EFFECTS OF ERGOMETRINE (ERGONOVINE) ON THE ISOLATED ATHEROSCLEROTIC HEART OF THE CHOLESTEROL-FED RABBIT

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Ergometrine usually depresses the S-T segment as in coronary insufficiency, when injected intravenously in rabbits with experimental coronary atherosclerosis and in patients with effort angina, but not in normal animals and man. To explain this difference, we carried out Langendorff perfusion studies in 32 normal and 29 atherosclerotic isolated rabbit hearts. Preliminary tests with ergometrine were done to ensure that advanced coronary atherosclerosis had developed in the rabbits fed a cholesterol diet; pathological examination of the heart after perfusion confirmed the result of the final test with ergometrine. Before drugs were perfused, the basal rate of coronary flow was greater, the heart rate was slower and the contractile amplitude was smaller in the atherosclerotic than in the normal hearts; nitroglycerin markedly increased flow in both normal and atherosclerotic groups. Ergometrine consistently caused a reduction in contractile amplitude with negligible changes in heart rate in both normal and atherosclerotic hearts. On coronary flow, however, the effects of ergometrine differed significantly in these groups; in doses of between 0.2 and 0.4 mg., the average decrease in flow was 8% in normal and 22% in atherosclerotic hearts. The effect was more variable in normal hearts and an increase in flow sometimes occurred. The difference in the response of normal and atherosclerotic hearts was particularly striking when ergometrine was given during recovery from a reduction of coronary flow which had been induced by vasopressin. Ergometrine then uniformly increased flow in the normal, but usually had the opposite effect in the atherosclerotic heart. In normal and atherosclerotic hearts, cardiac effects of vasopressin were similar. Tachyphylaxis to vasopressin, but not to ergometrine, was observed.

Ergometrine injected intravenously produces transient S-T segment depression characteristic of coronary insufficiency in patients with coronary artery disease (Stein, 1949; Stein and Weinstein, 1950, 1953; Rinzler, Stein, Bakst, Weinstein, Gittler, and Travell, 1954), and in rabbits with experimental coronary atherosclerosis (Rinzler, Travell and Karp, 1955; Rinzler, Travell, Karp, and Charleson, 1956). Such electrocardiographic changes after ergometrine are not seen in man or animal with a normal coronary circulation.

Although effects of ergometrine on heart rate and amplitude had been investigated in the normal frog (Davis, Adair, Chen, and Swanson, 1935),

rabbit (Brown and Dale, 1935) and cat (Rothlin, 1935), we found no reports concerning the actions of this drug directly on the coronary circulation. We therefore investigated the mechanism of the electrocardiographic effects of ergometrine by perfusion of the isolated heart in normal rabbits and in those rendered atherosclerotic by cholesterol feeding.

It was found that the atherosclerotic isolated heart can be satisfactorily perfused and serves like the normal heart for studying drug effects on coronary flow, heart rate, and amplitude of contraction. Furthermore, satisfactory pathological detail of the coronary arteries is obtained on section of the heart after perfusion. Preliminary reports (Karp, Penna, Rinzler, and Travell, 1956; Karp, Travell, and Rinzler, 1956; Travell, Karp, and Rinzler, 1957) have been made of what we believe are the first perfusion studies of the isolated atherosclerotic heart. The present

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report deals with the methods, the state of the isolated atherosclerotic heart, and the effects of ergometrine on this preparation.

METHODS

Animal Care.—Male Dutch rabbits (*Loryctolagus Cuniculus Domesticus*) were housed in single cages in an air-conditioned room. They were placed first on a stock diet for 10 days, and if conjunctivitis, coryza, or diarrhoea developed, they were given sulphamethylthiazole (Sulmet-Lederle) intraperitoneally every other day for 3 doses of 200 mg. each. Two groups of rabbits were then started at the same time either on the stock diet or on a similar diet with added cholesterol. The diets were fed ad libitum.

