drug has been utilized. The precise mechanism of the agonist-antagonist action of isoproterenol and propranolol for the beta receptor is under further investigation.

Summary. Trypsin dispersed chick embryo heart cells have been grown in tissue culture to produce rhythmic contraction cell clusters. Isoproterenol, a specific beta adrenergic stimulating drug, has been shown to increase the rate of contraction of these cells. This effect persists for 20-30 minutes and can be removed by washing the cells with fresh control media. Propranolol, a specific beta adrenergic blocking agent, blocks the stimulating action of isoproterenol. It appears likely that stimulation of beta adrenergic receptors of the Ahlquist classification can be used to explain these findings. These receptors apparently develop in the absence of adrenergic nerve endings.

ADDENDUM. At a recent symposium on the structure and function of heart muscle, Wollenberger described his unpublished observations(16) on the effects of adrenergic stimulating and blocking drugs on heart cells in tissue culture which are in general agreement with this report.

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Effects of Acutely Induced Ischemic Heart Failure on Myocardial High Energy Phosphate Stores. (31681)

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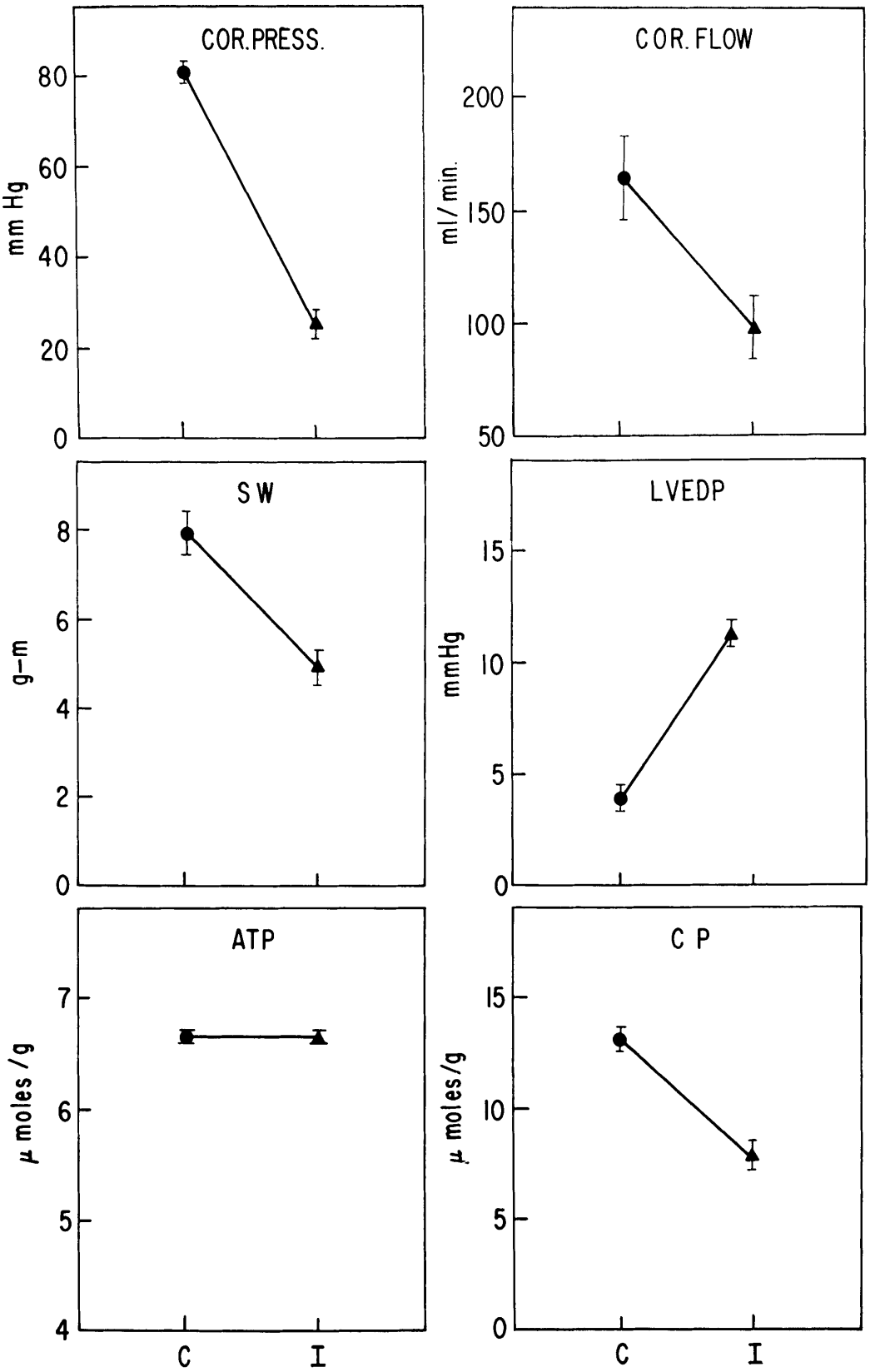
When severe myocardial ischemia occurs, a deterioration of cardiac contractile function takes place. It is generally assumed that the primary metabolic consequence of a severe impairment of oxygen delivery consists of a shift from aerobic to anaerobic metabolism (1-3), with an attendant reduction in the production of high energy phosphate compounds(3). This reduction is thought to be the ultimate cause of the breakdown of cardiac function(3-7). It was the purpose of the present investigation to test this hypothesis and specifically to determine whether there is a relation between the store of high energy phosphate compounds available to the myocardium and the depression of myocardial function produced by acute ischemia. Severe myocardial ischemia was produced by abruptly reducing coronary arterial flow in anesthetized dogs. Biopsies of the left ventricle were obtained before and during the development of heart failure, and correlations were made between the sequential changes in left ventricular function and the high energy phosphate stores.

