Phosphocreatine Content of Mammalian Cardiac Muscle.* (20616)

GEORGE FAWAZ AND ELIAS S. HAWA.

From the Department of Pharmacology, School of Medicine, American University of Beirut, Beirut, Lebanon.

The values reported in the literature for phosphocreatine in mammalian cardiac muscle vary widely. For the intact rat heart the published figures, expressed in terms of phosphocreatine-P, vary from 5.7 to 12 mg % (1-4). For the intact guinea pig heart and cat heart, the figures are 5.2 and 6.0 mg %, respectively(5,6). For the intact dog heart, the figures vary from 4.2 to 9.0 mg %(6-8). For the isolated dog heart (heart-lung preparation) the published values range from 6.2 to 21.5 mg %, depending on the experimental conditions(8-10). Wollenberger(8) and Pollack *et al.*(9) state that the phosphocreatine content of the heart in the intact animal is considerably below that of the normal heart-lung preparation. Mommaerts(11) states, without citing references, that cardiac muscle may contain one-tenth as much phosphocreatine as striated muscle, and he gives the value 2×10^{-6} mole per g for cardiac muscle, *viz.* 6.2 mg % phosphocreatine-P.

In connection with our studies on the effect of drugs on the metabolism and performance of the isolated dog heart (heart-lung preparation), we found it necessary to redetermine the "resting" values of phosphocreatine in mammalian heart muscle. At the same time we have made determinations of the corresponding values for the labile "nucleotide" phosphorus.

Methods. Pentobarbital anesthesia was used in all animals including those designated for heart-lung preparations. Artificial respiration was applied before opening the chest. Heart-lung preparations were performed by the conventional procedure except that the blood donors were anesthetized with chloroform. A sample of heart muscle was cut from the left ventricle and quickly immersed in liquid air or in a mixture of CO_2-snow and ether (temp. —60°C). When liquid air was used the frozen tissue was ground into a fine powder in a mortar chilled by adding successive portions of liquid air to make sure that the tissue did not thaw. The powdered material was then transferred to a previously weighed Erlenmeyer flask containing 5% trichloracetic acid using a spoon and a wide-necked funnel that had been dipped in liquid air. The flask was shaken to mix the contents and then reweighed. The volume of 5% trichloracetic acid employed was adjusted to give a dilution of 1:10, reckoning the water content of the tissue as 80%. Not more than 10 minutes was allowed to elapse between the fixing of the powder with acid and the time when the protein-free filtrate was neutralized. During this period of time all operations were carried out at a temperature which did not exceed 2°C. Inorganic phosphate and phosphocreatine were determined by the method of Fiske and Subbarow(12) and the labile "nucleotide" phosphorus was calculated by subtracting the sum of the phosphocreatine and inorganic phosphorus from the value for the total inorganic phosphorus obtained after hydrolysis for 10 minutes in 1 N HCl at 100°C. In a subsequent publication we are discussing the extent to which labile "nucleotide" phosphorus represents adenosine triphosphate. Equally satisfactory results were obtained by the use of the more available CO_2-snow. After the tissue was fixed in a CO_2-snow-ether mixture it was dried with filter paper and weighed quickly in a closed weighing bottle. It was then ground in a mortar with sufficient CO_2-snow to prevent thawing. Towards the end of the grinding, a small amount of coarsely ground glass was added to ensure fine pulverization. The powder was then transferred quantitatively into an Erlenmeyer flask containing the calculated amount of cold 5% trichloracetic acid.

Results. It can be seen from Table I that the phosphocreatine values for intact dog hearts obtained with the CO_2-snow procedure

* This study has been supported by a grant from the American Heart Assn.

TABLE I. High-Energy Phosphate Content of Intact Mammalian Hearts. Heart tissue removed under pentobarbital anesthesia with artificial respiration. All values are expressed as mg phosphorus per 100 g fresh tissue.

Species	No. of animals	Method of freezing	Phosphocreatine-P, mean ± S.E.	Inorganic-P, mean ± S.E.	Labile "nucleotide"-P, mean ± S.E.
Dog	19	CO_2-snow	18.2 ± .88	19.64 ± .68	37.1 ± .7
"	18	Liquid air	19.9 ± .84	20.14 ± .72	39.5 ± .98
Cat	6	"	15.04 ± 1.45	18.13 ± 1.34	34.3 ± .62
Rabbit	5	"	14.23 ± 1.66	19.32 ± 1.17	33.9 ± 1.2
Rat	6	"	9.0 ± .0	23.6 ± .33	29.9 ± .97

TABLE II. High-Energy Phosphate Values on Same Sample of Frozen Dog Ventricle Obtained by Two Methods: One involves grinding the frozen tissue with liquid air or CO_2-snow prior to treatment with trichloracetic acid, the other omits the grinding with liquid air or CO_2-snow.