Stock and cholesterol diets were commercially prepared (Vi-D-Co). The stock diet comprised: rolled oats 7.5 g.; ground corn 27.6 g.; dried skim milk 7.5 g.; grey shorts 7.5 g.; 17% dehydrated alfalfa 37.2 g.; bone meal 2.0 g.; calcium carbonate 1.5 g.; mineral salt 1.0 g. Either 2% cholesterol with 6% corn oil, or 2% cholesterol alone, was added to the stock diet. When the rabbits nibbled pellets prepared with corn oil, these crumbled into a powder which could not be ingested. A firmer pellet was made by coating the stock pellets with crystalline cholesterol dissolved in ether.

Rabbits may contract coccidiosis if the drinking water becomes contaminated with faecal matter. Water was supplied from hanging bottles with metal drinking spouts. Bottles, feed containers, and cages were sterilized by steam once a week. No insecticide sprays were used. Removable pans beneath the wire mesh floor were washed with a disinfectant solution of substituted dimethylbenzyl ammonium chloride (Hytron).

Ergometrine Test.—Serial ergometrine tests on cholesterol-fed and control animals were done after 6 to 8 weeks on the diets and thereafter usually at weekly intervals until the test became positive. The general procedure has been described (Rinzler *et al.*, 1955, 1956). Rabbits were anaesthetized with pentobarbitone sodium (Nembutal Sodium Veterinary Abbott) 15 mg./kg. intravenously, or 10 mg./kg. in some atherosclerotic rabbits. The corrosive action of this alkaline solution caused sclerosis of ear veins and created technical difficulties on repeated testing until we reduced the pH slightly by adding dilute hydrochloric acid just before injection and also diluted the dose with an equal part of 0.85% sodium chloride.

The general condition of the cholesterol-fed rabbits was usually good, although they were sluggish in behaviour and tended to lose weight gradually after the initial period of a few weeks on the diet (Rinzler *et al.*, 1956). Thus, when placed on the diet, the average weight of the 29 cholesterol-fed animals was 1.87 kg. (1.47 to 2.29 kg.) and 1.67 kg. (1.07 to 2.73 kg.) when sacrificed for coronary perfusion. In contrast, the average weight of the 32

control rabbits initially was 1.76 kg. (1.27 to 2.47 kg.), and 1.93 kg. (1.19 to 2.63 kg.) when the experiment was terminated.

Control electrocardiograms were taken before, and 5 and 10 min. after, pentobarbitone injection. Lead II and a precordial lead approximating to V_4 were taken. Ergometrine maleate 0.05 mg./kg. was injected intravenously, and leads II and V_4 were repeated after 1, 3, 5, 7 and 10 min. or longer, depending on the duration of the effect. Depression of the S-T segment, if it occurred, was usually most marked 3 to 5 min. after injection, and as a rule lasted 15 to 30 min. but in some instances 1 to 2 hr. The ergometrine test is positive if the S-T segment is depressed transiently by 0.5 mm. or more below the isoelectric level.

One or two days after the ergometrine test became positive, the atherosclerotic heart was isolated and the coronary circulation perfused. In control rabbits which were fed on the stock diet for approximately equal periods of time, the heart was similarly isolated and perfused.

Perfusion.—A modification of the basic Langendorff perfusion apparatus (Anderson type) was used; the modified apparatus has been described by Garb, Penna, and Scribaine (1955), and is shown in Fig. 1. For the operation, rabbits were anaesthetized with pentobarbitone sodium 30 mg./kg. intraperitoneally. We followed Garb's surgical technique for isolating the heart, with minor modifications. In the atherosclerotic rabbit, care was needed not to perforate the often rigid and fragile aorta when the cannula was inserted. The heart was suspended in air by the cannula and amplitude of contraction was recorded from the apex of the heart. Perfusion with oxygenated Ringer-Locke solution (NaCl 7.0 g./l.; NaHCO_3 2.1 g./l.; KCl 0.42 g./l.; CaCl_2 0.21 g./l.; MgCl_2 0.2 g./l.; and dextrose 1.8 g./l.; pH 7.2 to 7.4 at 37°) at a pressure of 52 cm. of water was started as soon as the cannula was connected with the perfusion system. The aortic valve remained closed at this perfusion pressure, and the solution flowed into the coronary arteries. The perfusate dripped from the pulmonary artery and was not recirculated. Water at 37° was circulated between the double walls of the glass chamber. The heart rate and amplitude of contraction were read from an ink writing kymograph. The rate of coronary flow was measured by a flowmeter with a glass ball (Emil Greiner Co.); readings on an arbitrary scale were converted to ml./min. by reference to the calibration curve of the flowmeter for water. In analysing the data, diastolic values were used for coronary flow.