Methods. Preparation. Twelve mongrel dogs weighing 15.0 to 26.4 kg were anesthetized with an average dose of sodium pento-

TABLE I

	Control								Ischemia								
Exp	LVEDP, mm Hg	C.O., ml/min	Co F, ml/min	Co P, mm Hg	SW, g-m	ATP, μmoles/g	CP, μmoles/g	~ P, μmoles/g	LVEDP, mm Hg	C.O., ml/min	Co F, ml/min	Co P, mm Hg	SW, g-m	ATP μmoles/g	CP μmoles/g	~ P μmoles/g	Time, sec
1A	4.5	1380	88	86	8.9	6.96	10.0	17.0	13.0	1101	42	40	7.8	6.20	8.2	14.4	135
1B	4.0	1190	65	80	9.6	6.45	11.5	17.9	10.0	883	22	19	6.6	7.16	5.2	12.4	47
2A	1.0	1232	190	91	11.6	7.85	15.9	23.7	12.0	683	155	32	6.0	6.54	10.3	16.8	45
3A	2.0	910	218	63	6.9	5.38	12.4	17.8	13.0	832	100	19	4.5	4.61	7.1	11.7	100
3B	5.5	988	218	60	7.1	5.40	14.1	19.5	15.0	988	90	7	5.7	4.79	3.4	8.2	80
4A	8.0	700	235	76	4.4	5.37	12.2	17.6	12.5	542	155	26	2.9	6.14	12.2	18.3	42
5A	4.5	960	310	86	9.0	5.13	11.1	16.2	10.0	666	187	23	5.5	6.09	6.7	12.8	32
5B	9.5	1809	330	84	13.8	5.35	11.9	17.3	15.0	1428	215	54	9.3	6.40	10.4	16.8	25
6A	4.5	1155	162	63	7.4	4.90	15.8	20.7	13.5	765	138	21	3.9	5.33	8.8	14.1	30
7A	1.0	666	90	80	6.2	6.91	12.1	19.0	6.5	384	10	14	3.8	5.85	4.3	10.1	43
8A	1.0	870	133	84	8.5	8.23	18.4	24.6	9.0	580	119	21	5.4	7.30	5.5	12.8	60
8B	2.0	660	164	82	6.0	6.63	14.3	20.9	14.0	400	150	33	3.2	8.21	7.3	15.5	58
9A	7.0	845	55	93	8.6	8.90	13.2	22.1	11.5	542	51	38	4.8	9.77	11.8	21.6	12
9B	6.0	812	58	82	7.1	8.72	14.8	23.5	12.0	407	53	32	2.8	9.43	9.0	18.4	30
10A	3.0	901	260	77	6.4	4.98	9.0	14.0	8.0	701	70	14	4.5	5.02	7.6	12.6	45
11A	3.5	800	135	82	8.4	7.33	13.7	21.0	11.0	614	105	18	5.4	7.65	8.7	16.3	39
11B	2.5	768	230	82	7.0	7.35	14.8	22.2	10.0	544	180	19	4.7	7.78	10.3	18.1	22
12A	3.0	676	80	94	6.7	7.46	13.8	21.3	7.5	416	10	24	3.7	6.35	6.7	13.1	29
12B	2.5	780	95	93	7.4	7.08	12.9	20.0	11.0	364	15	24	3.2	5.91	7.1	13.0	28
Mean	3.9	952	164	81	7.9	6.65	13.2	19.8	11.3	676	98	25	4.9	6.66	7.9	14.6	47
± S.E.	.5	64	19	2	.5	.29	.5	.6	.6	62	14	3	.4	.32	.6	.7	7

