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Response of Rabbit Aortic Strips Under Pulsed-Tension to Catecholamines.* (30248)

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A currently popular approach to the *in vitro* study of pharmacology of blood vessels utilizes the spiral-cut rabbit aortic strip. This preparation was described in detail by Furchgott(1). Experiments are usually carried out using an isotonic lever arm, weighted to deliver a fixed tension to the muscle, and the contractions are recorded on a kymograph. With this direct approach many observations have been made on the pharmacologic response of vascular smooth muscle to catecholamines and their derivatives(1,2,3), alpha or beta blocking agents and antihypertensive drugs(2,3,4,5,6) and cocaine(6). Other workers have investigated the effects of varying ion concentrations and pH(7,8) and other local anesthetics(9) on the responsiveness of this preparation.

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Although most investigators have carefully controlled temperature, ionic concentration, pH, oxygenation and physical tension, apparently none has subjected the arterial strip to the rhythmical stretching which normally occurs *in vivo*. Stretching the tissue, however, does appear to be of importance as suggested by several authors. Speden(10) pointed out that the sensitivity of the strip to catecholamines is dependent upon the stretched length of the strip. Wurzel *et al*(4,11) found that their preparations returned to baseline more rapidly if the tissue was gently stretched after stimulation with catecholamines. Similar results were obtained by Furchgott *et al*(6) by adding extra weight to the lever. This procedure permitted repetition of determinations within 10-20 minutes after washout of norepinephrine and the restoration of responses to small doses of tyramine was more consistent. Recently, Moreci(12) has shown that rhythmical stretching can effect the metabolic

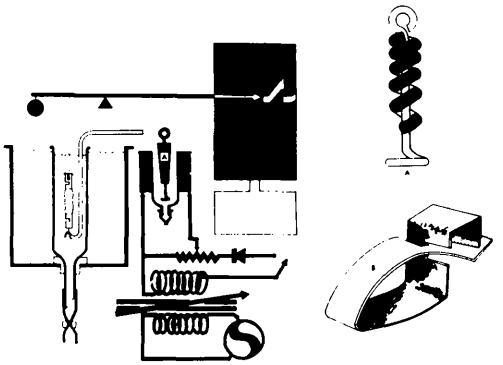

FIG. 1. Schematic drawing of a single unit of the 6-unit apparatus described in the text. Shown here is the muscle chamber, water bath, lever arm assembly, pulsing solenoid and energizing circuitry. Both the solenoid plunger, *A*, and the special muscle clip, *B*, are redrawn in detail to the right.

flux of Na^+ and K^+ in arterial tissue. Furthermore, Daniel(13) has called attention to the fact that stretch alone can depolarize smooth muscle and that there is an important interdependence between spread of electrical activity (depolarization), the contractile mechanism and chemical mediators. Because of this substantial literature, indicating an important relation between stretching and tissue responsiveness, metabolic ion transfer and depolarization, we have modified our apparatus to simulate normal cardiac pulsation by repetitive stretching of the arterial strip, Manion *et al*(14).

Methods. Six 50 ml glass tissue chambers were mounted, 3 at each end, in a semi-rigid plastic dishpan water jacket, in such a way that the drain spouts could be reached from below. Water, maintained at 37.5°C, with a Blue M Model 1120 water bath was supplied to the water jacket by a small, 2 gal/min pump. The water returned to the heating bath from the water jacket through a common sink drain installed in the bottom of the water jacket. An appropriate superstructure was constructed with aluminum rod to support the lever arms, oxygenating tubes and mountings for the electromagnets.

Lever arm assembly. Isotonic recording levers were balanced with counterweights to deliver a net static tension of 4 g to each strip. The stretching mechanism, consisting of a small solenoid electromagnet, was mounted near the pivot on the side opposite the tissue attachment. Specially designed solenoid cores (A, Fig. 1) were made by winding identical lengths of 14 gauge brass wire with 16 gauge soft iron wire. The iron wire was wrapped around the brass core in a differential spiral so that the finished core behaved as if it were a tapered soft iron rod when placed in the field of the solenoid electromagnet. This design was demonstrated to deliver an even pull through a wide range of physical displacement in the electromagnetic field of the solenoid. The brass center of the magnet core extended 2.5 cm below the winding of the solenoid and was fashioned into a small loop which dipped into a pool of mineral oil for damping. The electromagnet was constructed by winding a 1 ml disposable polyethylene syringe barrel with approximately 1000 turns of 32 gauge varnished copper magnet wire. The syringe barrel was sealed at the bottom to retain the mineral oil used for damping. This system provided a repetitive pulsing of the tissue replicating as nearly as possible the normal arterial pulse curve *in vivo* as demonstrated in Fig. 2.