It usually required about 15 min. for stabilization of baselines after starting the perfusion with Ringer-Locke solution. While this was continuously perfused, drugs were injected into the system close to the heart by means of a syringe attached to the upper end of a fine polyethylene catheter, the lower end of which opened within the bulb of the aortic cannula. The total time of injection was approximately 15 sec.

FIG. 1.—Diagram of the modified Langendorff perfusion apparatus.

for each dose. This method simulates the peak concentration of an intravenous injection in the intact animal. Each drug was injected in a volume of about 1 ml., and was followed at once by Ringer-Locke solution in a second syringe to make the volume 2 ml. and to wash the drug through the catheter. The capacity of the catheter was 0.3 ml. Control injections of 2 ml. of Ringer-Locke solution were made during every experiment. The effects of drugs were observed for at least 4 min., and longer if the effects lasted longer. Ringer-Locke solution alone was then perfused for 4 to 5 min. before the next dose or drug was given, except when ergometrine was injected while vasopressin acted. Continuous records were taken throughout the experiment, which usually lasted from 2 to 3 hr.

Drugs.—The drugs perfused in this study were: ergometrine (U.S.P. ergonovine maleate); purified arginine-vasopressin free of oxytocin, supplied by Dr. Vincent du Vigneaud as a solution (0.8 u./ml.)

recently assayed by the blood pressure effect in the rat and without any preservative; and nitroglycerin, as 0.65 mg. hypodermic tablets dissolved in Ringer-Locke solution just before injection. In preliminary experiments, we determined the approximate threshold and maximal doses of these drugs for the isolated rabbit heart.

Pathology.—After perfusion was ended, the heart was fixed in formalin. For pathological study, cross-sections at 3 levels (base, centre, and apex) were stained with haematoxylin-eosin, Weigert (for elastin) and oil-red-O (for fat). In the ergometrine-positive rabbits with atherosclerosis of the small coronary arteries, the arterial changes were roughly graded as follows: 1+ subintimal foam cells without encroachment on the lumen of any artery sectioned; 2+ subintimal plaques extending partially into the arterial lumen; and 3+ obliteration of the lumen by subintimal atheroma in one or more arteries.

RESULTS

A total of 29 atherosclerotic hearts of cholesterol-fed, ergometrine-positive rabbits and 32 normal hearts of ergometrine-negative control rabbits were perfused with the various drugs.

Pathological Changes.—Most (96%) of the ergometrine-positive rabbit hearts showed advanced (2+ to 3+) intimal atherosclerosis of the small intramural arteries (Figs. 2 and 3); one heart showed 1+ atherosclerosis. The larger arteries seldom showed such changes. In the ergometrine-negative control hearts, atherosclerotic changes in the coronary arteries were not seen.

After perfusion, in both groups the myocardium appeared oedematous with separation of muscle bundles. However, in the non-perfused hearts of similar ergometrine-positive cholesterol-fed rabbits (Rinzler *et al.*, 1955, 1956), the myocardium showed diffuse fatty infiltration, spotty hyaline degeneration, and occasionally the fibrosis of old infarction (Fig. 3). Fragmentation of the elastic membrane beneath the subintimal plaques was also evident at many sites (Fig. 2).

Basal State of Isolated Heart.—Initially and before any drug, the coronary flow, heart rate, and amplitude of contraction were different in the normal and atherosclerotic hearts (Table 1). In the atherosclerotic hearts, the average rate of coronary flow was about 4 ml./min. (24%)

FIG. 2.—Photomicrograph of a small coronary artery showing lumen partially occluded by subintimal plaques (2+ atherosclerosis) and fragmentation of underlying elastic membrane at margins of plaques, as indicated by arrows (Weigert stain). In this ergometrine-positive rabbit (11 weeks on cholesterol diet), the heart was sectioned immediately after death and was not perfused.

greater, the average heart rate was 25 beats/min. (17%) slower, and the average amplitude of contraction was 6 mm. (19%) less than in the normal hearts. No correlation was evident between basal coronary flow and heart weight, which ranged from 6.7 to 12.2 g. for 9 atherosclerotic, and from 6.6 to 11.6 g. for 23 normal hearts.

