In Vitro Studies of the Coronary Arteries of Man and Swine as Demonstrated by a New Technic, Angioplethysmokymography

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A new in vitro technic for the study of isolated surviving arteries and veins is described. Observations of the reactivity of 21 human and 68 swine coronary arteries to histamine, acetylcholine, l-epinephrine and l-norepinephrine are reported. Histamine and acetylcholine uniformly caused vasoconstriction of the coronary arteries of man and swine. Three potential mechanisms of coronary artery vasoconstriction in these species are suggested. The blocking action of antihistaminic drugs on vasoconstriction due to histamine, and of atropine on vasoconstriction due to acetylcholine is demonstrated. It is suggested that combined therapy with these drugs may be useful in treatment of diseases of the coronary arteries.

Early in the course of an experimental approach to the study of the vascular system in rheumatic fever and allied hypersensitive states, the need became apparent for a method whereby segments of blood vessels might be assessed for their immediate reactivity to various stimuli. It was required that such vessels remain intact as to their constituent layers and at the same time be uninfluenced by extravascular stimuli.

Hitherto information relating to the responses of blood vessels has been accumulated from the application of a variety of technics1-27 which, for the most part, fall into four categories: (1) measurements by some variation of the Schultz-Dale1-3 technic of the reactivity in vitro of strips of arterial tissue2-3, 8-8; (2) organ perfusion technics8-12; (3) microscopic observations in vivo13-16; (4) histologic analyses.17-27 Each of these technical approaches has yielded definite, but limited, information.

The arterial strip technics have been most commonly employed. Since Meyer in 19062 utilized arterial strips suspended in an oxygenated bath for assessing by kymographic recording the effect of epinephrine on isolated arterial tissue, and Schultz in 19073 recorded his observations of spasmodic arterial contracture in sensitized animals, arterial tissues have been tested for contractility. Generally the Dale apparatus4 has been used, employing longitudinal strips of arterial tissue,1, 2, 3 narrow cross sections of arteries united in chain formation by sutures2, 5, 6 and long spiral strips of arterial wall.7, 8 Each variation in this technical approach is useful for the direct testing of the smooth muscle of the arterial wall to pharmacologic and antigenic stimuli. None yields, however, information on what happens when the agent is contained within intact vascular endothelium, because through incision of the vascular wall the agent under test is brought into direct contact with all three vessel coats.

The second technical approach, perfusion of organs, is also a commonly employed physiologic method for evaluating the effects of substances on the vascular system.9-12 By these perfusion technics such indirect evidence as
variation in volume of the whole organ, variations in pressure required to start a flow through an organ, and variations in the rate of flow through that organ is adduced as indicative of alteration in the caliber of the vessels. Such methods, however, reflect not only changes in arterial caliber, but also the response of the capillaries, the veins and, indeed, of the entire organ. The errors inherent in the organ perfusion technic for the study of arterial reactivity were early recognized.

Microscopic observations of tissues and organs in vivo, using a variety of methods, is the third commonly used experimental method. These observations may be made by study of the skin or fingernail; by micromanipulation methods; or by the mount-chamber technic of Abell and Clark. Each of these methods has yielded valuable information relative to the reactivity of the capillary bed, arterioles and venules, yet none can give us decisive information relative to the reaction of the arteries and veins per se.

The fourth experimental approach, histologic analysis, has recently been given new impetus for its application as a measuring stick of the changes that occur in vessels. This approach, by making permanent alternative changes which must be interpreted in retrospect, is limited in its application.

No one of these experimental methods fulfilled the dual requirements considered prerequisite to the study contemplated, namely, (1) that the vessels remain intact as to their constituent layers, and (2) that they must be uninfluenced by extravascular stimuli.

This paper is intended to describe in detail the construction and operation of the apparatus finally adopted and to report the results obtained by its use when such stimuli as histamine, acetylcholine, l-epinephrine, and l-nor-epinephrine, were applied to the isolated surviving coronary arteries of man and swine.

**APPARATUS**

The apparatus was designed to record changes in the volume of vascular segments per unit of time; hence its name, angioplethysmokymograph (APKG).

The apparatus was constructed as a unit of four sections: (1) a plethysmographic chamber, in which a section of artery or vein is mounted to demonstrate but not to record changes in vascular volume; (2) a system which makes possible perfusion of the vascular segment with various solutions under constant conditions of pressure, oxygenation and rate of flow; (3) a temperature control system for maintaining, at a constant temperature, the vessel, the plethysmographic chamber and the perfusates; (4) a recording device, which registers photographically the changes in volume of the arterial segment per unit of time.