The tissue mounting assembly consisted of stainless steel surgical wire and specially con-

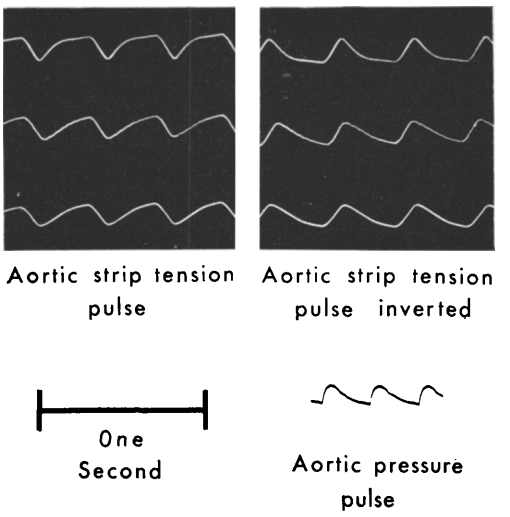

FIG. 2. Aortic strip pulse contours recorded on a fast kymograph. The record on the left of simultaneous tracings from 3 aortic strips has been inverted on the right to demonstrate the similarity between the mechanically induced pulse contour and the naturally occurring aortic pressure pulse.

structed tissue clamps. Short lengths of 26 gauge stainless steel suture wire were fashioned into a chain for flexibility. The clamps were made of spring stainless steel and were a miniaturized adaptation of the common battery clip (B, Fig. 1). These clamps measured 3 mm wide by 5 mm long and delivered the total force to the tissue across the straight line of the jaw. One-half the jaw was serrated with very fine teeth (*ca.* 0.2 mm deep) to hold the tissue securely. The linear force required to pull tissue from a clip was 40 g or more.

Magnetic actuating assembly. A pulsed current to the electromagnets was supplied by introducing a Hamlin DRG-1 magnetic reed switch, (110 amp-turn pull-in and a 36 amp-turn drop-out) in the magnetic power circuit. The magnetic reed switch was activated by 3 carefully selected permanent magnets of near equal strength mounted on the spindle of a 50 RPM induction motor. This provided 150 actuations per minute with fixed, constant on-off time. The voltage to the solenoids was consistently monitored during experimentation with a Simpson volt meter. Arcing of the reed switch from the high inductive load was reduced by placing a 50 peak input voltage, 200 milliampere silicon rectifier in series between the switch and the inductive load. Power to the pulsing circuit was provided by a Variac isolated by a constant voltage power transformer from the line current. Each electromagnet was wired in parallel with the switched and rectified output of the power supply. In addition, the individual electromagnets were wired in series with their own 10-ohm variable resistor to provide fine control of the current through the electromagnet and thereby permitted critical equilibration of the pulsed-tension on all 6 aortic strips. The total increase in tension applied by pulsing was 3.3 g $\pm$ 0.1 g as determined mathematically, and confirmed by experimentation.

Test solutions. The aortic strips in all experiments were kept completely submerged in Krebs-Ringer bicarbonate solution, aerated with 95% O_2 + 5% CO_2. Oxygenation was maintained at a high rate to insure complete saturation of the bath solution and the pH of the solution was recorded before and during use. The pH of the Ringer's was adjusted to 7.3 $\pm$ 0.1. To avoid the introduction of an antioxidant all test solutions of epinephrine and norepinephrine were prepared in fresh Krebs-Ringer bicarbonate solution which had been cooled to 2°C and washed for 20 minutes with N_2 to remove as much O_2 as possible prior to dilution. During use these solutions were kept refrigerated and sealed. During an 8- to 14-hour experimental period the activity of the solutions remained stable.

Preparation of aortic strips. The aortic strips were prepared according to the method of Furchgott(1). Precautions were taken during the cutting of the strips to ensure that the temperature remained at 37°C $\pm$.5°C. All strips, as they were cut, were immediately connected to their own coded tissue clamps and the place of origination (particular level of the aorta) of each strip was recorded. The strips were then mounted in their respective chambers and pulsed for 30-60 minutes until a level baseline was established. A uniform priming dose of norepinephrine (.025 μg) was then added to each muscle chamber and the contractions were recorded. The chambers were then washed and a dose response curve was determined for each strip.

At the end of each experiment the length of the relaxed strip (4 g static tension) was measured and a correction factor was calculated to bring each series of responses to a standard strip length of 2 cm.

Results. General effects of stretch. The effects of stretch were examined in 48 aortic strips from 6 rabbits at a pulsing frequency of 150 per minute and a 0.6 mm stretch per 2 cm strip. Responses to norepinephrine were uniform in shape and rapid in onset. In this experiment cross comparison was made on aortic strips from one rabbit by first pulsing half the strips while running a dose response curve and then reversing the procedure.

