Relation Between Work and Labile Phosphate Content in the Isolated Dog Heart

By ALBERT WOLLENBERGER, PH.D.

Starling heart-lung preparations of the dog (with only minor differences in heart rate) were subjected to changes in work load to study their effect on the amount of labile phosphate in the left ventricle. While increasing the work load three to fourfold by increasing output caused no change, doubling the work load by increasing the resistance was accompanied by a marked reduction in labile phosphate. The data suggest that the heart expends phosphate bond energy chiefly in overcoming resistance rather than in systolic emptying.

IT HAS long been known from studies in the isolated dog heart that the rate of cardiac oxygen consumption depends on arterial blood pressure to a much greater extent than on cardiac output. This finding was recently confirmed by Katz and Alella and co-workers in the dog heart in situ. Sarnoff and associates, using a new heart preparation, the isolated dog heart supported by a dog, came to a similar conclusion.

The renewed interest in the factors determining the metabolic rate of the heart has prompted us to present the results of experiments performed some years ago with the intent of finding what effects changes in arterial blood pressure and in cardiac output might have on certain high-energy phosphates in the myocardium that are essential for muscular activity. In our opinion, these results, conform with the findings made in the investigations on the physical determinants of myocardial oxygen consumption and complement them from the biochemical point of view.

METHODS

Starling heart-lung preparations of dogs in the postabsorptive state were used. They were prepared and activity was measured as previously reported. The work performed by the left ventricle was computed as the product of its minute output and the mean systemic arterial blood pressure. The kinetic energy imparted to the blood entering the systemic and coronary circuits (acceleration work) was neglected, except in the preparations with a high output in which it was calculated by the equation: work = \(\frac{m v^2}{2 g}\), where \(m\) = weight of blood ejected, \(v\) = mean linear velocity of blood during ejection, and \(g\) = acceleration by gravity. Values of \(v\) were obtained by dividing the volume of blood ejected by the cross-sectional area of the arterial cannula at its narrowest point and of the orifices of the coronary arteries, respectively, and by the duration of ejection. The latter was estimated from tracings of the pulmonary arterial pressure.

During the preliminary part of the experiments the level of blood in the venous supply reservoir (venous inflow level) was maintained at about 110 mm above the opening of the inferior vena cava, and the arterial resistance was held constant at 80 mm Hg. Under these conditions, which in this laboratory are standard for the heart-lung preparation of the dog, the work done by the left hearts amounted to approximately 0.7 Kg M./min. Variations in work load were then brought about by changing the venous inflow level or the arterial resistance. After the hearts had been subjected to the altered conditions of work for at least 10 min.—a period considered to be ample for the establishment of a new steady metabolic state—a piece of left ventricular muscle from the apical region was cut off and immediately immersed in liquid nitrogen. The frozen muscle was analyzed, according to procedures outlined or referred to previously, for the following acid-extractable high-energy phosphate fractions: the orthophosphate liberated after 30 min. of molybdate-catalyzed acid hydrolysis at room temperature ("labile" phosphate), and the orthophosphate liberated additionally by 1 N hydrochloric acid at 100 C. in 7 min. ("7-minute" phosphate). The phosphate freed by the treatment with acid molybdate solution is often taken to originate entirely from phosphocreatine. However, in extracts of mammalian tissues, other labile phosphate compounds contribute in varying degrees to this phosphate fraction. In the heart of the well-oxygenated, unpoisoned, heart-lung preparation, phosphocreatine probably accounts for not less than...
75 per cent of the "labile" phosphate. The "7-minute" phosphate is customarily assumed to represent the easily hydrolyzable phosphate groups of nucleotides, in the present instance probably chiefly adenosine triphosphate and adenosine diphosphate. It has been pointed out by Fawaz and Hawi that some breakdown of labile phosphate probably occurs in heart muscle when the tissue is extracted in the original frozen state, as was the case in the present experiment, instead of being extracted as frozen powder. However, this weakness in analytic procedure should not obviate the significance of differences between values of labile phosphate obtained under different experimental conditions.

RESULTS

As can be gathered from the data in table 1, the deviations produced from the "standard" conditions of work, prevailing during the preliminary part of the experiments and in the control runs, were large in all cases. In the first 2 experiments listed in the table, the venous inflow level was raised by 200 mm., causing large increases in stroke volume and minute output. There was a concomitant minor increase in arterial pressure. The work done by the left ventricles exceeded that in the control preparations.

TABLE 1.—Energy-rich Phosphate Fractions in Hearts of Heart-Lung Preparations Differing in Work Performance

<table>
<thead>
<tr>
<th>Dog *</th>
<th>Weight of ventricles (Kg.)</th>
<th>Time of survival (min.)</th>
<th>Inflow and resistance</th>
<th>Heart rate</th>
<th>Mean systemic arterial pressure</th>
<th>Total output of left ventricle</th>
<th>Coronary flow</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls†</td>
<td>±S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>13.4</td>
<td>92</td>
<td>86</td>
<td>362†</td>
<td>83</td>
<td>12</td>
<td>156</td>
<td>187</td>
</tr>
<tr>
<td>65</td>
<td>10.1</td>
<td>67</td>
<td>75</td>
<td>825†</td>
<td>77</td>
<td>15</td>
<td>144</td>
<td>117</td>
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<td>47</td>
<td>9.7</td>
<td>77</td>
<td>75</td>
<td>133</td>
<td>12</td>
<td>10</td>
<td>142</td>
<td>177</td>
</tr>
<tr>
<td>152</td>
<td>10.5</td>
<td>74</td>
<td>60</td>
<td>144</td>
<td>169</td>
<td>12</td>
<td>152</td>
<td>184</td>
</tr>
<tr>
<td>51</td>
<td>7.5</td>
<td>54</td>
<td>118</td>
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<td>12</td>
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<tr>
<td>153</td>
<td>11.4</td>
<td>60</td>
<td>71</td>
<td>42</td>
<td>42</td>
<td>12</td>
<td>130</td>
<td>30</td>
</tr>
</tbody>
</table>

