Rate of Change in Myocardial Glycogen and Lactic Acid Following Arrest of Coronary Circulation

By H. L. Conn, Jr., M.D., John C. Wood, M.D., and Guillermo S. Morales, M.D.

Comparative studies of glycogen content, rate of glycolysis, and lactic acid accumulation were carried out over a 2 hour period in potassium-arrested hearts excised from dogs with and without preliminary infusion of glucose and insulin. Glycogen consumption occurred at 60 to 100 per cent of the expected rate for 60 min., but was subsequently much less in both groups of animals. Lactic acid accumulation apparently was a critical factor with respect to continued metabolism after 60 min. Reperfusion of the coronary circulation after 90 min. of circulatory arrest revealed damage to the coronary microcirculation.

The maximal duration of anoxia that can be tolerated by the arrested heart has not been critically defined. However, 30 to 60 min. seems to represent the approximate limit usually compatible with survival in normothermic dogs. The factors governing this time limit have not been identified. Under anoxic conditions, myocardial energy requirements presumably must be satisfied by glycolysis. Cardiac glycogen stores, therefore, merit consideration as a limiting factor. Calculations based on oxygen consumption of the perfused arrested heart would lead one to predict that glycolysis might sustain metabolism in the nonperfused heart for about 30 to 50 min.—a figure closely agreeing with the empirically determined "critical period" for interruption of coronary blood flow. Albeit perhaps fortuitous, this agreement further stimulated our interest in the possibility that available glycogen might be a limiting factor in myocardial survival during anoxia; it also invited the question as to whether the safe period of arrest might be extended, provided that glycogen stores could be augmented. As a first approach to the over-all problem, we attempted to answer: How does the actual rate compare with the predicted rate of glycogen utilization in the arrested nonperfused dog heart? How does preliminary preparation with glucose and insulin infusion affect initial myocardial glycogen content and subsequent glycogen utilization with time? Are factors other than glycogen content important in determining the safe period of arrest?

Methods

A total of 32 experiments was performed. Control studies were carried out in 17 adult mongrel dogs lightly anesthetized by intravenous sodium pentobarbital and sacrificed by intravenous injection of 10 ml. of 20 per cent potassium citrate. The heart was promptly removed. A sample of left ventricular myocardium was taken immediately after the sacrifice. A control sample was taken from the heart during the perfusion period. The glycogen content was determined in the laboratory immediately after the samples were taken. The results are expressed as milligrams of glycogen per 100 grams of fresh myocardium. The samples were then placed in a vacuum desiccator and stored at room temperature until the time of assay. The glycogen content was determined by the method of Dubois et al. (1956).
TABLE 1.—Effect of Insulin on TCA Extractable Glycogen Content*  

<table>
<thead>
<tr>
<th>Untreated dogs</th>
<th>After glucose and insulin administration</th>
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<tbody>
<tr>
<td>(mg. %)</td>
<td>(mg. %)</td>
</tr>
<tr>
<td>0</td>
<td>30' 60' 120'</td>
</tr>
<tr>
<td>0</td>
<td>30' 60' 120'</td>
</tr>
<tr>
<td>628</td>
<td>138 — —</td>
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<tr>
<td>479</td>
<td>290 43 31</td>
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<tr>
<td>400</td>
<td>231 52 19</td>
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<tr>
<td>319</td>
<td>270 45 25</td>
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<tr>
<td>450</td>
<td>304 107 38</td>
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<tr>
<td>266</td>
<td>117 80 26</td>
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<tr>
<td>479</td>
<td>306 81 53</td>
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<tr>
<td>570</td>
<td>302 134 49</td>
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<td>260</td>
<td>82 31 18</td>
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<td>227 69 30</td>
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<td>639 337 133</td>
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<tr>
<td>649</td>
<td>521 280 188</td>
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<td>574</td>
<td>— 229 123</td>
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<td>290</td>
<td>189 107 47</td>
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<tr>
<td>564</td>
<td>350 137 39</td>
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<tr>
<td>429</td>
<td>154 66 25</td>
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<tr>
<td>475</td>
<td>281 122 59</td>
</tr>
</tbody>
</table>

* Values are for glycogen content of cardiac muscle in 17 control studies and 15 studies in which the animals were pretreated with glucose and insulin.

Results

The results are summarized in table 1. The zero time myocardial content was 475 ± 133 mg./100 Gm. wet weight (mean ± S.D.). During the subsequent anoxic period, glycogen content declined progressively: at 30 min. the mean value was 281 ± 143, at 60 min. 122 ± 78 (26 per cent of the initial value) and at 120 min. 59 ± 50 (12 per cent of the initial value). It may be noted that by 1 to 2 hours glycogen depletion was severe in many hearts, the mean being elevated by a relatively few high values.

