Modification of Rabbit Aortic Strip Technic for Catecholamine (4-point) Assay and Pharmacological Studies.*† (26208)

M. WURZEL, T. PRUSS,‡ W. WEISS AND G. D. MAENGWYN-DAVIES
Dept. of Pharmacology, Georgetown University Schools of Medicine and Dentistry, and Food and Drug Administration, U. S. Dept. of H. E. W., Washington, D. C.

In recent years catecholamine studies have been frequently performed on the isolated rabbit aortic strip, which gives a graded contractile response. It has been pointed out repeatedly that the rabbit aortic strip might serve as a reliable preparation for quantitative determinations(1-4). However, the waiting periods both for relaxation before starting drug applications and between a series of doses are many hours; in addition, this organ loses sensitivity after contraction by a supramaximal chemical stimulus. These factors limit seriously both the number of doses which can be applied to any one strip as well as the constancy of successive responses. The fully relaxed state, though, can quickly be reached by introduction of a small change in technic and consistent responses obtained by application of a dose at intervals as short as 7 minutes. With this modification, the rabbit aortic strip becomes suitable for a variety of uses.

Methods. A strip of rabbit aorta is set up as described by Furchgott and Bhadrakom (1). From a segment of thoracic aorta cut spirally, 4 pieces, 3 mm wide and 3 cm long, are obtained. Preparations, fresh and refrigerated for 24 hours, respond equally well. A strip is suspended in a 30 ml organ bath at 37°C in Krebs bicarbonate Ringer solution of pH 7.4 and aerated with 95%O_2-5% CO_2. The contractions are recorded on a smoked-paper kymograph with a light frontal writing lever at an amplification ratio of 1:20. The tissue is stretched with 2 to 2.5 g weight. For initial relaxation, the aortic strip is repeatedly, gently stretched by pulling down the writing lever. The organ is permitted to adjust to its resting length by tapping the bar to which the lever is attached, and the kymograph is started to mark a segment of baseline. While the drum is stopped, a dose of drug is added and the organ permitted to contract for 3 minutes during continuous tapping. The drum is started again to mark the 3-minute contraction height by a short horizontal line. The tissue is washed with drug-free Ringer solution, and 1 minute later gently stretched several times. The strip returns consistently to its resting baseline. Fresh, aqueous solutions of sympathomimetic amine salts are made up daily and stored at 0°C during use.

Results. 1. Assays of catecholamines. a) A graded dose-response relationship can be obtained easily. In a typical experiment, 5 doses, each twice the amount of the previous one, of l-epinephrine bitartrate 0.2 μg/30 ml bath (0.007 μg/ml), 0.4, 0.8, 1.6 and 3.2 μg cover the range from minimum to maximum response. Such doses, applied regularly at 7-minute intervals, can be repeated on the same strip throughout the day. b) Assay by alternating doses. A dose-response relationship as described under a) is obtained separately with a standard l-epinephrine solution and with the unknown solution to be tested. A dose of the known solution is chosen which will give about 50% of maximum contraction and is alternated with slightly more and slightly less effective doses of the unknown until a close match is obtained. c) 4-point log dose-response, parallel line assay. Having obtained dose-response series of the standard l-norepinephrine bitartrate monohydrate and unknown solutions, as described under b), a low (S_L) and a high dose (S_H) of the standard solution were selected to induce contractions to approximately 30 and 70%, respectively, of the maximum response. Similarly active low

* Aided in part by grant from N.I.N.D.B., U.S.P.H.S.

† The authors wish to express their thanks for the compounds used to Dr. M. L. Tainter, Winthrop-Stearns, Inc., to Dr. F. P. Luduena, Sterling-Winthrop Research Institute, to Dr. H. D. Moed, N. V. Philips-Duphar, and to Dr. I. H. Slater, Eli Lilly and Co.

‡ In partial fulfillment of requirements for Degree of Doctor of Philosophy in Pharmacology from Georgetown University.

TABLE I. Aortic Strip Responses of First Assay.

Dose:	.80 μg (S_L)	3.2 μg (S_H)	1.1 ml (U_L)	4.4 ml (U_H)
Replications		Responses (mm)		
1	9.0	28.0	10.0	25.0
2	11.5	30.0	8.0	26.0
3	7.0	31.0	8.0	26.0
4	11.0	30.0	9.0	24.0

(U_L) and high (U_H) doses of the unknown solution were selected, so that $\frac{S_H}{S_L} = \frac{U_H}{U_L}$.

In the first assay, 4 replications of each of the dose levels, S_L, S_H, U_L, and U_H, were applied to the aortic strip. The order of application of the doses was essentially random. Dose levels and responses of this first assay are given in Table I.

The potency estimate of the unknown, in terms of the standard, is 59.3%. The 95% fiducial limits for the potency are 52.9 and 66.4%, calculated as described by Finney (5). A statistical analysis of variance of the data shows a significant departure from parallelism of the 2 dose-response lines ($p < 0.05$). This non-parallelism is probably due to extension of the high dose of the unknown solution (U_H) beyond the linear portion of the log dose-response curve. Estimate of potency and fiducial limits for this assay should therefore be considered as close approximations rather than as exact estimates.

A second assay was run with a ratio of high to low dose of 3 to 1, rather than 4 to 1, the ratio of the first assay, to increase the likelihood of obtaining responses within the linear portion of the log dose-response curve.

The 4 doses were again replicated 4 times, but in this assay a latin-square design was used to predetermine the order in which the doses were applied to the aortic strip. Any effect on responses due to the order in which each dose was applied would, therefore, be cancelled out. It also became possible to measure an order effect on responses, should it exist. Responses to each of the 4 doses and the latin-square design used in the second assay are shown in Table II.

