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Parallel assay of prostaglandin-like activity in rat inflammatory exudate by means of cascade superfusion

Pharmacological activity was found in inflammatory exudates from rats treated with carrageenan, and was predominantly attributable to the presence of E-type prostaglandins. Some of this work has been briefly reported to the British Pharmacological Society (Willis 1968). Novel features of the experimental methods used are presented here in more detail.

Cascade superfusion for the detection and assay of prostaglandins has been used previously (Ferreira & Vane, 1968; Gilmore, Vane & Wyllie, 1968). However the modifications described below allow reduced flow rates and thus increased sensitivity for the repeated parallel assay of small amounts of prostaglandin-like activity.

Isolated tissues were suspended in chambers formed from non-wettable polypropylene and superfused in series with a stream of Tyrode solution delivered at constant rate by a roller pump (Watson Marlowe MHRE). Tyrode in the reservoir was gassed with air and contained atropine (10^{-6}M), mepyramine (10^{-6}M) and methysergide bimaleate (5×10^{-7}M). The entire cascade was enclosed in a Perspex-fronted box maintained at near 37° and saturated with water vapour from a humidifying device (Fig. 1). This arrangement permitted prolonged survival of up to four tissues with superfusion rates of only 2 to 4 ml/min. Standard prostaglandins (PGs) and extracts in Tyrode solution (0·8 ml or less) were injected into the inlet side of the silicone rubber roller pump tubing. Responses of the tissues were recorded on a kymograph by pendulum levers (Paton 1957) with lengthened writing arms (Schild 1947).

The principal isolated tissue used was the rat stomach strip as prepared by Vane (1957). It was found that a prolonged settling period of several hours resulted in a steady base-line and high sensitivity to prostaglandins (0·5 to 1 ng of PGE_2). This tissue was often used in conjunction with the chick rectum (Mann & West, 1950, Ferreira & Vane, 1967). It was found that under the conditions described, the Tyrode superfused rectum from chicks of 150–200 g responded in a selectively sensitive manner to E-type prostaglandins. This tissuewhile virtually equi-

sensitive to PGs E_1 and E_2 was at least 100 times less sensitive to $PGF_1\alpha$ and 20 times less sensitive to $PGF_2\alpha$.

Other tissues used have been the gerbil (*Meriones shawii*) colon, the guinea-pig proximal colon and rat colon (see review by Bergstrom, Carlson & Weeks, 1968).

