Preliminary Notes

PN 10027

Activation of α-glucan phosphorylase and related metabolic changes in dog myocardium following arrest of blood flow

A recent report by KLARWEIN, LAMPRECHT AND LOHMANN[1] makes it evident that the warm-blooded heart responds to sudden ischemia with a rapid shift in the pattern of its metabolism. Marked increases in the activity of α-glucan phosphorylase *a* (EC 2.4.1.1) and in the levels of glucose 6-phosphate, α-glycerophosphate, and lactate are among the changes which were noticed by these authors in the dog heart after 0.5 min of ischemia. The publication of these findings is prompting us to present results, taken from the draft of a doctoral thesis (E.-G. K.), which agree qualitatively with some of the data of KLARWEIN *et al.*, but differ from them in several important quantitative respects.

Pieces of the left ventricular muscle of adult dogs, whose thorax and pericardium were opened under pentobarbital (Nembutal, kindly donated by Dr. R. K. RICHARDS, Abbott Laboratories, North Chicago, Ill.) anesthesia and positive pressure breathing, were instantly frozen *in situ* by being forcefully compressed to a layer maximally 1.5 mm in thickness by means of a pair of aluminum tongs precooled to about −190° (ref. 2). Simultaneously with this manoeuvre, executed at the time designated as 0 sec, the aorta ascendens was severed. Additional pieces of the left ventricle, which continued to contract, were frozen by the same method approx. 5,

TABLE I

PHOSPHORYLASE *a* CONTENT OF THE LEFT VENTRICLE OF THE DOG HEART BEFORE AND AFTER PRODUCTION OF MYOCARDIAL ISCHEMIA

Portions of the frozen ventricular muscle specimens weighing 50–100 mg were reduced to microscopic particles by a 10-sec treatment in the "Ultraturrax" disintegrator* in the presence of 20 vol. of an ice-cold solution, 0.02 M in NaF and 0.002 M in EDTA and containing 1 mg of washed Dowex-1 X8 (200–400 mesh, Cl^- form) per mg muscle[3]. Phosphorylase activities were measured according to CORI AND ILLINGWORTH[3] in the 8-fold diluted supernatant of the centrifuged particle suspension. The orthophosphate liberated by phosphorylase was measured by the method of FISKE AND SUBBAROW[5]. The figures for phosphorylase *a* activity, representing the results of experiments on 4 dogs, are the means ± standard deviations.

Time (sec) after sectioning the aorta, means (ranges in parentheses)	*Phosporylase a (% of total phosphorylase activity)*
0 (0)	0.0 ± 0.0
5.4 (3.7–6.5)	11.0 ± 1.8
14.7 —	54.8**
19.5 (18.3–20.0)	45.3 ± 5.0
32.8 —	35.1**
67.5 (61.7–72.0)	20.2 ± 7.7

* Janke und Kunkel, Staufen i. Br., West Germany.
** 1 dog.

20, 35, and 65 sec thereafter. The time of compression of the tissues in the cooling tongs was determined to the nearest 0.1 sec from the electrocardiographic tracings.

Analyses of the frozen muscle specimens revealed, first of all, that phosphorylase *a* activity at 0 time was nil (Table I). Thus it appears that in the well-oxygenated cardiac ventricle of the anesthetized and resting dog all of the enzyme is present in the *b* form. Rapid conversion to the *a* form occurred upon arresting the blood flow. 15 sec after cutting the aorta the phosphorylase *a* content of the muscle had risen to a peak value of 55% of the total enzyme and began then to decline. Prior administration of dichloroisoproterenol (obtained through the courtesy of Dr. K. K. CHEN, Eli Lilly and Company, Indianapolis, Ind.) in concentrations blocking the phosphorylase-activating effect of epinephrine and sympathetic nerve stimulation[6] reduced the activation of the enzyme. Total phosphorylase (*a* + *b*) content remained unchanged throughout the period of the experiment at values averaging 12.9 μmoles P per g heart per min (415 Cori units/g).

In preliminary experiments it had been ascertained that the amount of Dowex-1 added to the solution used for extracting phosphorylase from dog-heart muscle did not alter the total phosphorylase activity. It was found, however, in confirmation and extension of an observation made by HAUGAARD *et al.*[3], that the ion-exchange resin, which is added for the purpose of removing AMP, lowers the yield of the enzyme from rat and guinea-pig heart muscle. Using an alternative means of avoiding activation of phosphorylase *b* by AMP extracted from the tissue or formed in the extract, namely, high dilution (400–800 ml/g tissue) of the extract[3], phosphorylase *a* was found, as in the experiments in Table I, to be absent in non-ischemic dog-heart muscle.

Activation of α-glycan phosphorylase in the ischemic ventricles was accompanied

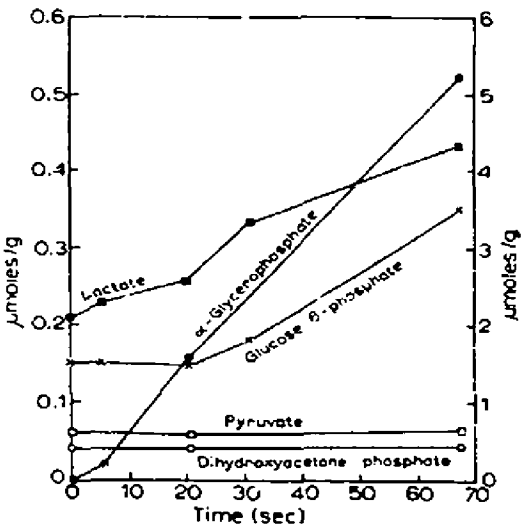

Fig. 1. Mean levels of end products and of some intermediates of glycolysis in the left ventricle of 4 dogs before and after the onset of myocardial ischemia. Pieces of the frozen muscle samples weighing 300–500 mg were pulverized and extracted at 0° with 5 vol. of 0.5 N $HClO_4$–0.00125 M EDTA solution. Following neutralization with 4 N KOH the metabolites listed in the graph were determined by enzymic methods[7]. The right-hand scale of the ordinate refers of the tissue content of lactate, the left-hand scale to the content of the other metabolites.

