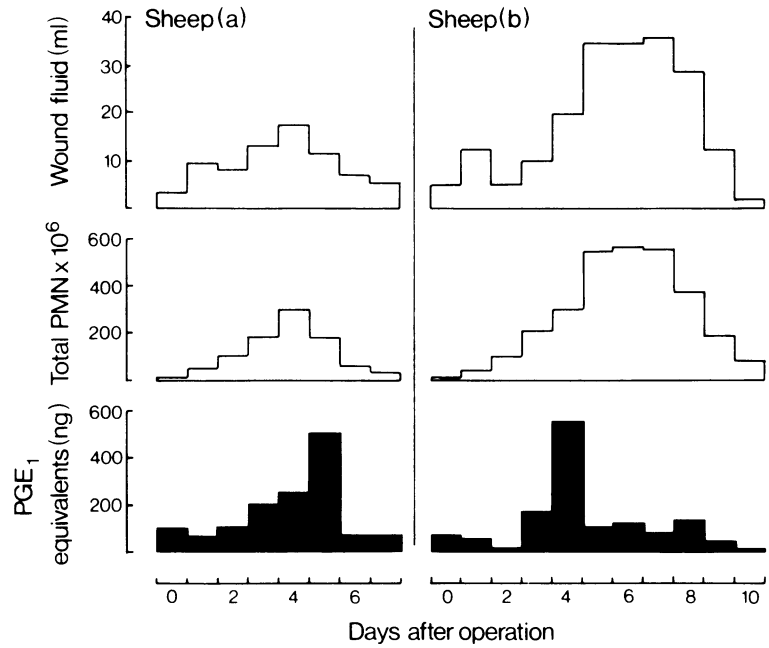

Figure 1 Volume, total polymorphonuclear leucocyte (PMN) count, and total prostaglandin E_2-like activity (expressed in ng quantities in terms of prostaglandin (PG) E_1 equivalents) of wound fluid from a mild aseptic surgical lesion in each of two sheep.

animals was associated with a low total prostaglandin level.

Discussion Previous (unpublished) experiments have shown that mixed populations of sheep blood leucocytes can produce prostaglandins when incubated and that this production can be increased tenfold by adding dead staphylococci to the cultures. Granulocytes from the rabbit peritoneal cavity behave similarly with respect to *B. pertussis* (Higgs & Youlten, 1972). It may well be that the dominant variable in producing the prostaglandin peak is not the total number of polymorphs in the wound, but the change in stimulus to prostaglandin synthesis by these cells.

The cell population in the wound fluid may not accurately represent the polymorph accumulation in the surrounding tissues. Biopsies do not, however, indicate that high numbers of polymorphs are present in the tissues at this time. Small numbers of monocytes, which, unlike polymorphs, are more obvious in the surrounding tissues than in the wound fluid, are beginning to accumulate. Were the prostaglandin peak to be related to the accumulation of monocytes, it might be expected that the rise would be prolonged and progressive.

An alternative explanation of the peak might be that prostaglandin removal or destruction is briefly diminished for some reason. It is, perhaps, surprising that prostaglandins remain in such large quantities in the wound fluid, and are not almost wholly removed by the blood, for equilibration of small molecules between wound fluid and blood appears to be good. On the other hand some degree of stasis is suggested by the P_{CO_2} and P_{O_2} levels of the wound fluid, which have been measured in other experiments. The restoration of prostaglandins following daily removal of wound fluid from the tissue space, indicates continuous active production.

Thin layer chromatography showed that the prostaglandin-like activity moved almost entirely with the prostaglandin E_2 standard (smooth muscle stimulating material which travelled with prostaglandin E_1 or $F_{2\alpha}$ was detected in trace amounts in only a few of the samples assayed). Incubated sheep mixed leucocytes produce predominantly E series prostaglandins. Velo *et al.* (1973) suggested that prostaglandin E_2 increases vascular permeability, but there was no correlation between the total prostaglandin content or concentration, and the volume of wound fluid. Of course, increased permeability may facilitate the removal of wound fluid if the tissue pressure

builds up adequately. In these experiments tissue fluid pressure is certainly positive, but the lax tissue in which the chamber is implanted, and the large volumes of wound fluid recovered do not suggest that great quantities of wound fluid are being forced back into the blood vessels.

The sources of prostaglandin E_2 in this lesion are being studied. That it is unlikely to arise from the tissue acutely injured at operation is suggested by the delay in maximum prostaglandin production. Our findings that sheep mixed leucocytes produce prostaglandin E indicate that the polymorph population is likely to contribute. This production is minimal unless bacteria are present, and these were not found in the wound fluid. However, there is bound to be some material suitable for phagocytosis in the damaged tissue, if, indeed, phagocytosis stimulates prostaglandin production by leucocytes as Higgs & Youlten (1972) have suggested.

We are grateful to Dr L.J.F. Youlten and G.A. Higgs, Esq., for drawing our attention to the presence of prostaglandins in previous sheep wound fluid samples. P.J.K. is a Research Training Scholar of the Wellcome Trust.

References

ANGGARD, E. & JONSSON, C-E. (1971). Efflux of prostaglandins in lymph from scalded tissue. *Acta physiol. scand.*, **81**, 440-447.

EAKINS, K.E., WHITELOCKE, R.A.F., PERKINS, E.S., BENNETT, A. & UNGER, W.G. (1972). Release of prostaglandins in ocular inflammation in the rabbit. *Nature, New Biol.*, **239**, 248-249.

GREAVES, M.W., SONDERGAARD, J. & McDONALD-GIBSON, W. (1971). Recovery of prostaglandins in human cutaneous inflammation. *Br. med. J.*, **2** 258-260.

GRÉEN, K. & SAMUELSSON, B. (1964). Thin layer chromatography of prostaglandins. *J. Lipid Res.*, **5**, 117-120.

GREENWOOD, B. (1970). An implanted chamber and catheter for the collection of tissue (wound) fluid from the sheep. *J. Physiol., Lond.*, **210**, 118-119P.

HIGGS, G.A. & YOULTEN, L.J.F. (1972). Prostaglandin production by rabbit peritoneal polymorphonuclear leukocytes in vitro. *Br. J. Pharmac.*, **44**, 330P.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344-349.

VELO, G.P., DUNN, C.J., GIROUD, J.P., TIMSIT, J. & WILLOUGHBY, D.A. (1973). Distribution of prostaglandins in inflammatory exudate. *J. Path.*, **111**, 149-158.

WILLIS, A.L. (1970). Identification of prostaglandin E_2 in rat inflammatory exudate. *Pharmac. Res. Commun.*, **2**, 297-304.

(Received September 17, 1974)