Metabolism of Ischemic Cardiac Muscle

By JOHN P. KALTENBACH, PH.D., AND ROBERT B. JENNINGS, M.D.

The aerobic metabolism of homogenates of normal and ischemic left ventricle was compared in dogs at various time intervals (15 min. to 24 hours) after high ligation of the circumflex branch of the left coronary artery. Glucose and fructose-1,6-diphosphate were used as substrates. The injured tissue was taken from the posterior papillary muscle (PP) and the non-ischemic tissue from the anterior superior septum (LV). Both oxygen consumption and organic phosphorus ($\Delta$ organic phosphorus) were slightly decreased in PP at 15 and 30 min. after ligation. Between 30 and 60 min., there was a sharp decrease in oxygen consumption and $\Delta$ organic phosphorus falling to 40 to 50 per cent of control values at 60 min. A further decrease was observed at 180 min., and at 24 hours there was a complete loss of oxidative metabolism in the PP. A striking increase in organic phosphorus metabolism and oxygen consumption was observed in the non-ischemic portion of the myocardium 60 and 180 min. after ligation.

If a major branch of a coronary artery in the dog is occluded for 25 min. or more a number of myocardial cells in the ischemic area will be irreversibly injured. These injured cells are readily recognizable several hours later by the fact that they have become necrotic. The complete structural disorganization characteristic of necrosis is the only available absolute criterion that irreversible injury has occurred in any given cell. Conversely, ischemic cells that do not become necrotic are, by definition, reversibly injured. Normal, reversibly, and irreversibly injured cells are all indistinguishable by routine microscopic and chemical means immediately before and after the development of the irreversible state.

The delayed appearance of necrosis in the heart following onset of irreversible injury to a group of myocardial cells makes it difficult to study the events occurring in the genesis of the irreversible state. However, it is possible to perform such studies through use of an experimental infarct of predictable location and character that has recently been described. Samples of reversibly and irreversibly injured tissue can be obtained from this infarct without difficulty, and are used in the studies described in this paper. The aerobic metabolism of homogenates of normal, reversibly, and irreversibly injured myocardium is reported. Glucose and fructose-1,6-diphosphate have been used as substrates and the results correlated with concomitant changes in organic phosphate metabolism.

Methods

Adult mongrel dogs were used. They were housed in air conditioned quarters and allowed as much food (Bordens Dog Chow) and water as they wanted.

The animals were divided into a control group and an experimental group. All animals were anesthetized with approximately 25 mg./Kg. sodium pentobarbital (Nembutal) injected intravenously. Ten to 15 min. before operation, 50 mg./Kg. of procaine amide (Pronestyl), was injected intramuscularly. Oxygenation was maintained through an endotracheal tube by a Harvard model 1063 respirator pump at a rate of 3 to 4 L./min.

The control group consisted of 8 dogs, 5 males and 3 females. Four controls were killed immediately after anesthetization and 2 were killed 5 to 10 min. after anesthetization. Two were killed after 180 min. of anesthesia.

The experimental group consisted of 16 dogs, 6 males and 10 females. Four controls were killed immediately after anesthetization and 2 were killed 5 to 10 min. after anesthetization. Two were killed after 180 min. of anesthesia.

The experimental group consisted of 16 dogs, 6 males and 10 females. Homogenous myocardial infarcts were produced in the posterior papillary muscle of the left ventricle by ligation of the circumflex artery as described previously. Tissue was not used from any dog in which the cyanosis that followed ligation did not extend to within one centimeter of the apex posteriorly. This criterion was used because serial observations have shown that infarcts associated with less degrees of cyanosis do not necessarily include the entire posterior
Table 1

Control Values from Posterior Papillary Muscle (PP) and Left Ventricle (LV)

The data in Table 1 are the averages, with standard deviations, of six controls. The three hour controls represent two animals killed after 180 minutes of anesthesia. Medium G contains glucose as substrate, and Medium F contains fructose-1,6-diphosphate as substrate.