The plethysmographic chamber (A, fig. 1) was constructed from three standard pieces of glassware; a 0.2 ml. pipet graduated in 0.001 ml.; a T-shaped, 2 mm.-bore, three-way stopcock; and a T-shaped specimen chamber with its open ends flared to accommodate rubber stoppers (size 00). The horizontal arm of the T tube constituted the specimen chamber. The perpendicular arm of the T tube was attached to one arm of the three-way stopcock and a 0.2 ml. pipet was fused onto the opposite arm. The pipet was bent parallel to the specimen chamber to obviate any variation of the external pressure (about 20 mm. Hg) on the vessel segment as the column of fluid in the pipet varied in response to changes in vascular volume. Spinal and long intramuscular needles ranging in size from 13 to 19 gage and grooved near the ends, were employed as cannulas on which to mount the vessel segments. The points were filed from the larger needles, whereas the bevel on smaller needles was

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**Fig. 1.** A line drawing of the perfusion system to show the component parts. A. Plethysmograph showing an artery mounted in the specimen chamber at the bottom. It is filled with Tyrode's solution as indicated by the crosshatching. B. Aspirator bottle (4 liter capacity). C. Warming coil. D. Manifold. E. Modified T tube for injecting test doses into perfusate. F. Outlet line.
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partially retained to permit more ready insertion into small arteries.

The perfusion system (fig. 1) consisted of three aspirator bottles (B, fig. 1), each of four liter capacity, which were elevated above the specimen chamber to provide for the arterial segment an inflow pressure of approximately 80 mm. Hg. The volume of flow was controlled by the diameter of the cannulas and by a needle in the outflow line (F, fig. 1). A special manifold with three three-way stopcocks (D, fig. 1) made possible the use of different test solutions. The insertion into the inflow line of a small T tube with one arm cut short and covered with a rubber diaphragm (E, fig. 1) made it possible to inject small amounts of test solutions directly into the perfusate. The inflow and outflow lines were connected to the cannulas by needle adapters. The distal end of the outflow line was connected to an inverted Y tube elevated above the specimen chamber at a height equivalent to 30 mm. Hg to simulate the “capillary” end of the artery and to break the siphon effect which would otherwise exist.

A warming coil (C, fig. 1) was interposed in each of the lines running from the perfusion-fluid bottles to the manifold. These coils were kept close to the bottom of the bath and equidistant from the heating element to ensure a constant and uniform temperature. The rubber tube leading from the manifold to the input cannula was kept short, so as more accurately to relate the response to successive test solutions by minimizing the time-lag between a change of perfusate at the manifold and its first contact with the endothelium of the vessel under test. Each bottle of perfusate was kept oxygenated by a continuous flow of 5 per cent carbon dioxide in oxygen. The temperature control system consisted of a constant temperature bath (37.5 C. ± 0.01 C.).

Changes in arterial volume, measured in cu. mm., can be readily recorded by direct observation, but this method proved tedious. Accordingly, a more satisfactory and efficient technic for recording changes in vascular volume was achieved by incorporating a photographic unit in the system, as depicted in figure 2. This was done by recording at 15 second intervals on photographic paper* mounted in a slowly revolving drum camera the image of the column of fluid in the pipet. The curve of the pipet served as the “lens” of the optical system. This lens was focussed on the photographic paper and an exposure of about 0.8 second was effected by means of a slit in a disc which revolved in the light beam. This amount of exposure sufficed for recording the image and the 0.001 ml. graduations. Thus, the method, by recording the changes in volume per unit of time and the graduations of the standard pipet, provided at all times for accurate calibration of the apparatus. By using a drum camera which revolved once in two and one-half hours, the results of experiments were recorded as a continuous experience. The development of a recording system which would employ a photoelectric cell and a recording galvanometer was considered, but it was not pursued because of the inescapable disadvantage of the constant need for calibration of the recording system.

Procedure

1. Glassware and Perfusate. The perfusate, glassware and equipment were made ready by applying the precautions utilized in tissue culture. A variety of physiologic solutions were found acceptable for use. However, Tyrode’s solution* proved the most satisfactory.

2. Pharmaceutical Preparations. (a) Histamine diphosphate. A 1:1000 solution was supplied in 1 ml. ampules. Doses are expressed as the salt. (b) Acetylcholine chloride. Ampules contained 100.0 mg. Dissolved in distilled water for each experiment. (c) L-Epinephrine, synthetic. A 1:1000 solution (as bitartrate) was supplied in 1 ml. ampules. Doses are expressed as base. (d) L-Norepinephrine, synthetic. A 1:1000 solution (as bitartrate) was supplied in 4 ml. ampules. Doses are expressed as base. (e) Atropine sulfate. U.S.P., 0.5 mg. tabs. (f) Antihistaminic drugs. Of the many in use today only two were used: diphenhydramine hydrochloride N.R. (Benadryl) and tripeleunamine hydrochloride N.R. (Pyribenazine). These were employed in the concentrations indicated. No comparative studies were made.