by a rapid rise in lactate and α-glycerophosphate, while pyruvate and dihydroxyacetone phosphate remained at aerobic steady-state levels (Fig. 1). The rise in glucose 6-phosphate followed that of lactate and α-glycerophosphate with a delay of 20 sec. It may be noted that the aerobic steady state level of α-glycerophosphate in the dog ventricle is so low as to escape detection by the present analytical method. This might be related to the further finding that the mitochondria of the dog heart are capable of oxidizing α-glycerophosphate at a relatively high rate.

The arrest of blood flow and oxygen delivery to the heart, resulting presumably in quick cessation of aerobic ATP synthesis, entailed a rapid disappearance of phosphocreatine (0.5 μmole/sec/g muscle during the first 5 sec) and a concomitant rise in orthophosphate, while adenine nucleotide levels were hardly changed (Fig. 2). Of relevance to the problem of control of α-glycan phosphorylase activity in heart muscle is the observation (Fig. 2) that the concentration of orthophosphate (assuming the compound to be evenly distributed in the tissue) did not reach the value of the K_m for phosphorylase *a*, which is 7 mM (ref. 9), until 1 min of anoxia had elapsed. On the other hand, as noted also by KLARWEIN *et al.*[1], AMP is present in aerobically beating dog myocardium in a concentration (0.2 mM) allowing for more than half-maximum activity of phosphorylase *b*. Whether such activity is possible at the low aerobic orthophosphate concentration in the tissue remains at present a matter of conjecture, since the K_m value of orthophosphate for phosphorylase *b* is not known.

A word of comment is called for concerning the large discrepancy between the present value of 415 Cori units of total phosphorylase per g dog myocardium and the values for the total phosphorylase content of dog and rat heart muscle reported in the literature[1,6,10], which range from 6750–10 200 Cori units/g, thus exceeding the

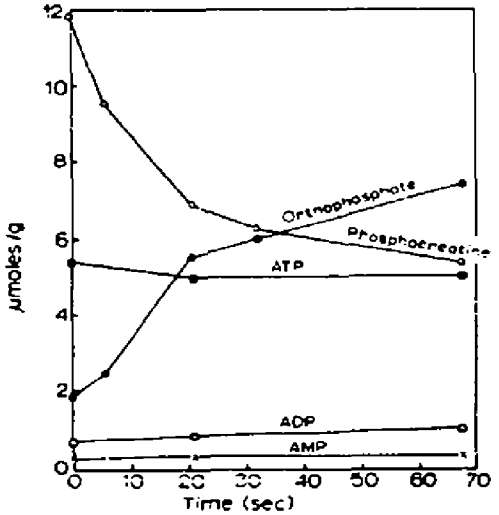

Fig. 2. Mean levels of principal high-energy phosphates and of AMP and orthophosphate in the left ventricular musculature of 4 dogs before and after the onset of myocardial ischemia. ATP, ADP, and AMP were determined by enzymic methods ("Test combination", C. F. Boehringer and Soehne, Mannheim) in the neutralized $HClO_4$ extract of the muscle (see Fig. 1). Orthophosphate and phosphocreatine, the latter as acid-molybdate-labile phosphate, were determined in the unneutralized portion of the extract by the method of WAHLER AND WOLLENBERGER[8].

figure of 4000 units/g given for rat skeletal muscle[6]. Recalculation of the total phosphorylase content from data on enzyme activity in the cardiac tissue extracts, presented in two of the publications just cited[8,10], yields values of 408 and 675 Cori units/g for dog ventricular muscle and whole rat heart, respectively, in excellent agreement with values obtained in the present and other experiments performed in this laboratory. It would be surprising indeed to find cardiac muscle to be richer in phosphorylase than skeletal muscle, since it is comparatively poorly equipped with other glycolytic enzymes. Moreover (see ref. 11), its glycogenolytic power *in vivo* does not compare to that of skeletal muscle.

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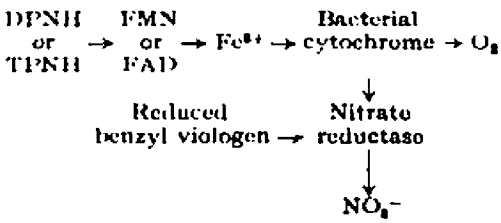
The purification of nitrate reductase of *Achromobacter fischeri*

The purification and properties of the nitrate reductase (cytochrome: nitrate oxidoreductase, EC 1.9.6.1) of *Achromobacter fischeri* have been described in an earlier communication[1]. Evidence was presented to show that the sequence of electron transport for nitrate reduction in *A. fischeri* is as follows:



The purified enzyme so obtained was strongly colored and showed absorption bands at 550 mμ, 520 mμ and 419 mμ in the reduced state. Although a certain amount of indirect evidence (inhibitor studies) was obtained which indicated that the terminal nitrate reductase from *A. fischeri* does not contain iron porphyrin, it was