To determine whether the higher level of basal coronary flow in the atherosclerotic heart showed a correlation with the maximal capacity of the coronary bed to dilate, nitroglycerin in graded doses was injected in 8 experiments on atherosclerotic hearts, and, for comparison, in 7 experiments on normal hearts. Fig. 4 illustrates the typical effects of a maximal dose of nitroglycerin

TABLE I
INITIAL STATE OF ISOLATED PERFUSED HEART OF RABBIT

Figures in parenthesis indicate range.

Group	Total Hearts (No. of Expts.)	Coronary Flow (ml./min.)	Heart Rate (Beats/min.)	Amplitude of Contraction (mm.)
Normal	32	15.7 (9-28)	151 (98-200)	33.0 (7-79)
Atherosclerotic	29	19.5 (8-32)	126 (36-196)	26.3 (6-52)
<i>t</i> test		$p < 0.01$	$p < 0.01$	$p < 0.001$

FIG. 3.—Photomicrograph of an old myocardial infarct with adjacent occluded small coronary artery (3+ atherosclerosis) at arrow (Masson stain). The heart was perfused before it was sectioned (rabbit 18 weeks on cholesterol diet).

in a normal and an atherosclerotic heart. In both, a marked increase in coronary flow occurred; in the normal, flow rose to 33 ml./min., and, in the atherosclerotic heart, to 29 ml./min., with little change in heart rate and slight reduction in amplitude of contraction. The highest level of coronary flow achieved with the maximal dose of nitroglycerin in the 7 normal

hearts averaged 28.8 ml./min., and for the 8 atherosclerotic hearts 25 ml./min., a difference of 3.8 ml./min., or 13% higher in the normal group. In these small groups of animals, this difference was not statistically significant.

Ergometrine.—Effects of graded doses of ergometrine (0.1 to 2.0 mg.) were studied in 15 atherosclerotic and 14 normal hearts; the average changes in coronary flow, heart rate, and contractile amplitude are shown in Fig. 5, and illustrative individual experiments on normal and atherosclerotic hearts in Fig. 6. Maximal changes usually occurred within 1 min. after injection of the dose, with a return to the control level within 2 min. In any given heart duplicate tests with the same doses gave results which agreed well (Fig. 7A). For example, in 25 comparisons with 61 doses of ergometrine on 13 hearts the mean reduction in coronary flow was 20.1% and the average difference from the mean 2.8% (S.E. ± 0.35).

Coronary flow was the only parameter that showed a significant difference in the response of the normal and atherosclerotic heart to ergometrine. Thus (Table II), after doses of 0.2 to 0.4 mg. in 47 tests on normal hearts, the average decrease in coronary flow was 8% as compared with 22% in 50 tests on atherosclerotic hearts. The greatest decrease in flow observed in any normal heart was 52%, whereas in the atherosclerotic group 3 hearts showed a decrease of 97%, 98%, and 100%, respectively. Furthermore, in the dosage range of 0.2 to 0.4 mg. ergometrine sometimes caused a significant increase (15% to 55%) in coronary flow in the 14 normal hearts, whereas in none of the 15 atherosclerotic hearts was there an increase in flow greater than 8%, which is comparable to the occasional effect of a control injection of Ringer-Locke solution.

FIG. 4.—Response of perfused normal heart (Rabbit Y₁ at left) and atherosclerotic heart (Rabbit X₁₅ at right) to a maximal dose of nitroglycerin. Note higher basal coronary flow in the atherosclerotic heart, but similar drug effects in both: increased flow and reduced amplitude of contraction with minor changes in heart rate.

FIG. 5.—Average effects of graded doses of ergometrine on coronary flow, amplitude of contraction and heart rate in 14 normal (cross-hatched columns) and 15 atherosclerotic (black columns) perfused rabbit hearts. A control dose of Ringer-Locke 2 ml. was injected initially.