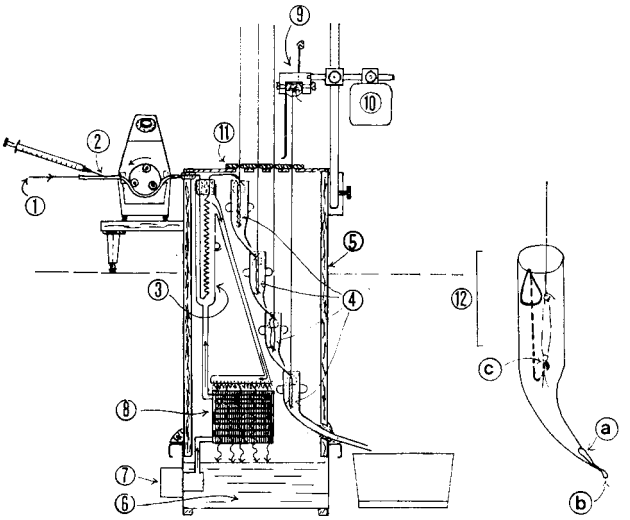

FIG. 1. The modified cascade apparatus. (1) Aerated atropinized Tyrode delivered at constant rate by a roller pump. (2) Extracts and standard prostaglandins injected through a small needle into the self-sealing inlet side of the silicone-rubber roller pump tubing. (3) Warming coil of fine polythene tubing. (4) Isolated tissues suspended in polypropylene chambers arranged in cascade. (5) Perspex-fronted cabinet constructed from marine plywood and sealed with polyurethane. (6) Water tank thermostatically maintained at 38°. (7) Pump. (8) Humidifying and air warming device. Warm water circulated through a series of finned tubes over which water returning from the warming coil trickles back to the tank. (9) Pendulum-type recording levers with lengthened frontal writing arms. (10) Vibrator. (11) Adjustable Perspex discs to seal top of the cabinet where strings pass to the recording levers. (12) Detail of isolated tissue chamber drawn from a polypropylene tube. (*a*) Open channel to provide free drainage of fluid to next tissue; (*b*) Rounded lip to avoid friction with thread; (*c*) Isolated tissue suspended from stainless steel hook clipped to side of the chamber.

The first source of prostaglandin-like activity was the oedema fluid from rat feet inflamed by carrageenan (Winter, 1962). A 1% suspension of carrageenan (Gelazone S.T.1, Whiffen) in saline was injected (0·1 ml) into the subplantar surface of a hind foot through a 26 gauge needle. Between 1 and $5\frac{1}{2}$ h later, groups of 5 to 10 animals were killed and the injected feet excised at the ankle joint. The oedema fluid was then squeezed out using a hand vice with rubber jaws, pooled in graduated centrifuge tubes and immediately extracted.

It could be argued that some or all of the pharmacological activity present in the fluid from the feet was due to physical damage of the tissue, inherent in this method. To obviate this the "carrageenan air bleb technique" was developed from the granuloma pouch of Selye (1954). In this way large volumes of exudate are recoverable for extraction and the fluid in the bleb is relatively free from contact with physically damaged tissue. This technique also permits the study of changes in levels of the pharmacologically active principles with time, although one should not assume that events are necessarily parallel in the air bleb and in the carrageenan-inflamed foot.

Rats were anaesthetized with ether, the skin of the back shaved, swabbed with cetrimide 1% in ethanol, and 10 ml of air injected through a bacterial filter (Millipore) with a 23 gauge sterile needle. 5 ml of a 2% carrageenan suspension in sterile

saline, warmed to about 37°, was then injected into the air pouch which extended along the dorsal part of the thorax. In this case a 19 gauge needle was used. In some experiments the carrageenan suspension was autoclaved for 15 min at 121° before injection. Groups of animals were killed at intervals up to 24 h after carrageenan injection and the fluid in the bleb swiftly and cleanly withdrawn into plastic syringes using 19 gauge needles.

For prostaglandin extraction the individual samples of bleb fluid were transferred to graduated centrifuge tubes, acidified to pH 3 with hydrochloric acid and extracted twice with an equal volume of ethyl acetate. This was removed under partial vacuum at 60° using a rotary evaporator and the dried material reconstituted in 0·5 to 1 ml of Tyrode solution before assay. The dried extracts were stored at 3° for up to 4 days whilst awaiting assay.

Table 1.

Source of activity	Activity extracted into ethyl acetate at pH 3 assayed as ng/ml of PGE_2*			
	Rat stomach strip	Gerbil colon	Chick rectum	Guinea-pig prox. colon
Oedema fluid from foot	3·7	3·9	2·4	—
	5·3	6·1	3·6	—
	6·0	7·9	8·3	—
Contents of sub-cutaneous air pouch	7·7	5·8	5·0	—
	8·0	8·1	8·5	—
	17·5	17·8	25·0	—
	26·7	20·7	31·0	—
	31·3	28·7	27·0	—
	34·3	34·3	38·3	—
	53·7	—	—	74·4
	82·3	—	—	85·2
	100·0	—	—	74·0

* Figures given are in ascending order after varying time intervals.

Parallel assay of activity in extracts recovered during carrageenan inflammation are shown in Table 1. These results suggest that activity in the extracts is mainly attributable to the presence of E-type prostaglandins. This assumption has been supported in further studies using thin-layer chromatographic separation.

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