Since the limits of the pointer excursion represented a function of the elastic properties of the strip, we measured the width of the excursion in experiments with 132 aortic strips from 22 different rabbits. From these experiments it appears that norepineph-

rine, epinephrine and angiotensin do not have a measurable effect on the elasticity of the rabbit aortic strip during, or after a stimulation as indicated by the constant character of the elastic response under pulsed-tension.

Specific effects upon the dose-response curve. During our early experiments it became evident that nearly every set of aortic strips, cut from a single artery, produced one or 2 strips of low reactivity to epinephrine and/or norepinephrine. Subsequently the order in which the aortic strips were cut from the aorta was recorded and the reactivities were compared. In 9 experiments on 54 aortic strips there was an obvious difference in the reactivities which were shown to be unrelated to mechanical or experimental errors (*i.e.*, no one bath gave consistently high or low readings). It was also observed that strips cut nearest the arch of the aorta were more reactive than the strips cut near the origin of the superior mesenteric artery. In 8 additional experiments on 48 aortic strips the difference in this reactivity was carefully recorded by establishing 7 point dose-response curves for each strip. From these data it was demonstrated that distal segments of aorta near the origin of the superior mesenteric artery were ¼-½ as sensitive as strips derived from the same aorta nearer the arch (Fig. 4).

The main effect of repetitive stretching was a quick, consistent recovery from contraction and return to baseline which permitted more determinations with each preparation and, in our hands, yielded more consistently reproducible results. Our minimal detectable threshold generally fell at, or below, the low end of the range (3-30 $\times$ 10^{-10}) described by Furchgott(1), but some animals were fairly resistant to norepinephrine (Fig. 3).

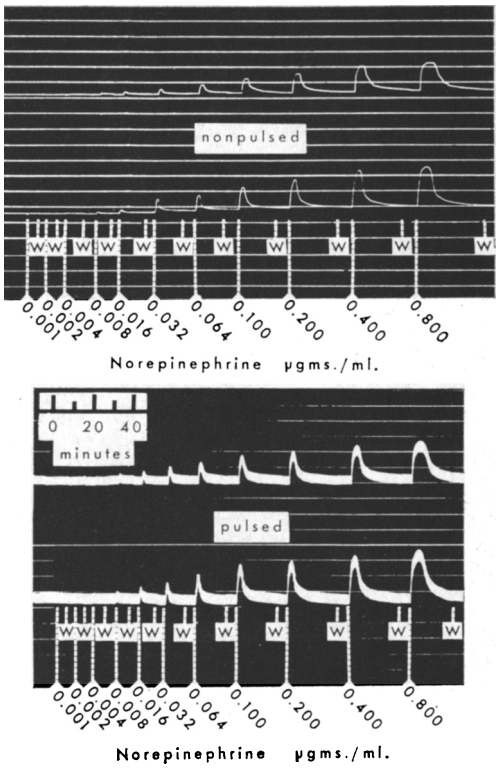

FIG. 3. Kymograph records of 2 aortic strips recorded simultaneously. In the upper tracing responses to graded doses of norepinephrine were obtained without pulsation. The same strips were then pulsed and again tested with norepinephrine. Note the more rapid and complete return of the pulsed tissue to baseline after washing (W), compared to the response of the non-pulsed aorta.

Discussion. In vivo, the aorta is normally stretched by the cardiac output. In the rabbit this occurs 125-300 times per minute. However, to our knowledge none of the previous experiments on aortic strips has provided any means of simulating this action. It is significant that Furchgott *et al*(6) and Wurzel *et al*(4,11) report that their preparations can be made to return to the base-line with greater speed following a contraction with catecholamine, by stretching the tissue. Furchgott and coworkers accomplished this by adding additional weight to the lever arm, while Wurzel and his colleagues obtained similar results by pulling on the tissue. It is interesting to note the observation by Furchgott and associates that the stretching procedure would allow experimentation to be recommenced within 10-20 minutes after wash-out of the norepinephrine. When this procedure was used the restoration of the responses to small doses of tyramine was reported to be more consistent than when it was not employed.

Stretching the tissue may be of some importance as suggested by the work of Speden(10) and Daniel(13). According to Speden the sensitivity of the strip to catecholamines is dependent upon the stretched