3. Preparation of Arteries for Testing. Coronary arteries were obtained from healthy swine within 20 minutes of death and from young human adults within three hours of a noncardiovascular death.†


† All human tissues studied were supplied by the Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, New York. Their cooperation in this study is hereby acknowledged.
These arteries were dissected from the surrounding tissue and each branch was ligated with silk thread. The segment for study was excised and transferred to a beaker of oxygenated Tyrode's solution at 37.5°C. The size of the cannula, already mounted in a no. 00 stopper, was determined by the diameter of the artery for study. The point of the cannula was passed through the specimen chamber to extend 1 cm. beyond the opposite opening, and thereupon it was inserted into the lumen of the arterial segment. The arterial segment in turn was fixed securely to the cannula by a silk ligature, and a second cannula was inserted and similarly attached by a ligature to the opposite end of the artery. The integrity of the arterial segment was tested by passing fluid through the artery from one cannula to the other. When perfusate was not observed to escape from the wall of the vessel, the cannulas and the artery were realigned within the chamber. Per fusate again was introduced to make certain that fluid did not escape due to the further manipulation. The plethysmograph was then filled with Tyrode's solution (as illustrated by the cross-hatching in fig. 1) by loosening one rubber stopper and tipping the chamber so that the air was displaced by fluid. With the stopper again firmly fixed in the mouth of the chamber, gentle suction applied to the sidearm of the plethysmograph by a syringe revealed any leaks, as shown by small air bubbles forming at the point of leakage. Care was taken throughout these operations to avoid traumatizing the arterial wall and to eliminate any air bubbles from within the plethysmographic chamber. The arterial length was adjusted to correspond with its original length, and the meniscus of the fluid in the pipet was adjusted toward the lower (proximal) end of the pipet. With these manipulations successfully completed, the stopcock was turned to connect the specimen chamber shown in figure 1.

**Fig. 2.** A line drawing of the recording apparatus showing the light and timing unit on the left and the drum camera on the right. The light and timing unit consists of a 6 volt single filament auto headlight bulb (G-E $1134)(A)$ mounted in a light-tight box. A disk $(B)$ revolving once in 15 seconds and driven by a synchronous clock motor $(C)$ flashes the light onto the drum camera by means of a slit. The drum camera consists of a standard kymograph drum $(D)$ mounted in a light-tight box. A gear drive $(E)$ revolves the drum. The plethysmograph is mounted on the face of the camera as shown above, with the specimen chamber submerged in the constant temperature bath to the depth shown in figure 1.
TABLE 1. Reactions of Swine Coronary Arteries to Histamine

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Reaction Produced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constriction</td>
<td>None</td>
</tr>
<tr>
<td>Histamine alone........</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td>Histamine + antihistaminic</td>
<td>0</td>
<td>40</td>
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</table>

TABLE 2. Reactions of Human Coronary Arteries to Histamine

<table>
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<th>Experimental Conditions</th>
<th>Reaction Produced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constriction</td>
<td>None</td>
</tr>
<tr>
<td>Histamine alone........</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Histamine + antihistaminic</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

1. The vessels were assessed as to their reaction to histamine in concentrations of $1 \times 10^{-8}$ to $1 \times 10^{-6}$. The results of these studies are tabulated in table 1 (swine) and table 2 (human). Histamine caused consistent vasoconstriction in the vessels examined. Having first established the uniformity of response to histamine, the blocking action of typical antihistaminic drugs was tested. In a concentration of 1 part per million, the antihistaminic drugs employed completely blocked (after three minutes exposure) the vasoconstrictive action of histamine. These studies are illustrated by experiment 754 (fig. 3).

2. The response of the vessels to acetylcholine in concentrations of $1 \times 10^{-8}$ to $1 \times 10^{-6}$ was recorded. These observations are tabulated in table 3 (swine) and table 4 (human). Acetylcholine, in the concentrations employed, uniformly caused vasoconstriction of the coronary arteries of both species. The effect of atropine (1 part in 10 million) was tested. In all instances, after three minutes exposure of the vessel to this concentration of atropine the vasoconstriction to acetylcholine was eliminated. Atropine caused no significant diminution in the vasoconstriction produced by histamine. These studies may be illustrated by experiment 727 (fig. 4).

