Pressure Measurements in Perfused Segments of Small Blood Vessels.* (19140)

VICTOR M. EMMEL AND DURWOOD J. SMITH.†

From the Departments of Anatomy and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

A plethysmographic method has recently been described(1,2) for evaluating *in vitro* the responsiveness of blood vessels during perfusion with various agents. The application of this technic to very small vascular segments (5 to 10 mm long and 1 mm diameter, such as obtained from dog mesentery) was not entirely satisfactory because of the difficulty in measuring the small volume changes involved. However, by measuring the rather large pressure differences which even these small specimens can develop, it is possible to extend the perfusion technic to vessels of any size which can be cannulated. The measurement of pressure responses has a further advantage in the fact that the pressure which a vascular segment can develop is independent of its length; whereas the volume change which it can undergo is, of course, directly proportional to its length. Hence, pressure responses provide a more satisfactory basis for quantitative comparisons between various specimens.

Materials and methods. The essential feature of this technic is that the specimen is tied between cannulae and placed within a fluid-filled chamber to which is attached an electrical pressure transducer of very small displacement (Fig. 1). The standard-taper bushing adapters were shortened to correspond to the medium length outer members into which they fit. The cannulae are appropriately sized hypodermic needles with ends squared and grooved. Perfusate flows by gravity at a constant pressure of about 35 mm Hg. An increase of vascular tone under these circumstances can produce no change in pressure within the vessel, but does produce a "negative" pressure in the external chamber. Since no significant volume change can occur, this response represents an essentially isometric contraction of the smooth muscle in the vascular wall. If the diameter of the vessel and thickness of the muscular portion of the wall are known, the muscular tension per unit cross-sectional area can be calculated (3). A Hathaway blood pressure recording system is currently employed with this apparatus, but any pressure transducer of small displacement together with a suitable recording device would be equally satisfactory. Temperature is maintained at 37.5°C. Test substances are injected into the perfusate through a T-tube in the perfusion line. In order to define the treatment to which the

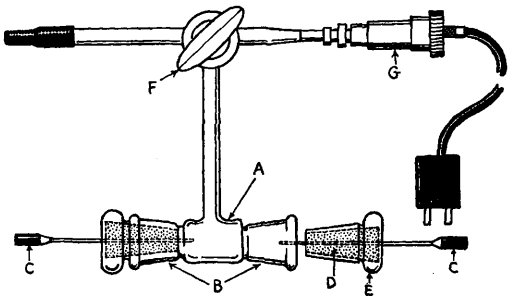

FIG. 1. Diagram of perfusion apparatus. Perfusion chamber (A) is formed by uniting 2 standard taper 14/20 ground glass joints (B). Cannulae (C) are inserted through rubber stoppers (D) placed within standard taper 14/35—10 bushing-type adapters. One cannula should be long enough to extend completely through the perfusion chamber. Silicone lubricant facilitates adjustment of cannulae. The T-stopcock (F) permits the chamber to be filled and placed in communication with an electrical pressure transducer (G).

* An abstract based on part of this work appears in *Anat. Rec.*, 1951, v109, 30.

† This work supported by grants from the Life Insurance Medical Research Fund (V.M.E.) and from the Masonic Foundation for Medical Research and Human Welfare, the Hochstetter Fund, and the National Heart Institute of the National Institutes of Health, Public Health Service, No. H 702 (R) (D.J.S.).

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2. Smith, D. J., and Syverton, J. T., *Circulation*, 1951, in press.

3. Burton, A. C., *Am. J. Physiol.*, 1951, v164, 319.

specimen is subjected it is necessary to know both the intravascular concentration and the time of arrival of the test substance. These depend essentially on the perfusion rate and the dimensions of the perfusion line between the injection site and the tip of the inflow cannula. They can, therefore, be evaluated by injecting a standard volume of a dye (0.05 ml of 1% congo red) and then collecting and colorimetrically analyzing closely spaced samples of perfusate issuing from the inflow cannula. The system can thus be calibrated for various perfusion rates. Vasoactive substances to be tested are so diluted that test doses of 0.05 ml will contain the desired amount of the test substance.

Results and comments. This method can readily be applied to large vessels; but it is particularly useful in extending perfusion studies to vessels of very small caliber. It has thus far been applied to vessels as small as the carotid and renal arteries of the rat and arterial biopsy specimens obtained from the mesentery of the dog. Fig. 2 represents a typical result obtained from a dog's mesenteric artery perfused with Tyrode's solution and stimulated with epinephrine. It is apparent that the maximal intravascular concentration of epinephrine (0.8 γ/ml) has already traversed the vessel before the vasoconstrictor response begins. Increasing the dose above this value (up to 4.0 γ/ml) increases the duration but does not materially increase the height of the response. With some specimens similarly stimulated, maximal pressures approaching 300 mm Hg have been recorded. By using successively smaller doses it was determined that the threshold for response of this specimen was with an injected dose of 0.05 γ (corresponding to a maximal intravascular concentration of 0.04 γ/ml). Returning to Fig. 2 it can be determined that the interval between the onset of contraction and the time at which the concentration curve had reached a value of 0.04 γ/ml is 3.4 seconds. This interval is tentatively interpreted as the "latent period" for chemical stimulation. Using various test doses giving maximal intravascular concentrations of from 0.04 to 4.0 γ/ml the latent period was found in 20 determinations to be 3.7 ± .47 seconds. The factors which influence and contribute to this latent period are currently under investigation and will be considered in a subsequent report. This technic is also being used in a study of biopsy specimens from animals with experimental vascular disease.

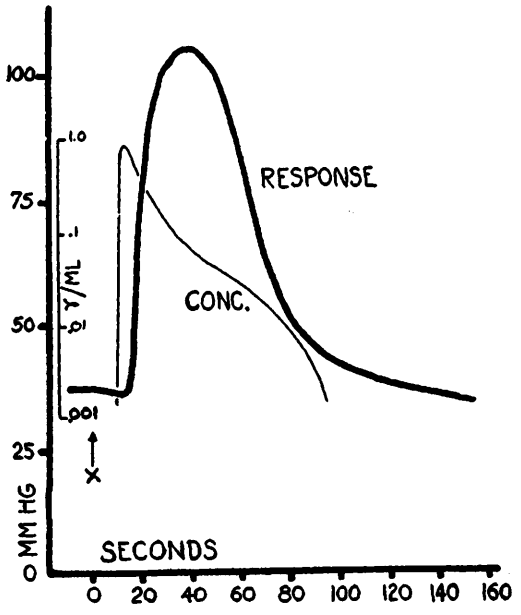

FIG. 2. Response of arterial segment (diameter 1 mm, length 7 mm) from mesentery of a dog. Perfused at a rate of 7.2 ml per minute with oxygenated Tyrode's solution. At X, 1.0 γ epinephrine in 0.05 ml injected into perfusion line. Concentration of epinephrine reaching specimen is shown by light line. Heavy line represents pressure response.

Summary. A method is described for recording *in vitro* pressure responses from perfused segments of small blood vessels. The method permits accurate evaluation of the relationships between dose and response. For arterial biopsy specimens from the dog's mesentery, maximal pressure responses of 200 to 300 mm Hg are obtained with test doses of epinephrine at an intravascular concentration of about 1.0 γ/ml. The threshold for response is about 0.04 γ/ml. The latent period for chemical stimulation of these specimens is approximately 3 to 4 seconds.

Received October 11, 1951. P.S.E.B.M., 1951, v78.