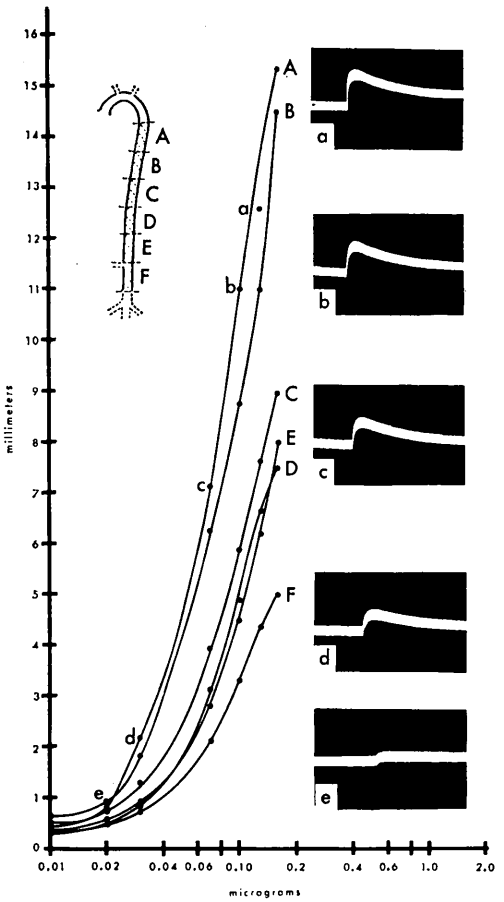

FIG. 4. Dose-response curves from 6 arterial strips cut from a single aorta. See insert for segment orientation. Capital (upper case) letters indicate response curves obtained from a particular segment. The kymograph tracings shown on the right labeled with small (lower case) letters show the individual responses of strips A to increased concentrations of norepinephrine.

length of the strip prior to the measurement of elasticity. This work is one of the first relating vascular elasticity and pharmacology. Others, including Zatzman *et al*(15), Hinke and Wilson(16), and Burton(17) have been more concerned with the elastic properties of arterial smooth muscle and have limited their attention largely to description and interpretation of the modulus of elasticity of aortic tissue. Zatzman and coworkers point out that investigation of the modulus of elasticity is characterized by two general approaches: *i.e.*, measurement of tension and relaxation in the given material with a single stretch or measurement of fatigue during repetitive stretching. Hinke and Wilson have measured fatigue following a continued stretch. However, their experiments with small (550 μ) vessels did not yield information comparable to ours because stretching of the vessel was derived from constant pressure of perfusate.

In our modification of the technique it is important that the iron core which delivers the force to the lever arm be constructed to deliver equal pull on the lever arm over a wide range in the position of the core in the solenoid. This difficulty is easily overcome by using a "tapered" core. The duration of current flow through the electromagnet is equally critical and demands careful attention. Actuation by a dry reed switch was found to be one reliable mechanical means for controlling current flow at 150 cycles per minute.

The differential sensitivity of the various portions of the aorta has been repeatedly tested to determine whether it might be the result of error introduced by using the static resting lengths of the strips in our calculation. The difficulty lies in establishing a true resting length. Ideally, one should accurately measure the length of the strip in the bath as it relaxes from the mechanical stretching being applied to it at a rate of 2.5 beats per second. Although this may be done with planned modifications of the apparatus, in the experiments described here we were able to measure only the stretched length under 4 g static tension at the end of each experiment. With these static readings we corrected for varying strip lengths to make interstrip comparisons meaningful.

In connection with our observations on differential sensitivity it seems significant to note that different parts of the aorta stretch with pulsation in different ways. When all strips are carefully cut to the same length (2 cm $\pm$ 2 mm), strips taken from the lower thoracic aorta, close to the superior mesenteric artery, were observed to stretch more with pulsation than their counterparts taken from near the arch.

If the observations of Speden(10), that increased stretching increases the response of arterial muscle to norepinephrine, apply here,

one would expect that the strips which were more easily stretched, and hence longer, would be more sensitive to norepinephrine. However, we were unable to confirm this in these experiments. Ideally each strip should receive a stretch that is exactly proportional to its length but with our apparatus this was not possible because we could not establish a resting length until the experiment had been completed. It may be, therefore, that the discrepancy between our findings and those of Speden are due, at least in part, to differences in technique. With further modification of the apparatus, to facilitate measurement of length changes during an experiment, we hope to find such a correlation.

Summary. A technique for studying the pharmacology of vascular smooth muscle has been described. This technique utilizes pulsed tension rather than the static tension customarily employed and has permitted the following observations: Contraction responses to the catecholamines have shown accelerated onset and recovery with increased uniformity of response. A differential sensitivity to norepinephrine has been shown to exist in segments taken from different parts of the same aorta. A progressive reduction in sensitivity to norepinephrine was observed from the arch to the origin of the superior mesenteric artery.

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Biological and Chromatographic Evidence for the Storage of Ethynylestradiol-3-Cyclopentyl Ether in Rat Brain. (30249)

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Unlike ethynylestradiol,* the oral administration of its 3-cyclopentyl ether is followed by storage of a considerable amount of estrogenic material in the body fat of the rat(1). The uterine growth-stimulating activity of orally administered estrogenic material stored

* The following trivial names and abbreviations are used in this paper: ethynylestradiol (EE) = 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17 β-diol; ethynylestradiol-3-cyclopentyl ether (EECPE) = 3-cyclopentyloxy-19-nor-17 α-pregna-1,3,5(10) -trien-20-yn-17 β-ol.