3. The reactions of the coronary arteries to L-epinephrine were recorded. In nearly
TABLE 3. Reactions of Swine Coronary Arteries to Acetylcholine

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Reaction Produced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constriction</td>
<td>None</td>
</tr>
<tr>
<td>Acetylcholine alone.......</td>
<td>97</td>
<td>0</td>
</tr>
<tr>
<td>Acetylcholine + atropine..</td>
<td>0</td>
<td>22</td>
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</tbody>
</table>

TABLE 4. Reactions of Human Coronary Arteries to Acetylcholine

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Reaction Produced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constriction</td>
<td>None</td>
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<tr>
<td>Acetylcholine alone.......</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Acetylcholine + atropine..</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Fully dilated vessels the drug caused a slight and transient vasoconstriction; whereas in those arteries which had during perfusion assumed a certain degree of tone, vasodilation was uniformly produced. The action of l-norepinephrine was studied in 11 swine coronary vessels which had assumed a certain degree of tone and in all instances vasodilation was recorded. Experiment 728 (fig. 5) illustrates the response of a coronary artery to l-epinephrine and l-norepinephrine.

DISCUSSION

Previously used experimental methods for the study of vascular reactivity have been re-
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viewed. A new* in vitro technic for the study of isolated surviving arteries and veins has been described, employing an angioflethysmokymograph. This method enables the investigator to assess the reactivity of blood vessels to stimuli received through the intact endothelium, while being uninfluenced by extravascular stimuli. Furthermore it eliminates the reactivity of the endothelium of the capillary bed which may well have differing contractile properties.31

Histamine consistently caused vasoconstriction of the coronary arteries of man and swine studied. These observations are in agreement with the results reported by Barbour32 (coronary rings of ox), Cruickshank and Subba Rau33 (coronary rings of man, dog, and ox), and Anrep34 (coronary rings of ox and dog). Acetylcholine uniformly produced vasoconstriction of the coronary arteries examined. Previous studies of the effect of acetylcholine on the isolated surviving coronary arteries of man and swine have not been found in a search of the literature, although they have probably been accomplished.

In those arteries, which had during perfusion assumed a certain degree of tone, l-epinephrine and l-norepinephrine uniformly produced vasodilation. These results are in agreement with those reported by Barbour32 (coronary rings of ox), and Cruickshank and Subba Rau33 (coronary rings of dog and ox). The correlation between the type of reaction produced by l-epinephrine and degree of vasodilation of the specimen artery is discussed in detail in a previous paper.35

Our results present further evidence to support the view that (in man and swine) the adrenergic nerves are the vasodilator fibers of the coronary arteries, while the cholinergic nerves are the vasoconstrictor fibers. Furthermore the histaminergic nerves† in man and

swine are probably also vasoconstrictor in action. It seems reasonable to postulate that if coronary artery vasoconstriction does occur in man and swine, there are at least three potential mechanisms: (1) vasoconstriction mediated by cholinergic innervation, or (2) vasoconstriction by histaminergic innervation, both mechanisms probably of reflex origin, (3) or direct action of increased blood histamine‡ on the coronary arteries.

The results of our experiments indicate that atropine (1 part per 10 million) blocks completely coronary artery vasoconstriction due to acetylcholine, and that representative antihistaminic drugs block completely coronary artery vasoconstriction due to histamine and in addition diminish the vasoconstriction caused by acetylcholine.

Realizing the difficulties encountered in translating experimental results to clinical problems, we feel it pertinent to suggest that combined therapy with these drugs may be useful in treatment of disease states associated with evidence of reflex coronary artery vasoconstriction or with a significant increase in the blood histamine level.

**SUMMARY**

1. A new technical approach for studying isolated surviving blood vessels with intact constituent layers and independent of extraneous extravascular influences is described.

2. The reactions of 21 human and 68 swine coronary arteries have been studied in vitro by this method with the following results: (a) Acetylcholine uniformly caused vasoconstriction in the concentrations employed. (b) Histamine consistently caused vasoconstriction in the concentrations employed. (c) l-Epinephrine caused vasodilation in the arteries which had assumed a certain degree of tone

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* An apparatus similar in some respects to this one was described by Gorrill,30 but no experimental results were presented.

† The experimental evidence recently presented by von Euler34 has reemphasized the possible existence of a histaminergic section of the autonomic nervous system associated anatomically with the postganglionic sympathetic fibers and activating peripheral structures by the release of histamine. Whether vasoconstriction of these arteries to histamine is due to innervation by histaminergic nerves or is due to a direct effect of the histamine on these smooth muscle cells is so far undetermined.

‡ It is of interest to note that the blood histamine level is raised during certain types of stress. This has been demonstrated in anaphylaxis,37 38 anoxia (cat39 and dog40) and pulmonary embolism.41
but in the fully dilated specimens produced an insignificant degree of vasoconstriction. (d) Norepinephrine uniformly caused vasodilation of the vessels examined (swine).

3. Atropine (1 part per 10 million) blocked completely coronary artery vasoconstriction due to acetylcholine. Representative antihistaminic drugs (1 part per million) blocked completely coronary artery vasoconstriction due to histamine.

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