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Phosphorylation in Cardiac Muscle from Failing and Unfailing Heart-Lung Preparations.*† (21218)

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(Introduced by M. H. Seevers.)

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Little is known about the sequence of biochemical events that leads to failure of heart muscle either in the intact animal or in isolated preparations. In an attempt to elucidate the cause of failure, most of the studies have been centered around the various enzymatic steps in the breakdown of carbohydrate and the concomitant generation of energy, primarily because more is known of these biochemical pathways. One approach to the problem of whether or not failure is associated with a defect in energy production has been the analysis of the high-energy phosphate stores of the normal and failing heart(1,2). The results of these studies have indicated that in failure, cardiac muscle retains its normal level of phosphocreatine and adenosine triphosphate. However, the level of the high-energy phosphate compounds of the heart is in reality merely the balance between the generation and the utilization of these compounds. Thus, an inhibition of both generation and utilization might still result in normal levels of high-energy compounds. It was therefore decided to study in a more direct way the ability of failed and unfailed cardiac muscle to generate energy-rich bonds.

Methods. In the control group of animals, mongrel dogs were anesthetized and immediately sacrificed, or the failed heart from the dog heart-lung preparation was employed. The animals sacrificed immediately were anesthetized with ether or pentobarbital, while preliminary operations on the heart-lung preparation were performed under pentobarbital anesthesia. The failed hearts in all experiments were obtained from the dog heart-lung, prepared by a modification of the Krayer-Mendez method(3). Failure in these animals was either spontaneous or pentobarbital induced. The competance index as described by Wollenberger(1) was used to evaluate the degree of failure. Those preparations having indices of from 0.8-1.0 were considered to be unfailed, while those with indices of from 0.0-0.2 were taken to be failed. In both failing and unfailing animals the left ventricle was rapidly excised and placed in 0.25 M sucrose solution. Mitochondria were then prepared by a modification of the method of Langemann *et al.*(4). Operations from this point on were carried out in ice baths in order to maintain a temperature of from 3-5°C. The tissue was then minced, rapidly weighed and a 10% homogenate prepared in isotonic sucrose using an all-glass Potter-Elvehjem homogenizer. The homogenate was centrifuged for 5 minutes at 700 x g in a refrigerated centrifuge. The residue was discarded and the supernatant fluid was centrifuged at 12000 x g for 15 minutes. The resulting residue was resuspended in 0.25 M sucrose and recentrifuged at the same speed. The final

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residue was made to volume with sucrose and added to the reaction mixture. The total volume in the Warburg flask was 1.8 ml. The final concentrations of the components of the reaction mixture, added in the order given, was as follows: 1.7 x 10^{-2} M Na-glycylglycine, pH 7.4, 2.2 x 10^{-2} M K-phosphate buffer, pH 7.4, 1.7 x 10^{-2} M KCl, 1.3 x 10^{-2} M $MgCl_2$, 2.8 x 10^{-3} M Na-adenosine triphosphate, 3.1 x 10^{-3} M glucose, 5.6 x 10^{-4} M disphosphopyridine nucleotide, 1.4 x 10^{-2} M Na-pyruvate, 2.2 x 10^{-3} M Na-malate, 9.2 x 10^{-6} M cytochrome c, 0.1 ml yeast hexokinase 3a(5), 60 units; 1.4 x 10^{-2} M Na-fluoride, 0.4 ml mitochondria in 0.25 M sucrose containing approximately 0.80 mg nitrogen. 8 x 10^{-3} M Na-succinate or 1.2 x 10^{-2} M α-ketoglutarate were also used as substrates. The sidearm contained 0.2 ml of 50% trichloroacetic acid to stop enzymatic activity at the end of the desired incubation period. Center well contained 0.2 ml of 2 N K-hydroxide, the gas phase was air and incubation temperature 25°C. Equilibration time was 5 minutes and incubation time varied from 10-30 minutes depending upon activity. Most experiments were run in triplicate. Oxygen uptake was measured by standard manometric methods and concomitant inorganic phosphate uptake as described(6). Phosphate to oxygen ratios (P:O) were then calculated from these values. Lindberg and Ernster(7) have substantiated the fact that the mitochondrial preparation when used with the hexokinase trapping system is a valid method of determining adenosine triphosphate synthesis.