<table>
<thead>
<tr>
<th></th>
<th>mg N/Gm. Tissue</th>
<th>µg TOTAL P/mg N</th>
<th>µg INORG. P/mg N</th>
<th>µl O2/hour/mg N at 37°C</th>
<th>µg ORG. P/hour/mg N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL S</td>
<td>PP</td>
<td>LV</td>
<td>medium G</td>
<td>medium F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.2±1.57</td>
<td>27.9±1.85</td>
<td>13.3±1.66</td>
<td>134.4±25.4</td>
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<tr>
<td>3 HOUR</td>
<td></td>
<td>24.4</td>
<td>22.0</td>
<td>139</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>CONTROL S</td>
<td>LV</td>
<td></td>
<td>19.6±18.02</td>
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<td></td>
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<td>24.4</td>
<td>22.0</td>
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<tr>
<td></td>
<td></td>
<td>25.2</td>
<td>21.8</td>
<td>134</td>
<td>134</td>
</tr>
</tbody>
</table>

Papillary muscle. Three to five dogs were studied at each desired time interval: 15, 30, 60, and 180 min. Two animals were killed 24 hours after ligation. After the desired period of ischemia had elapsed, the heart was quickly excised, and the segment containing the posterior papillary muscle cut out of the left ventricle.

Tissue samples were taken from the upper two-thirds of the papillary muscle after the chordae tendineae and endocardium were removed. Non-ischemic left ventricular tissue was obtained from the anterior superior portion of the interventricular septum.

Samples of control and injured heart weighing between 250 to 325 mg. were used. The tissues were weighed on a torsion balance and then homogenized (Potter-Elvehjem glass homogenizer) for approximately one minute in 2.0 ml. of 1.15 per cent KCl. Prior to homogenizing, the muscle tissue was placed on the inside rim of the homogenizer tube and quickly minced with a pair of iris scissors. This facilitated homogenization of the muscle. Following homogenization, additional 1.15 per cent KCl was added to make the homogenate to a 5 per cent concentration. Twenty minutes were allowed from the time the heart was removed from the animal until the Warburg flasks were placed in the bath.

Standard Warburg technic was used in the manometric experiments. In experiments labeled Medium G, the flasks contained: 0.56 mM glucose, 0.12 mM nicotinamide, 0.002 mM ATP, 5.4 x 10^{-4} mM DPN (Pabst), 0.8 mL of 0.0667 M phosphate buffer, and 1.0 mL of 5 per cent KCl (1.15 per cent) homogenate. Total volume was 3.0 mL. Experiments labeled Medium F contained 0.008 mM fructose-1,6-diphosphate in place of the glucose. The flasks were incubated for one hour at 37° C with air as the gas phase and alkali in the center-well for absorption of carbon dioxide. The data obtained is a measure of glucose and/or fructose-1,6-diphosphate oxidation since in the absence of added substrate there was a negligible oxygen uptake (less than 3 µl O2/hour/mg. nitrogen). In addition, oxidation occurs to only a slight extent when these substrates are added without cofactors (less than 17 µl O2/hour/mg. nitrogen).

Total and apparent inorganic phosphorous were determined according to LePage. The apparent inorganic phosphorus includes the true inorganic fraction and the phosphate of creatine phosphate. The organic phosphorus values were obtained from the difference between total and inorganic phosphorus, correcting for tissue, reagent blanks and zero time controls. Organic phosphorus values were also calculated from the change in inorganic phosphorus. The results of both methods agreed well. All determinations were made in duplicate or quadruplicate.

Kjeldahl nitrogen determinations were made in duplicate on an aliquot of each homogenate. A thin section from the middle of the posterior papillary and a section of non-ischemic left ventricle were always taken for histological examination. A complete autopsy was performed on all animals. No significant naturally occurring diseases or abnormalities were noted.