LVEDP = Left ventricular end-diastolic pressure; C.O. = Cardiac output; Co F = Left coronary arterial flow; Co P = Coronary perfusion pressure; SW = Stroke work; ATP = Adenosine triphosphate; CP = Creatine phosphate; ~ P = Total ATP + CP; Time = Time from onset of ischemia to failure.


COR.PRESS.
mm Hg
80
60
40
20
0
COR. FLOW
ml/min.
200
150
100
50
SW
g-m
8
6
4
2
0
LVEDP
mmHg
15
10
5
0
ATP
μ moles/g
7
6
5
4
C P
μ moles/g
15
10
5
0
C
I
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I

FIG. 1. Average values (±SE) of hemodynamic and biochemical variables at control (C) and during ischemic heart failure (I). Cor. press. = left coronary perfusion pressure; cor. flow = left coronary arterial flow; SW = left ventricular stroke work; LVEDP = left ventricular end-diastolic pressure; TAP = adenosine triphosphate; CP = creatine phosphate.

barbital of 34 mg/kg administered intravenously. Respiration with 100% O_2 was maintained with a Harvard respiratory pump. The heart was exposed through a bilateral thoracotomy, and the left main coronary artery was cannulated through the left subclavian artery with a Gregg cannula which was connected by a polyvinyl tube to a cannula in the right subclavian artery. Left coronary arterial flow was measured with an extracorporeal electromagnetic flow probe* and regulated by means of a screw clamp on the polyvinyl tubing leading to the Gregg cannula. Phasic and mean aortic flow were recorded by a flow transducer about the ascending aorta. Flow probes were calibrated with gravity flow. Coronary arterial pressure was measured with a Statham P23Db transducer connected to a T-tube on the Gregg cannula. Left ventricular and aortic pressures were measured through cannulae inserted into the left ventricular apex and left femoral artery respectively and attached to Statham P23Db transducers. Temparature was monitored with a thermistor probe† in the right atrium and maintained between 35°C and 37°C with a heating pad. Heart rate was maintained constant by electrical stimulation of the right atrium.‡ Mean aortic pressure was maintained constant with a reservoir bottle attached to both femoral arteries.

After a control myocardial biopsy was obtained, left coronary arterial flow was abruptly reduced to approximately 50% of control. A subsequent myocardial biopsy was obtained when progressive myocardial depression, as defined by the development of a progressive elevation of left ventricular end-diastolic pressure (LVEDP) and fall in stroke work, was firmly established. The Gregg cannula circuit was then opened and the heart allowed to recover. In 7 dogs, following return to normal function, the effects of ischemia were determined a second time.