Results. Table I summarizes results obtained when unfailing dog cardiac muscle was studied. The P:O ratios in this group ranged from 2.04 (Exp. 4) to 3.07 (Exp. 7), average of 8 experiments 2.28. There seemed to be no difference in generation of high-energy bonds in tissues where the competance index was determined (Exp. 1-4) as compared with the values obtained from the myocardium of animals anesthetized and immediately sacrificed (Exp. 4-8). Nor did the nature of the anesthetic agent seem to alter the P:O ratio. Table II shows the results obtained when the dog heart-lung preparation was failing, mitochondria prepared from excised ventricle, and

TABLE I. Oxidative Phosphorylation in Cardiac Muscle from Unfailed Heart.* Eight experiments.

Anesthetic	Comp. index	Uptake: Oxygen†	Uptake: Inorg. phos.‡	P:O§
Pentobarbital	1.0	8.67	18.4	2.14
		9.37	20.0	2.14
		9.00	20.0	2.22
	.9	5.30	12.0	2.27
		4.64	9.6	2.08
	1.0	5.90	12.8	2.17
		5.54	12.0	2.16
		6.35	12.8	2.00
	.9	5.66	11.2	1.98
		5.75	12.0	2.09
		5.86	12.0	2.05
	—	6.27	14.4	2.30
		6.73	12.8	1.90
	—	7.97	16.8	2.11
		8.17	16.8	2.09
		8.00	16.8	2.10
	—	4.02	12.0	2.98
		3.53	11.2	3.17
Ether	—	5.54	14.4	2.60
		6.64	16.0	2.40

* Pyruvate plus malate substrate.
† Microatoms.
‡ Micromoles.
§ Ratio of uptake of micromoles of inorganic phosphate to uptake of microatoms oxygen.

ability of tissue to synthesize high-energy bonds assayed. Here the P:O values range from 2.16 (Exp. 9) to 2.83 (Exp. 11), average 2.42. No difference was found between drug and non-drug failing animals. In comparing Table I and Table II there appears to be no difference in ability to generate energy as measured by means of the P:O ratio. Use of other substrates gave results similar to those obtained with pyruvate and malate, *i.e.*, no significant difference between failing and unfailing muscle.

Discussion. Wollenberger(1) found similar phosphocreatine and adenosine triphosphate concentrations in hearts from normal and from failing heart-lung preparations. Greiner(2) analyzed papillary muscles that were moderately hypodynamic and found no fall in energy-rich compounds. The adenosine triphosphate level was decreased only in muscles which showed more than a 50% decline in systolic force. Furchgott *et al.*(8) indicated that normal guinea pig auricles have the same high-energy phosphate compound concentra-

TABLE II. Oxidative Phosphorylation in Cardiac Muscle from Failed Heart. Eight experiments.

Failure	Comp. index	Uptake		P:O
		Oxygen	Inorg. phos.	
Pentobarbital	.0	8.00	18.4	2.30
		5.98	12.0	2.01
		6.97	15.2	2.17
	.0	6.14	14.4	2.34
	.0	5.91	16.0	2.71
		5.54	14.4	2.60
		4.29	13.6	3.17
	.1	5.43	12.0	2.21
		5.42	12.8	2.36
		5.27	12.0	2.28
	.0	6.42	16.0	2.49
		5.68	17.6	3.10
		6.25	16.8	2.69
Non-drugs*	.0	8.36	19.2	2.41
		8.19	18.4	2.24
		7.83	19.2	2.45
" †	.0	9.76	23.2	2.38
		11.70	23.2	1.99
		11.20	24.0	2.14
	.0	6.62	16.0	2.41
		5.50	13.6	2.47

* Failed by occluding aorta.
† Spontaneous failure.

tions as either failing auricles or failing auricles reversed with strophanthin. All of the above data indicate that levels of high-energy compounds are not significantly different when muscles of failing and unfailing preparations are compared. The data, in no way, rule out the possibility that both production and utilization might be impaired in the hypodynamic muscles. Our work would suggest that the defect in the failing preparation is not one of energy production. It would also seem to indicate that future investigation into the mechanism of action of the cardiac glycosides at sites of energy production will not prove fruitful.

In the interpretation of our data one should bear in mind that we are measuring oxidative phosphorylation in the presence of a fortified system. Any deficiency in quantity of substrate or other essential metabolite in a failing heart might well interfere with energy production. The evidence presented indicates that under optimum conditions the phosphorylative reactions in both failing and unfailing muscle are operating efficiently.

Summary. This investigation has demonstrated that there is no difference in phosphorylating ability, *i.e.*, enzyme concentration or activity, between normal cardiac muscle and similar tissue obtained from dog heart-lung preparations. These findings can be considered support for the thesis that if energy metabolism is concerned in failure, the defect is probably not one of production of energy-rich compounds.

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