Results

The results obtained in the control dogs, using homogenates of posterior papillary muscle (PP) and anterior superior septum (LV), are summarized in Table 1. The chemical data given in the first portion of this table show that there were no significant differences in the tissue levels of nitrogen, and total and inorganic phosphorus in these 2 different parts.
of left ventricle. The metabolic data obtained from these tissues after 1 hour incubation are also given in table 1, and show that there were no significant differences in oxygen consumption or organic phosphorus levels in these 2 tissues with glucose (G) or with fructose-1,6-diphosphate (FDP) as substrates. As would be expected, with glucose, which may act as a phosphate acceptor, there was a net increase in organic phosphorus (P + 43) after 1 hour incubation, whereas with FDP there was a net decrease in organic phosphorus (P − 47). The absence of any significant differences in the chemical and metabolic data obtained from the posterior papillary and the remainder of the left ventricle correlates well with previous observations on electrolyte and enzyme levels and indicates that these different parts of left ventricle are chemically and metabolically indistinguishable.

The three-hour control animals were included in table 1 in order to show the negligible effect of anesthesia in these experiments.

The oxygen uptake of homogenates of the non-ischemic left ventricle and the ischemic posterior papillary following various periods of ischemia are shown in figure 1. The oxygen consumption of the posterior papillary muscle was slightly decreased at 15 and 30 min. with both substrates. However, between 30 and 60 min. there was a sharp decrease in oxygen consumption in the injured muscle, reaching levels of 30 to 50 per cent of the control values 60 min. after ligation. A further decrease in oxygen consumption was observed at 3 hours, and at 24 hours the results indicate a complete loss of oxidative metabolism in the injured tissue. At both 1 and 3 hours, there is a consistently greater reduction in oxygen consumption with glucose than with FDP, but both are zero 24 hours after ligation.

The oxygen consumption of the non-ischemic left ventricular tissue is slightly increased with both substrates at 1 hour, and is 130 per cent of normal at 3 hours. By 24 hours, the oxygen consumption has returned to near the initial control level.

The amount of organic phosphorus present in the posterior papillary and left ventricle at various time intervals after ligation is shown in figure 2. The posterior papillary muscle shows a decrease in organic phosphorus, and this is a reflection of the concomitant increase in the tissue level of inorganic phosphorus. The change in organic phosphorus is considered a function of the change in inorganic phosphorus levels, as there were no significant variations of total phosphorus between the controls and the injured posterior papillary after various periods of ischemia.

The change in organic phosphorus after 1 hour incubation using homogenates of left ventricle and ischemic posterior papillary muscle is plotted in figure 3. The posterior papillary muscle shows a progressive decrease in organic phosphorus during the first hour, post-ligation, and by 3 hours, with both substrates, less than 50 per cent of the initial organic phosphorus is still intact and unsplit. The non-ischemic left ventricle shows a corresponding increase in organic phosphorus.
levels at 1 hour after ligation. This is most evident in the presence of glucose which may act as a phosphate acceptor. With FDP as a substrate, a comparable increase at 1 hour is noted, but at 3 hours the organic phosphorus has returned to the initial value. Except for the drop in organic phosphorus with FDP at 3 hours, the general shape of these curves is in accord with oxygen consumption data plotted in figure 1. It should be noted that the increased phosphorylation and oxygen consumption of the non-ischemic left ventricle at 1 and 3 hours correlates with an increase in tissue organic phosphorus level (fig. 2).

Discussion

The marked decrease in the in vitro phosphorylation and oxygen consumption of homogenates of ischemic posterior papillary muscle 60 min. after ligation of the circumflex branch of the left coronary artery is highly significant statistically (p < .01) and is noted with both substrates studied. The general exponential shape of the curves of decreasing oxygen consumption with time is also similar with both substrates. A further decrease in phosphorylation and oxygen consumption is noted after 180 min. of ischemia, and the posterior papillary muscle is metabolically dead 24 hours after ligation.

The period between 30 and 60 min. after ligation is of particular interest as this is the time during which there is the greatest decrease in the production of organic acid soluble phosphate and in the oxygen consumption of homogenates of the injured myocardium. The marked decrease in activity during this time interval indicates that the injury occurring in the ischemic myocardial cells at this time is of such proportions that homogenates of these cells can no longer maintain their metabolic activity even in the presence of excess substrate, co-factors and potassium.

