The technique of superfusion

J.H. Gaddum

Commentary by

J.R. Vane

Jack Gaddum was one of the giants of pharmacology in the 1950's. Along with Harold Burn, George Brownlee, Willie Bain, A.D. MacDonald and Gladwin Buttle, he dominated meetings of the British Pharmacological Society. This paper illustrates several of his attributes including conciseness, clarity and directness of approach.

These were the days when isolated organs such as the guinea-pig ileum and the rat uterus were used not only to assay solutions of known mediators such as histamine but also for the serendipitous discovery of unknown but potentially important pharmacological activity.

This paper on "the technique of superfusion" is widely cited as the beginning of the method. His idea of dribbling a suitable solution over the surface of an isolated tissue derived from Finkleman (1930). Important aspects of this paper were the coining of the word "superfusion", the front page illustration of the superfusion chamber and Gaddum's simple trick of stopping the normal flow of fluid in order to replace the drops with the test solution. This added even further to the improvement in sensitivity over and above the isolated organ bath that was commonly used.

Of substantial importance in those days was to reduce the coefficient of friction between the writing lever and the smoked drum, normally achieved by regularly tapping the bench. Here, however, Gaddum deliberately used a stream of bubbles through the warming jacket in order to cause a slight vibration of the whole apparatus which could be optimised by finding the appropriate height for the apparatus on a vertical bar.

Much of this paper is concerned with substance R, a slow reacting substance which he discovered was released from a superfused piece of small intestine and which contracted a rat uterus superfused below it. He then began experiments to try to identify substance R released from the perfused mesenteric bed of the rat into fluid which then superfused the rat uterus. He used various chemical manoeuvres and enzymes such as trypsin or chymotrypsin to try to identify the class of chemical to which substance R belonged. In a classical statement typical of Gaddum, he says "substance R is not histamine, because histamine does not cause contraction of the rat's uterus". He also distinguished it from acetylcholine, 5-hydroxytryptamine, bradykinin, oxytocin and substance P by the use of various manoeuvres, including dialysis and antagonists. He concluded that the properties of substance R were similar to those of kallikrein and warned that the appearance of substance R may complicate experiments on the pharmacological activity of extracts of intestine.

Interestingly, Rod Flower and his colleagues (1991) took up the challenge of substance R. Because of the preciseness of Gaddum's work and of his description of it, they were able totally to reproduce the formation of this unknown slow reacting substance in the perfused mesentery of the rat, even to reproducing the exact time course of its appearance. They concluded that it was a kininogenase enzyme, distinguishable from plasma or urinary kallikrein but did not elucidate the exact nature of the protein.

The significance of Gaddum's paper was several-fold. First, it popularised the technique of superfusion, leading to a substantial increase in the sensitivity of bioassays. It also formed the basis for my own work on cascade superfusion (Vane 1964), a technique which allowed more accurate identification of substances in solution by simultaneous differential bioassay. Overall, this is a gem of a paper, especially because it describes a simple technique in such minute detail that it can be reproduced easily by others.

Bioassay continued to have a major importance over the next 40 years or so, especially in identification of more unknown substances. The advent of electronic pen recorders and of powerful transduc-

ers further increased the sensitivity of bioassay, dispensing with the need for a vibrator or for tapping the bench! The whole discovery of endothelium-derived relaxing substance (EDRF) and its eventual identification as nitric oxide in the 1980's would not have been possible without the type of superfusion bioassay technique described in this paper by Gaddum and developed further by me.

References

DOUGLAS G.J., WILLIAMS K.I. & FLOWER, R.J. Characterisation of Gaddum's substance R. (1991). *Br. J. Pharmacol.*, **102**, 926-930.

FINKLEMAN B. (1930). On the nature of inhibitions in intestine. *J. Physiol. (Lond)* **70**, 145-157.

VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmacol.*, **23**, 360-373.