FIG. 6.—Illustrative experiments showing graded responses of flow —, rate . . . , and amplitude ---, to 0.1 mg., 0.2 mg., and 0.4 mg. of ergometrine in (A) *normal* and (B) *atherosclerotic* hearts. Note also marked dilator effect of nitroglycerin after ergometrine in (A), and profound constrictor effect of vasopressin in (B).

Fewer tests were conducted with the smaller (0.1 mg.) and larger (1 mg. and 2 mg.) doses of ergometrine, and with these doses differences in the response of coronary flow in the normal and atherosclerotic groups were not statistically significant. However, for the small dose (0.1 mg.) in 19 tests on normal hearts, there was an average decrease of 17% in coronary flow, and in 20 tests on atherosclerotic hearts an average decrease of only 12%. With the largest doses (1 mg. and 2 mg.) the trend was toward a reversal of the vasoconstrictor effect; thus, in 11 tests on normal hearts coronary flow increased 6%, whereas in the atherosclerotic hearts it decreased only 2% (Fig. 5).

Amplitude of contraction decreased after all doses of ergometrine, and to about the same extent in normal and atherosclerotic hearts (Fig. 5, Table III). The % change was similar in both groups even though the average control amplitude for the atherosclerotic hearts was approximately one-half that for normal hearts before each dose of ergometrine. For example, after the 0.4 mg. dose the average decrease in amplitude in normal hearts was 44% from a level of 17.6 mm., and 43% from a level of 9.3 mm. in atherosclerotic hearts.

Graded responses in amplitude of contraction were obtained for doses of 0.1 mg., 0.2 mg., and 0.4 mg. of ergometrine. The average decrease for

TABLE II

COMPARISON OF EFFECTS OF ERGOMETRINE AND VASOPRESSIN ON CORONARY FLOW

Ergometrine effects were studied in 14 normal and 15 atherosclerotic hearts. Vasopressin effects were studied in 9 normal and 9 atherosclerotic hearts. Maximal change within 1 min. after injection is expressed in % of control (pre-injection) value. When duplicate tests were done, the average for each heart was used.

Coronary Arteries	Ringer-Locke 2 ml.		Ergometrine 0.2 to 0.4 mg.		Vasopressin 0.08 u.	
	Control Flow	Maximal Change	Control Flow	Maximal Change	Control Flow	Maximal Change
	ml./min.	%	ml./min.	%	ml./min.	%
Normal	15.2	-4.5	13.0	-8.1	12.6	-90.4
Atherosclerotic	21.0	-3.9	17.6	-22.0	17.5	-82.3
<i>t</i> test	$p > 0.8$		$p < 0.05$		$p > 0.8$	

TABLE III

EFFECT OF GRADED DOSES OF ERGOMETRINE ON CARDIAC CONTRACTION AND RATE
IN 14 NORMAL AND 15 ATHEROSCLEROTIC RABBIT HEARTS

Maximal change within 1 min. after injection is expressed in % of control (pre-injection) value. When duplicate tests were done, the average for each heart was used.

Drug and Dose	Amplitude of Contraction				Heart Rate			
	Control Level		Maximal Change		Control Level		Maximal Change	
	Normal	Athero.	Normal	Athero.	Normal	Athero.	Normal	Athero.
	mm.	mm.	%	%	Beats/min.	Beats/min.	%	%
Ringer-Locke 2 ml. ..	21.3	15.3	-0.1	-2.5	138	113	-1.5	-0.4
Ergometrine 0.1 mg. ..	24.6	13.9	-16.4	-19.9	141	115	+1.6	-1.8
0.2 ,, ..	21.4	11.6	-26.3	-30.3	132	113	+4.5	-2.5
0.4 ,, ..	17.6	9.3	-43.6	-42.9	150	115	+0.9	-2.8
1-2 ,, ..	16.7	8.7	-13.1	-22.6	138	114	+3.5	+2.1

FIG. 7.—*Atherosclerotic hearts*. Flow —, rate . . . , and amplitude ---. (A) Note reproducible effects of duplicate injections of ergometrine on all three parameters; also typical profound effect on flow of an initial dose of vasopressin, given after two doses of ergometrine. (B) Note limited reduction in flow after ergometrine as compared with initial dose of vasopressin, and tachyphylaxis to vasopressin when same dose was repeated three times.