Cellular disorganization at many different levels could explain the decrease in phosphorylation and oxygen consumption associated with this injury. An early and perhaps primary lesion may be in the membrane of the myocardial cells, or in the energy processes involved in maintaining the integrity of this membrane as the loss of enzymes, substrate, and cofactors from the cell to the general circulation through an injured membrane could easily lead to the decreased metabolism found in the homogenates. The loss of enzymes to the general circulation following necrosis of well differentiated cells is an amply documented phenomenon first demonstrated in heart muscle with glutamic oxaloacetic transaminase. Decreased levels of this enzyme in the posterior papillary muscle have since been shown to occur 40 to 70 min. after ligation of the circumflex artery and it is not unreasonable to expect that some other enzymes specifically involved in phosphorylation and aerobic glycolysis of glucose and FDP may also promptly begin to leave the irreversibly injured cells. Co-factor loss probably also occurs, but if this is a key phenomenon in producing the observed changes, ATP and DPN should not necessarily be included as they are present in excess in the in vitro experiments. Another type of cellular disorganization that could occur and lead to the defective homogenate metabolism is the inactivation of enzyme by prolonged ischemia. Such a process is a possibility but has not yet been demonstrated at this early time in this or in any other analogous situation.

Figure 2

*Figure 2*

*Tissue organic phosphate content (ordinate) after various periods of ischemia (abscissa).*

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The fact that carbohydrate metabolism in muscle is closely related to electrolytes has been shown by several investigators, particularly Hastings, Weinhouse, Haugaard and co-workers. However, a previous study of the electrolyte levels of the ischemic posterior papillary muscle showed only a slight decrease in tissue potassium at 1 hour, along with a slight increase in water, sodium, and chloride. The changes are so small as to be readily compensated for by the electrolytes of the incubation media, and are therefore not considered to be causally related to the observed decreased metabolism in vitro of the posterior papillary. As a matter of fact, the decreased intracellular potassium may well be the result of the failing metabolic ability of the myocardial fibers.

The metabolic results obtained after 1 hour of ischemia when the posterior papillary tissue is known to be irreversibly injured show a greater decrease in oxygen consumption with glucose than with FDP (fig. 1). The curves on PP in figure 3 show that by 1 hour after ligation, the phosphorylation of glucose has virtually ceased and that glucose, therefore, is apparently not being metabolized. FDP, on the other hand, is already phosphorylated and can continue through the glycolytic system. This is shown by the greater amount of organic phosphorus split into inorganic phosphorus with FDP as substrate than with glucose (fig. 3). Consequently, the oxygen uptake at 1 and 3 hours with FDP as substrate is greater than it is with glucose. Moreover, the decrease in organic phosphorus noted in vitro with non-ischemic left ventricle utilizing FDP as substrate, and the concomitant increased phosphorylation of glucose reflects the need for such phosphorylated metabolites for subsequent enzymatic degradation and oxidation.

The decreased tissue level of organic acid soluble phosphorus in the ischemic posterior papillary muscle, which is apparent at 1 and particularly at 3 hours, is a reflection of the fact that oxidative phosphorylation is probably not occurring intra-cellularly in vivo any better than it occurs in the homogenates.

The metabolic results obtained between zero and 30 min. are the most difficult to evaluate biochemically chiefly because of the nature of the biological process involved in the production of ischemic injury to the myocardium. The first irreversibly injured cells are detectable about 25 min. after ligation of the circumflex artery and the number of such cells present in the ischemic portion of the posterior papillary muscle increases with time up through one hour after ligation. The slight decrease in oxygen consumption and phosphorylation that is observed in the 30-minute animals, while not statistically significant, does approximate the beginning of the necrotic process in the injured myocardium, and it is believed it would be more marked if more irreversibly injured cells were present.