A statistical analysis of variance of the data shown in Table II, given in Table III, confirms the existence of a real log dose-response parallel line relationship. It demonstrates that each replication gives essentially similar average aortic strip responses and that the order of doses does not show a demonstrable influence on these responses.

TABLE III. Analysis of Variance of Data of Table II.

Source of variation	Degrees of freedom	Mean square	F-ratio
Total variation	15	—	—
Regression	1	1164.14	Sig. at $p < 0.001$
Drugs	1	92.64	" " $p < 0.01$
Parallelism	1	11.68	Not sig.
Replications	3	3.52	" "
Order of doses	3	1.85	" "
Error	6	6.23	—

Fiducial limits were calculated by combining the last 3 sources of variability for the experimental error. Estimate of potency from the second assay is 49.5%, and 95% fiducial limits are 31.4 and 77.4%. d) Assay of l-norepinephrine added to normal urine. l-Norepinephrine bitartrate monohydrate from an aqueous solution as well as from a solution in normal urine is adsorbed on alumina(6,7). It is then eluted and the 2 eluates, tested on the rabbit aortic strip, induce contractions closely agreeing with each other. Normal urine without added norepinephrine is processed and applied in the identical manner; this control solution causes no significant contractions. It is therefore suggested that the rabbit aortic

TABLE II. Aortic Strip Responses of Second Assay.

	Order of administration of doses and responses							
Replications	1	mm	2	mm	3	mm	4	mm
1	S_L	12.0	S_H	31.0	U_L	10.0	U_H	22.0
2	S_H	32.0	U_H	20.0	S_L	10.0	U_L	9.0
3	U_L	8.0	S_L	12.0	U_H	28.0	S_H	29.0
4	U_H	26.0	U_L	7.5	S_H	30.0	S_L	13.0

$S_L = .90$ μg $S_H = 2.70$ μg $U_L = 1.33$ ml $U_H = 4.00$ ml

strip could be used for assaying catecholamines in urine.

2. Pharmacodynamics of sympathomimetic amines can also be conveniently studied.

Dichloronorepinephrine hydrochloride (DCN) (50, 100 and 200 μg/30 ml bath) potentiates contractile responses to norepinephrine. These doses of DCN by themselves do not contract the strip significantly. However, in their presence, norepinephrine (0.027 μg/ml) gives much higher contractions. Control doses of norepinephrine in absence of DCN are given alternately. With increasing doses of DCN, increasing potentiation is observed.

3. Inactivation of sympathomimetic amines can also be demonstrated on the aortic strip. Ten ml of Krebs bicarbonate Ringer solution containing 10 μg/ml of phenylephrine hydrochloride are shaken with a 5 cm long, cleaned, longitudinally cut strip of rabbit intestine at 37°C. Samples (0.32 ml) are removed at 10-minute intervals starting at zero-time. Phenylephrine disappears from the medium within 30 minutes. Rabbit intestine homogenates are also effective. Inactivation of epinephrine and norepinephrine could also be shown using rabbit intestine and other tissues.

Discussion. Reports in the literature(1-4) invariably state that an initial relaxation period of 2 to 3 hours for the rabbit aortic strip is necessary to acquire sufficient sensitivity and a constant baseline. Use of nitrite to shorten the initial waiting period(8-10) did not serve its purpose. Nitrite might even be objectionable because of the unnecessary additional complexity in design of the experiment.

By application of gentle stretching, initial waiting period can be shortened to 5 to 10 minutes instead of the usual 2 to 3 hours; therefore, deterioration of the tissue need not be feared. Relaxation between 2 doses is also easily obtained by stretching; thus, every 7 minutes a new dose can be applied. In this way the rabbit aortic strip can be used for a variety of purposes as conveniently as the frog rectus abdominis muscle.

By the cumulative method of drug applications, concentrations for responses ranging from minimum to maximum extend approximately to 4– (from 10^{-9} to 10^{-5} M), whereas by the non-cumulative method concentrations extend only to slightly more than one-order of magnitude; in other words, the non-cumulative method gives a much steeper regression line. Therefore, we find the rabbit aortic strip appropriate for precise bioassay of sympathomimetic amines, and for study of their pharmacodynamic features. The described technic could, no doubt, be applied to laboratory diagnosis of pheochromocytoma.

Summary. 1. Gentle stretching of the rabbit aortic strip produces rapid relaxation, and single doses can be applied at intervals as short as every 7 minutes. 2. Use of this preparation for a variety of purposes is described, including a 4-point bioassay of catecholamines and pharmacological studies, as exemplified by potentiation and inactivation.

1. Furchgott, R. F., Bhadrakom, S., *J. Pharmacol. Exp. Therap.*, 1953, v108, 129.

2. ———, *Pharmacol. Rev.*, 1955, v7, 183.

3. Bevan, J. A., *J. Pharmacol. Exp. Therap.*, 1960, v129, 417.

4. Williamson, A. W. R., Moore, F. D., *Am. J. Physiol.*, 1960, v198, 1157.

5. Finney, D. J., *Statistical Method in Biological Assay*, Hafner Publishing Co., New York, 1952, Chap. IV.

6. Von Euler, U. S., Orwén, I., *Acta Physiol. Scand.*, 1955, Suppl. 118, 1.

7. Kärki, N. T., *ibid.*, 1956, Suppl. 132, 7.

8. Redleaf, P. D., Tobian, L., *Circ. Res.*, 1958, v6, 185.

9. Yamabayashi, H., Hamilton, W. F., *Am. J. Physiol.*, 1959, v197, 993.

10. Mallov, S., *Circ. Res.*, 1959, v7, 196.

Received September 19, 1960. P.S.E.B.M., 1960, v105.