FIG. 8.—Responses of coronary flow — and of amplitude ---. (A) *Normal hearts.* Note coronary constrictor and negative inotropic effects of three doses of ergometrine (0.4 mg.) given during different phases of vasopressin action. (B) *Atherosclerotic heart.* Note coronary constrictor and negative inotropic effect of three doses of ergometrine (0.4 mg.) given during similar phases of vasopressin action. For comparison, control doses of ergometrine were given before and after the first dose of vasopressin. Heart rate was essentially unchanged in both *normal* and *atherosclerotic* hearts, and is not charted.

these doses in normals was -16.4% , -26.3% , and -43.6% , and in the atherosclerotic hearts, -19.9% , -30.3% , and -42.9% , respectively. However, with the larger doses (1 mg. and 2 mg.) the negative inotropic effect was less intense, namely, -13.1% and -22.6% in the respective groups (Table III).

The heart rate showed no consistent change after ergometrine (Fig. 5).

Vasopressin.—All hearts, normal and atherosclerotic, that were injected with ergometrine were tested with vasopressin; the dose of vasopressin ranged from 0.008 to 0.8 units. The effects of vasopressin showed striking differences from those of ergometrine.

Coronary flow was more profoundly reduced by the initial dose of vasopressin than by ergometrine both in normal and atherosclerotic hearts (Table II, Figs. 6B, 7 and 8). Thus, 0.08 units of vasopressin, maximal dose, decreased the average coronary flow by 90% in 9 normal hearts, and by 82% in 9 atherosclerotic hearts; this difference was not statistically significant (Table II).

Unlike ergometrine, successive identical doses of vasopressin yielded diminishing effects (Fig. 7B). This tachyphylaxis to vasopressin was observed for both atherosclerotic and normal hearts. The typical profound reduction in flow after the first dose of vasopressin was not blocked if ergometrine was given first (Fig. 7), nor did a prior dose of vasopressin modify the effect of ergometrine given after the vasopressin effects had finished (Figs. 6B and 8B). The reduction in coronary flow by vasopressin could be momentarily reversed by nitroglycerin.

Amplitude of contraction after vasopressin sometimes increased initially, but usually decreased if the reduction in coronary flow was profound and prolonged (Figs. 6B, 7 and 8A). In this respect, no essential difference was observed between atherosclerotic and normal hearts.

Heart rate after vasopressin usually decreased in both atherosclerotic and normal hearts (Figs. 6B, 7 and 8). Both contractile amplitude and heart rate returned to control levels more slowly than did coronary flow.

Ergometrine During the Action of Vasopressin.—When ergometrine (0.4 mg.) was given about 1 min. after vasopressin, when the coronary flow was most reduced, ergometrine caused no change in flow in either normal or atherosclerotic hearts (Table IV). However, when ergometrine was given while the vasoconstrictor action of vasopressin was wearing off, usually in 1.5 to 2 min. after vasopressin injection, the effect of ergo-

TABLE IV
EFFECT OF ERGOMETRINE ON CORONARY FLOW WHEN INJECTED DURING VASOPRESSIN CONSTRICTION IN 7 NORMAL AND 5 ATHEROSCLEROTIC HEARTS

Ergometrine given early, 0.25 to 1 min. after vasopressin, in column at right; other doses given later, 1.5 to 23 min. after vasopressin, during rising flow.

Heart	Total Doses Ergometrine (0.4 mg.)	Ergometrine-induced Flow Change during Vasopressin Action		
		Increased	Decreased	Unchanged
Normal	No. 10	No. 8	No. 0	No. 2
	12	1	7	4
Atherosclerotic	No. 10	No. 8	No. 0	No. 2
	12	1	7	4

metrine on coronary flow differed significantly for normal and atherosclerotic hearts (Table IV). In the normals, a transient rise in flow after ergometrine (8 doses) was uniformly superimposed on the rising slope of recovery from the vasopressin reduction (Fig. 8A). On the other hand, in the atherosclerotic hearts, only 1 of 8 similar doses of ergometrine caused any increase in flow; 6 of the doses caused a transient reversal of the vasopressin recovery curve, and in the remaining 1 the rising slope was temporarily flattened (Fig. 8B).