Following completion of the experiment 2 samples of left ventricular myocardium were obtained for determination of total creatine concentration. The biopsy technique and details of the chemical determinations have been presented(8,9). In brief, a bone rongeur was used to obtain approximately 50 mg samples of myocardium, and the sample was transferred to liquid nitrogen in less than 0.6 sec. The original biopsy was split and each pair was assayed in duplicate. The average of these determinations is reported. The standard deviations of the difference between these paired determinations were: creatine phosphate (CP) ± 0.93 μmole/g, ATP ± 0.75 μmole/g, inorganic phosphate (Pi) ± 0.91 μmole/g and creatine ± 1.5 μmoles/g.

Total creatine was determined by the alpha-napthol-diacetyl method(10), and CP and Pi concentrations were determined by the Furchgott and de Gubareff(11) modification of the Fiske and SubbaRow(12) techniques. ATP was assayed by a modification of the firefly luminescence technique of Strehler and McElroy(13) using firefly lantern extract.§ All statistical comparisons were made by the paired t test. Average values are reported ± SE.

Results. The results of all 19 experiments are summarized in Table I and Fig. 1. Recordings of a representative experiment are shown in Fig. 2. Coronary perfusion pressure was reduced from an average of 81 to 25 mm Hg causing a 40% reduction of coronary flow, from an average of 164 to 98 ml/min. Left ventricular failure occurred at an average of 47 seconds following reduction of coronary flow. This was evidenced by a significant ($p<.01$) fall in stroke work, from an average level of 7.9 ± 0.5 to 4.9 ± 0.4 g-m, and elevation in LVEDP from 3.9 ± 0.5 to 11.3 ± 0.6 mm Hg.

No change in left ventricular ATP stores occurred during ischemia, the values averaging

* Medicon Model M 4000.

† Yellow Springs Inst. Co., Yellow Springs, Ohio. Model No. 402.

‡ American Electronics Laboratories, Model 104A.

§ Sigma Chemical Co., St. Louis, Mo.

6.65 ± 0.29 μmoles/g during the control period and 6.66 ± 0.32 μmoles/g during ischemia. A comparison of the individual values of ATP between control and ischemia revealed that in no experiment did ATP fall by more than 2 standard deviations of the difference between paired determinations. CP declined significantly during ischemia from an average of 13.2 ± 0.5 μmoles/g during the control period to 7.9 ± 0.6 μmoles/g during ischemia ($p<.01$). A comparison of the individual values revealed that in 5 of the 19 observations the CP values were within two standard deviations of the differences between paired determinations.

Discussion. It has been demonstrated repeatedly that coronary ischemia, both in man and experimental animals, is associated with anaerobic metabolism as evidenced by the production of lactate(1-3). In the present study myocardial ischemia sufficient to cause progressive cardiac failure in less than one minute was produced. Despite this severe depression of cardiac performance, there was no detectable change in the concentration of ATP. Moreover, although there was a significant decline in the average CP concentration during ischemia (13.0 to 7.9 μmoles/g), the change in 5 of the 19 observations (Table I, nos. 1A, 4A, 5B, 9A and 10A) was less than 1.8 μmoles/g, *i.e.*, 2 standard deviations of the difference between paired determinations. In these 5 experiments the CP concentration decreased by an average of only 11.3% of control levels despite the presence of definite myocardial

