The use of isolated organs for detecting active substances in the circulating blood

J.R. Vane

Commentary by

J.R. Vane

By 1964 I was thoroughly committed to Gaddum's technique of superfusion (Gaddum 1953) and also to the principles of parallel pharmacological assay proposed by Gaddum (1959) as strong evidence for the identity of a compound. I introduced the idea of superfusing several tissues in cascade (generally up to six, arranged in two banks). Besides enabling the parallel assay of individually injected samples, this arrangement also allowed parallel and dynamic analysis of the active components present in a fluid stream taken from the outflow of a perfused organ.

Many different buffers such as Kreb's solution, Tyrode's solution, Locke's solution etc., are used to support the activity of isolated organs. Vane's solution was something entirely different! All of these artificial solutions were based on the concentrations of various salts in the blood, so why not use blood itself? This idea was the birth of what became known as the blood-bathed organ technique. An anaesthetised animal is heparinized and arterial blood is removed at a constant rate of 10-15 ml per minute (dogs, cats or rabbits). Lower rates were used from guinea-pigs (Piper, Collier and Vane 1967). The blood superfuses the cascade of tissues and is then returned intravenously to the animal.

I well remember my first experiment with a stream of blood as the bathing fluid for a rat stomach strip. There was a vigorous contraction of the strip which lasted throughout the experiment, making it exquisitely sensitive to relaxation by catecholamines. Over the years it has become evident that part of this contraction is due to 5-hydroxytryptamine, but 5HT antagonists do not totally remove it.

Plainly, when perfusate from an organ or blood from an animal is used for superfusion, substances can reach the tissues within a few seconds of generation or release. This element of "instantaneity" is an important aspect of cascade superfusion bioassay, in that it detects biological activity of chemically unstable compounds whose activity would otherwise be lost in an extraction process. Another important feature of the method is that it gives the maximum opportunity for serendipity! The dynamic nature of the assay also allows the measurement of the degree of inactivation of an infused substance across a particular vascular bed.

With a cascade including a rat stomach strip and a chick rectum the differential bioassay of adrenaline and nor-adrenaline could be made, for the chick rectum did not relax to nor-adrenaline whereas both tissues relaxed to adrenaline.

When a small dose of histamine was injected into a spinal cat, a guinea-pig ileum superfused in cascade with blood was contracted by the blood histamine some 20-30 seconds later but, then over the next few minutes, the rat stomach strip relaxed due to catecholamines liberated by histamine into the circulation from the adrenal medulla. Similarly, the prolonged release of histamine by intravenous injection of compound 48/80 could be illustrated on the guinea-pig ileum superfused with blood.

Over the years, I asked each new PhD student to find a new assay organ to add to the cascade. With a choice of rat stomach strip, a chick rectum, a rat colon, a cat jejunum strip, a rabbit aorta, a rabbit coeliac artery or bovine coronary artery, we were able differentially to detect many of the classical mediators such as adrenaline, nor-adrenaline, angiotensin II, bradykinin and the prostaglandins.

Cascade superfusion bioassay made possible the detection of the release of "rabbit aorta contracting substance" or RCS ($t_{1/2}$ < 5 min), the discovery of prostacyclin ($t_{1/2}$ = 3 min) (see Vane, 1983) and the identification of EDRF as nitric oxide ($t_{1/2}$ = 10 sec) (Palmer, Ferrige & Moncada, 1987).

As far as the blood-bathed organ technique is

concerned, over the next few years, we published many abstracts and more than 30 full papers using it to measure the release and fate of vasoactive hormones in the circulation. (Vane, 1969).

References

GADDUM J H (1953) Technique of superfusion *Br. J. Pharmacol.* **8,** 331-326.

GADDUM J H (1959) Symposium on catecholamines. I. Measurement of adrenaline, noradrenaline, and related compounds. Bioassay procedures. *Pharmacol Rev.* **11,** 241-249

PIPER P J, COLLIER, H O J & VAN J R (1967) Release of catecholamines in the guinea pig by substances involved in anaphylaxis. *Nature* **213,** 838-840

PALMER R M J, FERRIGE A G, MONCADA S (1987), Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327,** 524-526

VANE J R (1969) The release and fate of vasoactive hormones in the circulation *Br J Pharmacol* **35** 209-242

VANE J R (1983) Adventures and excursions in bioassay: the stepping stones to prostacyclin. Les Prix Nobel 1982, 181-206, Almqvist & Wiksell International, Stockholm, Sweden.