Method	No. of animals	Phosphocreatine-P, mean ± S.E.	Inorganic-P, mean ± S.E.	Labile "nucleotide"-P, mean ± S.E.
		Intact hearts		
Liquid air	11	18.14 ± 1.3	19.62 ± .97	37.37 ± .68
Trichloracetic acid	"	10.26 ± .89	27.88 ± .79	38.6 ± .65
		Failing isolated hearts		
Liquid air	6	25.23 ± 2.13	16.0 ± .11	37.81 ± .82
Trichloracetic acid	"	15.56 ± 1.78	25.68 ± 1.21	38.0 ± .14

are not significantly lower, as might possibly be expected, when compared with those obtained by using liquid air. It will also be noted that these values are higher than those reported by previous authors, being over 3 times as high as those of Cruickshank *et al.* (6) and twice as high as those of Pollack *et al.* (7) and of Wollenberger(8). Although the procedures used by these 2 latter authors included artificial respiration and also ensured quick freezing of the heart tissue, Pollack *et al.* put the frozen tissue through a meat grinder before extracting with acid, and Wollenberger ground the frozen tissue in ice-cold acid. It is quite possible that in both procedures the tissues were permitted to thaw before the trichloracetic acid had had time to diffuse thoroughly through the tissue and stop the enzymatic hydrolysis of the labile phosphocreatine. To test this type of procedure we carried out the experiment indicated in Table II. In this experiment a piece of frozen tissue was broken in two, one was powdered with liquid air or CO_2-snow as described above and the other was ground in ice-cold trichloracetic acid. The average phosphocreatine value of the 11 intact hearts was 18.1 mg %, as determined by our standard technic as compared with the value 10.3 mg % obtained when the frozen tissue was ground directly with ice-cold trichloracetic acid (Table II). Equally sharp differences were obtained with failing hearts: 25.2 mg % as compared with 15.6 mg %. It is thus clear that in dealing with labile compounds like phosphocreatine, the tissues should be powdered in the frozen state before extraction with acid.

Table III shows that the phosphocreatine content of the non-failing isolated dog heart is not higher than that of the normal heart in the intact animal. The lower results obtained by Pollack *et al.*(7) and Wollenberger(8) for the normal intact hearts as compared with the values they obtained for the isolated hearts cannot be explained by suggesting that the phosphocreatine in the intact heart is enzymatically more labile than in the isolated heart. It is seen from Table II that the phosphocreatine value of the failing isolated heart obtained by using their procedure (15.6 mg %) is significantly lower (Table II) than those obtained by using the standard procedure (25.2 mg %). It is possible, however, that in Wollenberger's experiments, the isolated hearts may have undergone some degree of failure because of the longer period of 82

TABLE III. High-Energy Phosphate Content of Normal and Failing Isolated Dog Hearts (Heart-Lung Preparations). Values are expressed as mg phosphorus per 100 g fresh tissue.

Time of survival in min.	Heart rate Initial	Heart rate Final	Systemic output in ml/min. Initial	Systemic output in ml/min. Final	Serum glucose, mg %	Phosphocreatine	Inorganic-P	Labile "nucleotide"-P
				Normal hearts				
30	192	192	744	708	20	14.6	20.1	35.5
"	168	164	684	624	74	21.6	18.3	35.1
"	144	140	792	888	41	18.2	13.1	33.2
"	156	140	804	708	66	20.9	13.1	38.8
"	176	164	552	552	27	20.5	20.4	30.1
"	144	144	780	732	223	24.5	13.7	33.2
"	156	120	720	792	50	13.7	19.3	31.8
				Mean ± S.E.		19.15 ± 1.47	16.86 ± 1.29	33.9 ± 1.07
				Failing hearts (pentobarbital)				
75	156	128	456	300	—	20.4	21.4	—
53	180	124	912	180	44	20.5	15.9	30.2
65	220	148	708	276	75	30.9	12.2	38.8
40	120	128	660	324	67	23.6	15.5	38.4
52	120	124	720	216	60	27.4	16.3	37.4
50	164	128	828	240	106	23.0	16.9	38.5
30	140	116	792	84	111	29.8	17.2	36.9
80	160	124	528	228	70	16.7	17.8	36.9
26	140	104	—	10	—	24.3	11.9	37.0
				Mean ± S.E.		23.5 ± 1.58	16.9 ± .99	37.8 ± 1.05

minutes which elapsed in his experiments after the heart was isolated. An indication of this is given by the fact that the average systemic output in his experiments was 413 ml, whereas in ours it was 711 ml, although the temperature of the blood, the venous inflow level, and the artificial resistance were not significantly different in both sets of experiments. However, the resistance of the venous inflow tube may have been different: in our case the tube was 8 cm long with an inner diameter of 0.6 cm.

Table III also shows that in barbiturate-induced failure the phosphocreatine content of the heart-lung preparation is higher than that of the intact heart. This finding is in harmony with that of Wollenberger, although the difference is not as striking as in his results. This constitutes further evidence that in barbiturate failure which is known to respond to digitalis, there is probably an interference with energy utilization rather than with energy production(13). It should be mentioned, however, that Greiner(14), using a different preparation, obtained results which indicate that the oxygenated, severely hypodynamic papillary muscle of the cat is characterized by a decrease in adenosine triphosphate content, and that digitalis treatment not only corrects the muscle failure but also restores the adenosine triphosphate level to normal.

