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COMMUNICATIONS

Production of an inflammatory exudate containing prostaglandins

It has been reported that an inflammatory exudate containing prostaglandins (PG) can be obtained from a subcutaneous air bleb containing a suspension of carrageenan (Willis, 1969a). PGs in the bleb fluid were identified as E type prostaglandins, mainly PGE_2 (Willis, 1969b). Since the release of lysosomal enzymes, measured as an increase in β-glucuronidase and acid phosphatase activity in the exudate, was parallel with the appearance of PGE_2-like activity in the bleb fluid, it was suggested that lysosomal phospholipases were released from leucocytes and that these phospholipases released arachidonic acid and other fatty acids from cell membrane phospholipids; arachidonate would subsequently be converted to PGs by tissue enzymes (Anderson, Brocklehurst & Willis, 1971). It has been shown that phospholipases will release Rabbit Aorta Contracting Substance and PGs from perfused lung tissue (Vargaftig & Dao Hai, 1972). Thus it would be expected that PG-like activity could be recovered from an air bleb containing phospholipase A. The enzyme preparation used was prepared from bee venom and was obtained from Sigma Ltd. Activity was 1180 U mg^{-1} protein.

Rats were anaesthetized with ether and their backs were shaved. Air (10 ml) and a 2% suspension of carrageenan (5 ml) were injected subcutaneously as described by Willis (1969a, b). At various times up to 24 h after the injection of carrageenan the rats were killed and the bleb fluid withdrawn. The bleb fluid was acidified to pH 3 with dilute hydrochloric acid and partitioned against three volumes of ethyl acetate. The non-aqueous phase was removed and evaporated under reduced pressure. The residue was then either reconstituted in Krebs solution and bioassayed or used for thin-layer chromatography in the A1 system of Gréen & Samuelsson (1964). After chromatography, the zone corresponding to PGE_2 was removed from the plate, eluted into Krebs solution and bioassayed. All samples were bioassayed on a rat colon and a rat stomach strip preparation (Vane, 1957; Regioli & Vane, 1964) superfused with Krebs solution at 34°. The responses of the preparations were recorded via isometric strain gauge transducers.

It was found that 24 h after the injection of carrageenan, PG-like activity equivalent to 100–200 ng of PGE_2 per ml could be recovered from the pooled exudate of a group of five rats. This activity co-chromatogrammed with PGE_2 and was thus taken to be due to an E type PG. During the first 8 h following the injection of carrageenan only low levels of prostaglandin-like activity could be recovered from the bleb fluid (usually only 10–60 ng ml^{-1} assayed as PGE_2), as shown in Table 1. Between 8 and 24 h after the carrageenan injection the level of prostaglandin-like activity rose to the final levels quoted above. These findings are consistent with those of Willis (1969a, b).

Table 1. *Yields of PGE_2-like activity in the exudate from air blebs injected with carrageenan and phospholipase A.*

Phlogistic agent	PGE_2-like activity in exudate * ng ml^{-1}					Mean	s.e.
Carrageenan 5 ml 2%	57	12·5	62·5	50	60	48·4	±9·21
Phospholipase A 5 ml (0·5 U ml^{-1})	200	100	150	100	100	130	±19·9

* Exudates were removed 4 h after the irritant was injected.

Each value refers to the prostaglandin content of the pooled exudate from a group of five rats.

When 5 ml of a solution of phospholipase A (0·5 U ml^{-1}) in 0·9 % saline was injected into the air bleb in place of the carrageenan suspension it was found that PGE_2-like activity equivalent to 100–200 ng ml^{-1} of the bleb fluid was present after 4 h (Table 1). This activity could be extracted into ethyl acetate and it co-chromatogrammed with PGE_2 as did the activity recovered from carrageenan air blebs. Pretreatment of the rats with aspirin (128 mg kg^{-1} orally) or indomethacin (4 mg kg^{-1} orally) reduced the levels of prostaglandin-like activity to an undetectable level.

A major drawback of the inflamed air bleb technique concerned the recovery of the exudate. There was so much resorption of fluid from air blebs containing carrageenan that all the exudate recovered often came from only one rat out of a group of five. Phospholipase A air blebs tended to give a more consistent yield from each rat but occasionally some rats in a group yielded little or no fluid. Thus it was necessary to use groups of at least five rats.

Thus as an exudate containing PG's can be created in an air bleb containing phospholipase A, it is suggested that this may offer an alternative method for the study of prostaglandin synthesis *in vivo*. This technique does, however, rely on the introduction of large amounts of a cell-damaging enzyme rather than activation of tissue enzymes by other phlogistic agents such as carrageenan.

The findings described above are consistent with the hypothesis that lysosomal phospholipases may be involved in the generation of prostaglandins in inflammatory states (Anderson & others, 1971). Non-steroidal anti-inflammatory drugs which have been shown to prevent the formation of PG are known to inhibit the microsomal enzyme chain referred to as PG-synthetase, which converts arachidonate or dihomo γ-linoleate to PGs (Vane, 1971). These drugs do not inhibit phospholipase, and they are not thought to interfere with the formation of fatty acid peroxides which are intermediate products in the synthesis of PGs (Samuelsson, 1972). The fact that these drugs prevent the production of PG in the present study, coupled with the purification steps before bioassay makes it reasonably certain that the activity was due to PGs. It follows that these PGs originated from the appropriate unsaturated fatty acids produced by hydrolysis of phospholipid.

The prostaglandins used in this study were obtained from Dr. J. Pike of the Upjohn Co. Kalamazoo; carrageenan was obtained from Marine Colloids Inc; phospholipase A from Sigma Ltd.

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