In these experiments, the dose of vasopressin ranged from 0.008 to 0.8 units, and showed no correlation with the direction of the ergometrine effect on flow during vasopressin action.

When ergometrine was given while vasopressin acted, the usual reduction in amplitude was seen regardless of whether coronary flow increased or decreased (Fig. 8). The heart rate showed negligible changes.

DISCUSSION

Basal State of Isolated Heart.—The finding that the atherosclerotic heart in its initial state, before any drugs were perfused, had a higher coronary flow than normal was unexpected. Since these observations were made, however, it has been reported (Gorlin, Brachfeld, Macleod, and Bopp, 1959) that in man the resting coronary flow determined by cardiac catheterization likewise is significantly higher in patients with coronary insufficiency (effort angina) than in normal subjects.

Wegria (1951) showed in the normal dog that blood flow is determined by the diastolic pressure at the coronary ostia and by the resistance of the coronary bed. Since the aortic (perfusion) pressure is constant in our isolated rabbit heart preparation, the observed differences in basal coronary flow must depend on differences in coronary resistance. Theoretical factors which might alter coronary resistance of the atherosclerotic heart in the direction of an increased basal coronary flow include: (1) an increase in collateral circulation during the course of atherogenesis with enlargement of the total coronary bed; (2) increased energy expenditure of the heart owing to impaired myocardial efficiency; (3) a hyperaemic response to localized areas of anoxic myocardium, mediated by the intrinsic neuro-humoral mechanisms of the heart; (4) yielding of the atherosclerotic vascular wall owing to fragmentation of the subintimal elastic membrane in the small coronary arteries; and (5) weakening of the extravascular support of the coronary bed owing to extensive damage to the myocardium.

In considering mechanical factors in the rate of coronary flow, Duguid and Robertson (1957) point out that it is the large coronary arteries which receive most of the impact of ventricular systole, and thus their functional state rather than the resistance of the terminal arterial bed largely determines the maximal capacity of the coronary bed to dilate. In the atherosclerotic rabbit heart, the large coronary arteries are relatively free of atherosclerotic plaques even when the intramural vessels are extensively occluded. It is not surprising, therefore, that the coronary dilator effect of nitroglycerin in the atherosclerotic rabbit heart is only moderately or little impaired. At the same time, lowered resistance in the smaller arteries would be compatible with a higher basal coronary flow in the atherosclerotic group.

The known depletion of myocardial catecholamines in coronary artery disease (Raab and Gigue, 1955) would explain the diminished amplitude and slower rate of contraction observed initially in the atherosclerotic, as compared with the normal isolated rabbit heart. Lee and Shideman (1959) have demonstrated in cats that a reduction in myocardial catecholamines by sympathectomy or reserpine does indeed cause diminished contractility of isolated cardiac (papillary) muscle.

Differences in Ergometrine Effects on Normal and Atherosclerotic Hearts. — Mechanisms by which ergometrine causes electrocardiographic changes typical of coronary insufficiency in man

and the intact rabbit with latent coronary artery disease, but not in normal persons or animals, are suggested by the results of this investigation.

The consistent and considerable negative inotropic effect of ergometrine, although equal in normal and atherosclerotic isolated rabbit hearts, may nevertheless play a role in the production of electrocardiographic changes in the intact atherosclerotic animal. In the isolated heart, ergometrine causes a similar percentage decrease in contractile amplitude for both groups, but the absolute level to which the amplitude falls is much lower for atherosclerotic hearts since the amplitude is initially less, only half that of the normals. If this reduction represents a deleterious effect on heart muscle with impaired oxygen utilization, it may well contribute to the S-T segment depression induced by ergometrine in the intact animal, especially when a reduction in coronary flow is superimposed.