Summary. 1. "Resting" values for the phosphocreatine content of the ventricles of several mammalian species are reported and an explanation is offered for some of the low values recorded in the literature. The necessary precautions are noted if maximal values are to be obtained. 2. The isolated dog heart (heart-lung preparation) does not contain more phosphocreatine or adenosine triphosphate than the intact heart provided that heart failure has not set in. If, on the other hand, failure is induced by barbiturates the level of phosphocreatine increases without a change in the labile "nucleotide" phosphorus. This constitutes further evidence that this kind of heart failure is not due to the lack of production of high-energy phosphate bonds.

1. Bodansky, M., *J. Biol. Chem.*, 1935, v109, 615.

2. Schumann, H., *Z. Gesamt. Exp. Med.*, 1939, v105, 577.

3. Shelley, W. B., Code, C. F., and Visscher, M. B., *Am. J. Physiol.*, 1943, v138, 652.

4. Kimura, T. E., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 419.

5. Eggleton, G. P., and Eggleton, P., *J. Physiol.*, 1929, v68, 193.

6. Cruickshank, E., McClure, G. S., and Mackin-

tosh, D. C., *Physiol. Rev.*, 1936, v16, 623.

7. Pollack, H., Flock, E., and Bollman, J. L., *Am. J. Physiol.*, 1934, v110, 105.

8. Wollenberger, A., *Am. J. Physiol.*, 1947, v150, 733.

9. Pollack, H., Flock, E., Essex, H. E., and Bollman, J. L., *Am. J. Physiol.*, 1934, v110, 97.

10. Burns, H., and Cruickshank, E., *J. Physiol.*, 1937, v91, 314.

11. Mommaerts, W. F. H. M., *Muscular Contraction*, 1950, Interscience, New York, p32.

12. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1929, v81, 629.

13. Visscher, M. B., *Blood, Heart and Circulation, AAAS Pub.*, 1940, v13, 176.

14. Greiner, T., *J. Pharmacol.*, 1952, v105, 178.

Received July 3, 1953. P.S.E.B.M., 1953, v84.

Electrocardiograms and Serum Electrolyte Levels in Hamsters with Adrenal Insufficiency and Hypercorticalism.* (20617)

LELAND C. WYMAN, GEORGE P. FULTON, FREDERICK N. SUDAK, AND GEORGE N. PATTERSON.
(With technical assistance of Joseph and Norbert Benotti.)†
From the Department of Biology, Boston University, Boston, Mass.

In previous reports, Wyman, Fulton, and Shulman have described the circulatory changes in the transillumined cheek pouch of the hamster, (*Mesocricetus auratus*) after adrenalectomy and during treatment of the normal animal with cortisone(1). Vasoconstriction occurred consistently during cortisone administration. Furthermore, occasional vasomotion, frequent vasoconstriction, bradycardia, and slow blood flow occurred prior to death from adrenalectomy. Zweifach and Chambers, however, described a loss of vascular tone and absence of vasomotion in the mesoappendix of the adrenalectomized rat(2). In view of the evidence that hyperpotassemia may stimulate smooth muscle(3,4), a possible explanation for the discrepancy might be significant differences in serum potassium levels in the 2 species after adrenalectomy. Snyder and Wyman(5) reported a rise in the serum potassium of over 100% in the adrenalectomized hamster as compared with reported elevations of less than 50% in the adrenalectomized rat. In order to test the possible relationship between vasoconstriction, cardiac changes, and serum electrolyte levels, sodium and potassium determinations were made and electrocardiograms taken for each day following adrenalectomy and every other day during cortisone administration.

Materials and methods. Twenty-four male hamsters, 80 to 100 g in weight, were adrenalectomized using a dorsolateral approach in a single operation. Purina Laboratory Chow and tap water were given *ad libitum* and the hamsters received the usual postoperative care. The 24 hamsters were divided into 3 groups of 8 animals each, and on every third day an electrocardiogram was taken and blood was drawn by heart puncture for electrolyte determinations on each group so as to provide data for each postoperative day for their period of survival. Three groups of 8 intact hamsters were similarly used for cortisone studies except the electrocardiograms and heart punctures were made every other day throughout the entire period. Each animal was given a daily injection of 5 mg cortisone acetate (Cortone, Merck)‡. *Electrocardiograms* were obtained by means of hypodermic

* This investigation was supported in part by a research grant from the National Heart Institute of the National Institutes of Health, Public Health Service.

† Messrs. Benotti of the Boston Medical Laboratory made the electrolyte determinations. The work has benefitted immeasurably by numerous conferences with Dr. Maurice H. Shulman. We are indebted to Dr. David Littman for advice in interpreting the electrocardiograms, to Mr. Frederick W. Maynard for technical photographic advice, and to Miss Lois L. Smith and Miss Roma Kagan for valuable assistance.

‡ Kindly supplied by Dr. Elmer Alpert, Merck and Co.