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Bioassay by cascade superfusion using a highly sensitive laminar flow technique

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Superfusion of tissues (Finkleman, 1930; Gaddum, 1953; Vane, 1964) is a widely used technique for the bioassay of biologically active substances. Recently, Ferreira & De Souza Costa (1976) described a sensitive superfusion method using a laminar flow technique. The tissues were superfused with Krebs at a low flow-rate and were protected from desiccation by suspending them in mineral oil. This technique permitted the detection of minute amounts of smooth muscle stimulating substances, such as prostaglandins (PGs), bradykinin and angiotensin II. The sensitivity of the technique was competitive with radioimmunoassays. However, the disadvantage of this method is that it is not possible to use a combination of different assay tissues arranged in a cascade (Vane, 1964) for simultaneous parallel bioassay. In this paper we describe a simple adaptation of the method of Ferreira & De Souza Costa (1976), which permits the use of at least two tissues, superfused in a cascade with the laminar flow technique.

A siliconized glass cylinder, with a sloping drain attached to the inside, was fitted into an organ bath surrounded by a water jacket at 37° (Fig. 1). The organ bath was then filled with gassed (95% O_2, 5% CO_2) Krebs, the level of which was adjusted with a syphon. The upper tissue was secured by knotting the lower thread in a small hole in the middle of the drain and the lower tissue was secured as described by Ferreira & De Souza Costa (1976). Both tissues were connected to Harvard heart/smooth muscle transducers and contractions were recorded on a Rikadenki pen recorder. Krebs solution was then delivered directly onto the thread above the upper tissue, at 0·3–0·4 ml min^{-1}, with a peristaltic pump (Verder, Vleuten, The Netherlands). The Krebs solution in the organ bath was replaced by prewarmed mineral oil, which was gently poured down the inside of the bath. The Krebs was displaced through the syphon until the oil-water boundary was below the lower tissue; the upper oil level was kept above the upper tissue by adjusting the syphon. To obtain a smooth flow over the tissues, the inner cylinder was carefully cleaned before each experiment and the tissues were tied to the transducers with thick threads (Leinenzwirn, 4, J. Phrimmer & Co., F.R.G.). In initial experiments, the upper tissue was secured by tying it to a small hook attached to the cylinder immediately above the drain. However, this was less satisfactory as, in some cases, the Krebs tended to run down the side of the bath instead of flowing completely over the lower tissue.

Test substances were dissolved in either Krebs or saline and injected in constant volumes with micropipettes (10–100 μl, Eppendorf or Finnpipette) directly

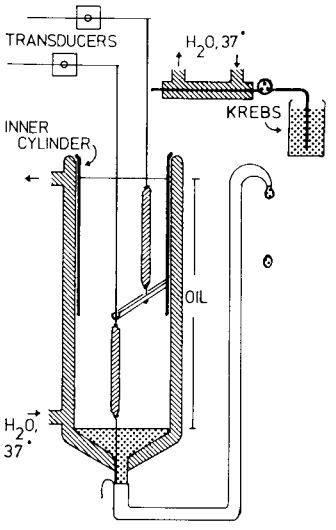

FIG. 1. Organ bath, containing an inner cylinder with a small drain. At the beginning of each experiment the level of the paraffin oil was adjusted with the syphon.

* Correspondence.

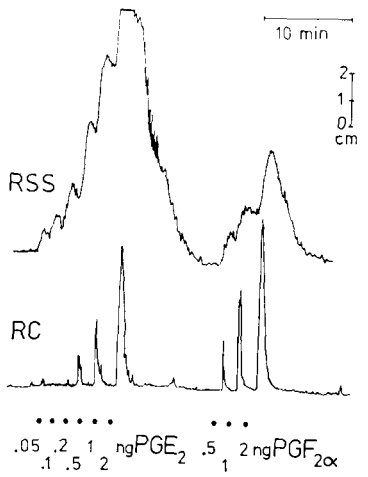

FIG. 2. Typical dose-response curves to prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The cascade consisted of a rat stomach strip (RSS) and a rat colon (RC), which were superfused with a laminar flow (0·38 ml min^{-1}). The test doses were given in 50 μl.

nto the Krebs flowing over the first tissue. This direct method reduces the time between injection and arrival at the upper tissue and facilitates the detection of highly unstable substances such as thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975; Bult & Bonta, 1976a).

An example of a typical experiment, using a rat stomach strip (Vane, 1957) and a rat colon (Regoli & Vane, 1964), superfused with Krebs, containing a mixture of antagonists (Gilmore, Vane & Wyllie, 1968; Bult & Bonta, 1976b) is shown in Fig. 2. The capability of this combination to discriminate between low doses of PGE_2 and $PGF_{2\alpha}$ is clearly demonstrated. The doses were administered within short time intervals, thus producing cumulative dose-response curves on the rat stomach strip (Bult & Bonta, 1976b). This tissue showed a greater sensitivity towards PGE_2 than towards $PGF_{2\alpha}$, while both PGs were almost equipotent on the rat colon. This confirms results obtained on a normal cascade (Vane, 1969), but the apparent sensitivity was about 20 fold greater. Using this technique, the stomach strip responded to 50–100 pg PGE_2 (flow rate 0·38–0·4 ml min^{-1}), whereas in an optimally sensitive normal cascade (flow rate 2·5 ml min^{-1}) the threshold dose of PGE_2 was 1–2 ng (e.g. Bult & Bonta, 1976a). The lowest dose of PGE_2 and $PGF_{2\alpha}$ which induced contractions of the rat colon was 500 pg. Greater sensitivity could probably have been achieved by using lower flow rates (cf. Ferreira & De Souza Costa, 1976).

This modification of the laminar flow technique permits superfusion of different tissues at a very low flow rate, and thereby diminishes the effect of dilution of the samples and increases the sensitivity of the assay. In contrast to the original technique described by Ferreira & De Souza Costa (1976), the present modification has the advantage that different assay organs can be superfused simultaneously. Thus, it is possible to discriminate between biologically active substances in very small quantities or to enhance the specificity of a bioassay after extraction and column chromatography of test samples. Preliminary results indicate that it is also possible, because of the low flow rate, to superfuse the tissues with blood from a rat and to detect the release of smooth muscle stimulating substances in this small animal *in vivo* (cf. Vane, 1964, 1969).

Very recently, Naylor (1977) has described a similar organ bath for use in a normal cascade with up to 5 tissues. It is possible that our apparatus might also be adapted for superfusion of more than 2 tissues.

We thank Mr M. J. P. Adolfs for excellent technical assistance. PGs were kindly supplied by Dr J. Pike (Upjohn, Kalamazoo, U. S. A.). H. B. is supported by the Foundation for Medical Research FUNGO, and M. J. P. is the recipient of a grant from the Nederlandse Vereniging tot Rheumatiekbestrijding.

December 21, 1976

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