On the average, the quantitative effect of ergometrine on coronary flow differs significantly in the normal and atherosclerotic groups at the dose range of 0.2 to 0.4 mg.; in the normals the reduction in flow was 8% as compared with 22% in the atherosclerotic. For the smaller and larger doses of ergometrine that we employed, the numbers of experiments were too small for differences to be statistically significant.

In the atherosclerotic heart, ergometrine quite uniformly causes a transient decrease in coronary flow. In the normal heart, the response is more variable; increases as well as decreases in flow occurred. The coronary dilator action of ergometrine in normal hearts is most consistently seen when the coronary bed is partially constricted by vasopressin. In contrast, under these conditions ergometrine has a predominantly constrictor effect in the atherosclerotic heart.

A reduction in coronary flow of about 25% has been shown not to cause electrocardiographic changes in the normal intact animal (Wegria, Segers, Keating, and Ward, 1949). On the other hand, in the presence of occlusive disease of the small coronary arteries and myocardial damage, a flow reduction of this magnitude may be critical and so contribute to anoxic changes in the electrocardiogram. The delay of several min. in the appearance of S-T segment depression after intravenous ergometrine in the intact atherosclerotic rabbit and in the patient with effort angina suggests that this effect depends on myocardial ischaemia secondary to constriction of the diseased coronary arteries, rather than solely on a direct action of the drug on the myocardium.

In contrast to the reproducible effects of a given dose of ergometrine, vasopressin induces tachyphylaxis in the isolated heart. Furthermore, the effects of vasopressin are essentially similar for normal and atherosclerotic isolated rabbit hearts. These observations suggest fundamental differences in the mechanism of action of these two drugs on the heart. One may speculate that the effects of ergometrine on coronary flow are mediated at least in part through mobilization of catecholamines, whereas vasopressin acts more directly on receptors. When catecholamines are depleted during atherogenesis, a direct action of ergometrine on receptors may be unmasked.

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REFERENCES

- Brachfeld, N., Bozer, J., and Gorlin, R. (1959). *Circulation*, **19**, 697.
 Brown, G. L., and Dale, H. H. (1935). *Proc. roy. Soc. Series B*, **118**, 446.
 Davis, M. E., Adair, F. L., Chen, K. K., and Swanson, E. E. (1935). *J. Pharmacol. exp. Ther.*, **54**, 398.
 Duguid, J. B., and Robertson, W. B. (1957). *Lancet*, **1**, 1205.
 Garb, S., Penna, M., and Scriabine, A. (1955). *Amer. J. Physiol.*, **180**, 103.
 Gorlin, R., Brachfeld, N., Macleod, C., and Bopp, P. (1959). *Circulation*, **19**, 705.
 Karp, D., Penna, M., Rinzler, S. H., and Travell, J. (1956). *J. Pharmacol. exp. Ther.*, **116**, 34.
 ——— Travell, J., and Rinzler, S. H. (1956). *Communications XX International Physiological Congress, Brussels*, p. 482.
 Lee, W. C., and Shideman, F. E. (1959). *Science*, **129**, 967.
 Raab, W., and Gigue, W. (1955). *Circulation*, **11**, 593.
 Rinzler, S. H., Stein, I., Bakst, H., Weinstein, J., Gittler, R., and Travell, J. (1954). *Proc. Soc. exp. Biol. (N.Y.)*, **85**, 329.
 ——— Travell, J., and Karp, D. (1955). *Science*, **121**, 900.
 ——— ——— and Charleson, D. (1956). *Amer. J. Physiol.*, **184**, 605.
 Rothlin, E. (1935). *Schweiz. med. Wschr.*, **65**, 947.
 Stein, I. (1949). *Amer. Heart J.*, **37**, 36.
 ——— and Weinstein, J. (1950). *J. Lab. clin. Med.*, **36**, 66.
 ——— (1953). *N.Y. State J. Med.*, **53**, 1454.
 Travell, J., Karp, D., and Rinzler, S. H. (1957). *Fed. Proc.*, **16**, 341.
 Wegria, R., Segers, M., Keating, R. P., and Ward, H. P. (1949). *Amer. Heart J.*, **38**, 90.
 ——— (1951). *Pharmacol. Rev.*, **3**, 197.