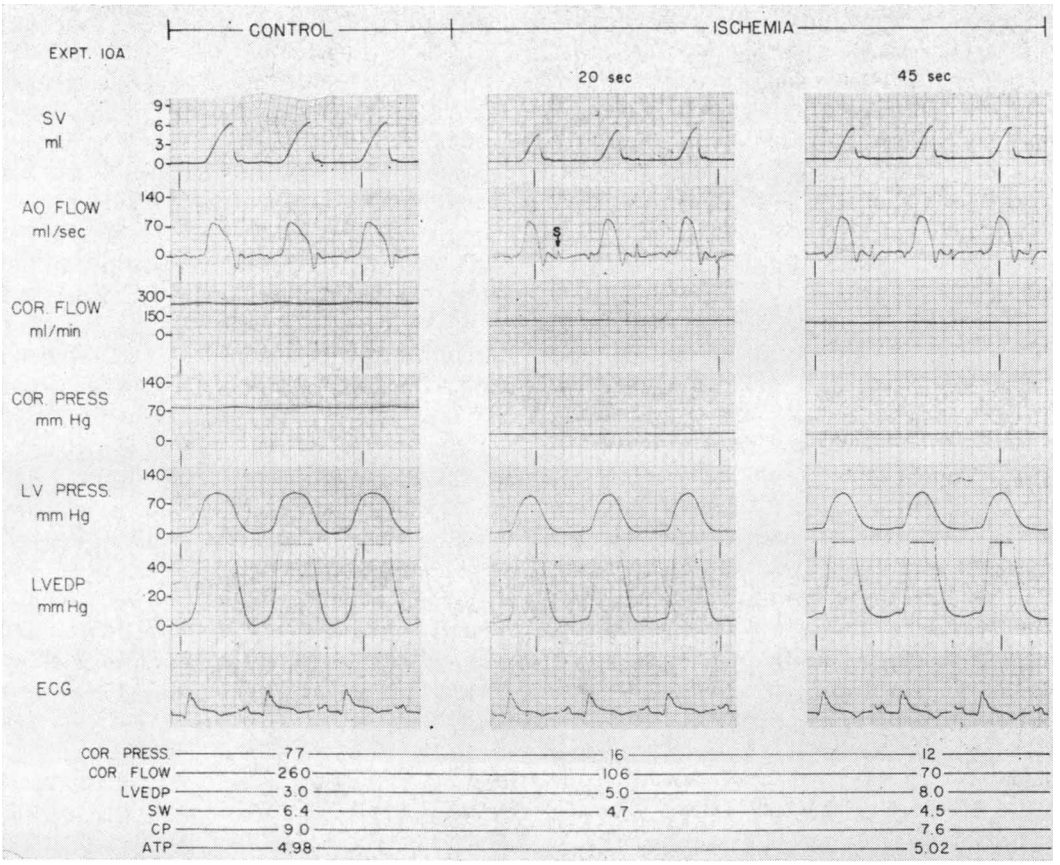

FIG. 2. Recordings from an experiment (Table I - 10A) typical of those in which ATP was unchanged and CP declined by less than 2 S.D. of the difference between paired determinations. SV = left ventricular stroke volume. Ao flow = aortic flow. S = stimulus artifact. Times shown are from the onset of ischemia. Other abbreviations as in Fig. 1.

failure. Moreover, Feinstein has shown that cardiac function can be normal in spite of a considerable depression of high energy phosphate stores(14). Several other studies have shown that ischemic heart failure will ultimately result in a reduction of myocardial ATP stores(4-8). However, the results of the present study show that there is no detectable alteration in myocardial ATP stores at the onset of ischemic heart failure. Thus it would appear from the present investigation and an earlier study on acute hypoxic heart failure carried out in this laboratory(8) that reduction of O_2 delivery to the myocardium can impair cardiac function without lowering total myocardial ATP levels.

The results of the present study would be compatible with a defect in energy storage as the cause of ischemic heart failure only if a very small undetectable fraction of the total ATP store, such as that used for the maintenance of membrane function, were depleted (8,15,16). On the other hand ischemia might interfere directly with excitation-contraction coupling or with the myofilaments themselves and in this manner alter the mechanical performance of the myocardium.

Summary. The effects of acutely induced ischemic heart failure on myocardial high energy phosphate stores were studied in dogs following an abrupt reduction (avg. 40%) of left main coronary arterial flow. Left ventricular biopsies were obtained before and during the onset of heart failure. Myocardial ATP stores were unchanged (control, 6.65 μmoles/g; ischemia, 6.66 μmoles/g) at a time when left ventricular end-diastolic pressure had risen from 3.9 to 11.3 mm Hg and left ventricular stroke work had fallen from 7.9 to 4.9 g-m. The average myocardial creatine phosphate (CP) stores fell from 13.2 μmoles/g to 7.9 μmoles/g. These results indicate that acute ischemic heart failure is not initiated by a detectable depression in total myocardial ATP stores.

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Complexometric Titrations Using Calcium Specific Electrodes.* (31682)

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Ionized calcium is believed to be essential for the execution of a number of physiological functions which include muscle contraction, blood clotting, and nerve impulse transmis-

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