The results obtained with homogenates of the posterior papillary at 15 min. are of interest in that they show that the metabolic systems involved in aerobic glycolysis are still intact after 15 min. of ischemic injury, and imply that recovery of these injured cells is still possible. These results also correlate well with the results of temporary ligation experiments which have shown that the ischemic...
myocardial cells are "reversibly injured" at this time interval, and that restoration of the blood supply to the ischemic cells is not followed by necrosis or any permanent changes in function. A particularly striking and totally unexpected finding was the increased phosphorylation and oxygen consumption noted both in vivo and in vitro in the non-ischemic portion of the left ventricle. Although these results are undoubtedly a reflection of increased enzymatic activity, it is not possible at this time to explain the mechanisms involved. The increased activity is not related to dehydration of the heart muscle, as the tissue waters of the left ventricles of dogs with 1 and 3 hour infarcts are normal. An increased synthesis of enzymes could also result in the increased metabolic activity, but if such synthesis occurs, the increased magnitude of enzyme protein is too small to be apparent by any significantly measurable increase in mg. N/Gm. of wet or dry heart. It should be observed that though the increased metabolic activity noted in control left ventricle was unexpected, it is not necessarily an irrational finding. The non-ischemic left heart is subjected to a tremendous work load following ligation, since at least 40, and sometimes 70 per cent of the total left ventricle is non-contractile within a minute after occlusion of the circumflex artery. The increased mechanical and metabolic activity of the left heart under these circumstances is an attempt to compensate for the functional loss of the ischemic tissue and there seems to be little doubt that this increased work load is mediated through a mechanism involving increased capabilities of energy production.

The aerobic glycolytic system studied in this paper was chosen on the basis that early disorganization of the cell following injury could be most easily discerned through observing a change in a system of interrelated reactions. The results of this approach showed some of the early alterations from normal occurring in the ischemic myocardial cell but yielded no definitive evidence as to the nature of the primary process that results in cellular disorganization. It is our present working hypothesis that the energy systems involved in maintaining the integrity of the semi-permeable membrane of the cell are injured first, and that the early metabolic changes observed in these experiments are secondary to this phenomenon. It is postulated that the injury of the cell membrane first becomes severe 20 to 25 min. after ligation of the nutrient artery, and that the progressive disorganization of the myocardial cell takes place subsequently.

Summary

The aerobic metabolism of homogenates of normal dog myocardium was compared to the metabolism of myocardial cells after reversible and irreversible ischemic injury. Glucose and fructose-1,6-diphosphate were used as substrates and the results correlated with concomitant changes in organic phosphate metabolism.

The metabolic activities of the irreversibly injured cells fell to 30 to 50 per cent of normal, with both substrates, between 30 and 60 min. after the onset of the ischemia. A further decrease to less than 15 per cent of normal was observed at 180 min., and at 24 hours there was a complete loss of oxidative metabolism in the ischemic posterior papillary.

A slight, but not statistically significant decrease in the activity of the homogenates of the injured muscle was observed with both substrates after 15 to 30 min. of ischemia when most of the cells were still reversibly injured.

A striking increase in organic phosphate metabolism and oxygen consumption (130 per cent of normal) was observed in the non-ischemic portion of the myocardium 60 and 180 min. after ligation.

Acknowledgments

We are grateful to Dr. Herbert Sommers for performing some of the surgery on the later animals in this series and to Benjamin Alexander, Zigmund E. Zudyk, and Vivian Newton for their competent technical assistance.
Summario in Interlingua

Le metabolismo aerobic de homogenatos de myocardio canin normal esseva comparate con le metabolismo aerobic de cellulas myocardial post reversibile e non-reversibile lesions. Glucosa e fructosa-1,6-di-phosphato esseva usate usate como substratos, e le resultatos esseva correlata con concomitante alterationes in le metabolismo de phosphat organique.

Le activitate de irreversibilmente lesionate cellulas descendeva a inter 30 e 50 pro cento del valores normal in ambe substratos a un periodo de inter 30 e 60 minutas post le declaration del ischemia. Un descendita addito usque a infra 15 pro cento del valores normal esseva observate al fin de 180 minutas, e post 24 horas il habeva occurritte un complete perdita de metabolismo oxydative in le ischemic musculo postero-papillari.

Un leve sol statisticamente non significative ruction del activitate de homogenatos de musculo lesionate esseva observate in ambe substratos al fin de 15 a 30 minutas de ischemia quando le majoritate del cellulas esseva lesionate de maniera ancora reversibile.

Un frappante augmento del metabolismo de phosphat organique e del consumption de oxygeno (130 pro cento del norma) esseva observate in le portion non-ischemic del myocardio CO e 180 minutas post ligation.

References

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