* Height above opening of the inferior vena cava.
† Mean of 6 control heart-lung preparations (±S.D. = standard deviation). For individual values, see Wollenberger.10
† The figures in italics lie outside the fiducial limits of the respective values for the control preparations (mean ± S.D. x t) at p = 0.02.
§ Including 0.472 Kg. M./min. acceleration work.
|| Including 0.724 Kg. M./min. acceleration work.

In the next two experiments (dogs 47 and 152), a large rise in systemic arterial pressure was brought about by doubling arterial resistance. Coronary flow increased greatly, but the total output was only slightly augmented. The heart rate remained unaltered. Due to the rise in arterial pressure, the work done by the left heart was almost doubled. This change was associated with a significant reduction in the concentration of labile phosphate; the levels in the left myocardium lying 40 to 45 per cent below those encountered under standard work conditions. The "7-minute" phosphate was not significantly changed.

In the third set of experiments, the hearts were induced, by means of limiting the venous blood supply, to perform negligible amounts of work, consisting essentially, for the left hearts, in maintaining the coronary circulation. In 1 heart-lung preparation (no. 53) the arterial cannula was closed; in the other 2 preparations arterial resistance was lowered just to the point where a trickle of blood began to pass into the systemic circulation. Uninfluenced by these manipulations, the systemic arterial pressures recorded were very low. The chemical determinations differ significantly from those found in the control preparations.

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nations in all 3 hearts revealed significant increase in the content of labile phosphate. It is not possible to say whether this increase was more specifically related to diminished output or to diminished arterial pressure. From the results of the preceding 4 experiments in table 1, the latter alternative seems to be more probable. In 1 heart (no. 51) a rather low beat frequency may have contributed to the accumulation of labile phosphate.14

**DISCUSSION**

While it would be premature to draw final conclusions from the small number of experiments presented, nevertheless the results may be considered indicative of a trend toward a lowering of the concentration of labile phosphate in the myocardium in response to a large increase in arterial resistance and pressure. This effect is not due to an increase in cardiac work per se since, at more or less constant heart rates, large increases in systolic discharge without a major rise of arterial pressure do not cause depletion of labile phosphate. Comparable observations have been made in skeletal muscle by Nachmansohn,16 who reports that breakdown of phosphocreatine is greater in isometric than in isotonic contraction. On the assumption that the metabolic change observed on raising arterial pressure reflects an acceleration of the utilization and not an impairment of the synthesis of energy-rich phosphate, it might be inferred that the heart expends phosphate bond energy chiefly in overcoming resistance, i.e., during the isometric contraction phase of systole, rather than in systolic emptying.

In previous studies dealing with the relationship between the work and the metabolism of the mammalian heart,1,3 attention was focussed mainly on oxygen consumption and the importance of arterial pressure as contrasted to the stroke volume was stressed. Biochemically speaking, the rate of oxygen consumption of a tissue is a function of the concentration of inorganic phosphate and of phosphate acceptors.16 Therefore, a decrease in the concentration of labile phosphates, implying a rise in the concentrations of inorganic phosphate and phosphate acceptors, can be expected to stimulate oxygen consumption. On this basis the present findings are in harmony with the results of the studies on the hemodynamic determinants of cardiac oxygen consumption and suggest an explanation for them.

**SUMMARY**

Preliminary findings made in the heart-lung preparation of the dog are reported, showing the amount of labile phosphate (probably mostly phosphocreatine) in the left ventricle to be markedly diminished following large increases in systemic arterial pressure. This effect is apparently not due to an increase in work per se, since there was no change in labile phosphate when, at more or less constant heart rates, large increases in cardiac output were produced without a major rise in arterial pressure. Elevated concentrations of labile phosphate were encountered in hearts doing very little work.

**SUMMARIO IN INTERLINGUA**

Datos preliminari es reportate ab observationes in canin preparatos cardio-pulmonar. Le quantitate de phosphato (probabilemente super toto phosphocreatina) in le ventriculo sinistre se monstra marcatemente diminuite post major augmentos del systemic pression arterial. Iste effecto es apparentemente non causate per le augmento del travalio per se, proque nulle alteration del phosphato labile esseva notate quando—sub conditiones de un plus o minus constante rapiditate cardiac—grande augmentos del rendimento cardiac esseva effectuate sin considerable alteraciones del pression arterial. Elevate concentrationes de phosphato labile esseva determinate in cordes con molto basse nivellos de travalio.

**REFERENCES**

1 Evans, C. L., and Matsuoka, Y.: The effect of various mechanical conditions upon the gaseous metabolism and efficiency of the mammalian heart. J. Physiol. 48: 378, 1915.


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