In contrast, initial myocardial glycogen content in the animals receiving preliminary glucose and insulin varied between 653 and 1,224 mg./Kg. of glucose and insulin averaged between 653 and 1,224 mg./Kg. of glucose infused animals exceeded that in the untreated group. At 30 min., the mean glycogen value was 552 ± 202 or 197 per cent of the comparable control group value. At 60 min., the mean glycogen was 391 ± 196 or 302 per cent of the comparable control group content. At 120 min., muscle glycogen averaged 238 ± 159 or 400 per cent of the untreated group average at this time. Each of these differences is significant at the 95 per cent level or better.

Figure 1 shows that, throughout the period of observation, the myocardial glycogen concentration in the glucose infused animals exceeded that in the untreated group. The slope of each curve, considered separately, shows that the rate of decline of glycogen in the untreated animals did not differ significantly from a straight line during the first 60 min. Thereafter, a highly significant (p < 0.001) fall in the rate of glycogenolysis oc-
curred. The curves also show that the rate of glyco- 
gen disappearance in the pretreated animals was sug- 
gestively but not significantly more rapid from 0 to 30 min. than from 30 to 60 min. The difference in the 
strips has a p value of about 0.1. As in the control 
periments, there was a relatively marked and sig- 
nificant decline in the rate of glycogen utiliza- 
tion after 60 min. (p < 0.025), in spite of an 
apparently adequate residual glycogen con- 
tent.

A comparison for each time interval of the 
relative slopes of the 2 curves (reflecting the 
comparative glycogen disappearance rate with 
respect to time in the treated and untreated 
animals) shows a slope significantly greater 
in the pretreated group from 0 to 30 min. (p < 0.025) and from 60 to 120 min. (p < 0.001), 
but a slope similar to the untreated group in 
the 30 to 60 min. interval. Use of the slopes of 
the 2 curves to determine mean glycogen con- 
sumption per unit time gives the quantitative 
results seen in table 2. Glycogen utilization by 
the pretreated animals averaged 10 mg./100 
Gm./min. versus 7 by the normal group from 
0 to 30 min. From 30 to 60 min., glycogen 
consumption decreased relatively more in the 
pretreated than in the control animals, so 
that the mean rate was 6 mg./100 Gm./min. 
in both groups. From 60 to 120 min., the rate 
of glycolysis averaged 3 mg./100 Gm./min. in 
the pretreated versus 1 in the control ani- 

Table 3 summarizes the results from the 10 
experiments in which the KOH extractable 
fraction and/or myocardial lactic acid con- 
tent were measured in addition to TCA solu- 
tible glycogen. These results indicate that: (1) 
In the untreated animals, the KOH extract- 
able glycogen was equivalent to approximately 
one-fifth of the TCA fraction; (2) prelimi- 
nary glucose and insulin infusion increased 
the KOH fraction little if at all; (3) the 
KOH extractable glycogen appeared to vary

*In the statistical analysis of differences, the in- 
fluence of incomplete experiments was taken into 
consideration. In addition to this factor, the actual 
time (average 3 min.) of TCA homogenization of the 
initial sample was considered in calculating glycogen 
utilization per unit time.

in only a random fashion during the 2 hours of 
observation; and (4) muscle lactic acid in- 
creased progressively with time, so that about 
three-fourths of the glycogen decline in any 
given period could be accounted for by the
TABLE 2.—Glycogen and Lactic Acid Content of Heart Muscle

<table>
<thead>
<tr>
<th>TCA</th>
<th>KOH</th>
<th>0'</th>
<th>30'</th>
<th>60'</th>
<th>120'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen fractions (mg. %)</td>
<td>0'</td>
<td>30'</td>
<td>60'</td>
<td>120'</td>
<td>0'</td>
</tr>
<tr>
<td>Untreated</td>
<td>707</td>
<td>630</td>
<td>337</td>
<td>133</td>
<td>80</td>
</tr>
<tr>
<td>Glucose + Insulin</td>
<td>649</td>
<td>521</td>
<td>280</td>
<td>188</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>574</td>
<td>—</td>
<td>229</td>
<td>123</td>
<td>137</td>
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<td>564</td>
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<td>137</td>
<td>39</td>
<td>163</td>
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<tr>
<td></td>
<td>429</td>
<td>154</td>
<td>66</td>
<td>25</td>
<td>91</td>
</tr>
<tr>
<td>Glucose + Insulin</td>
<td>1224</td>
<td>1056</td>
<td>878</td>
<td>592</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>653</td>
<td>340</td>
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<td>697</td>
<td>498</td>
<td>230</td>
<td>135</td>
<td>113</td>
</tr>
</tbody>
</table>

increment in lactate recovery. The differences between the 2 groups of animals were not statistically significant (fig. 2).

DISCUSSION

There is some controversy relating to the methods and significance of tissue glycogen measurements. On the basis of comparative studies of the TCA and KOH methods for extracting glycogen, Roe concluded that the former method isolates true glycogen whereas the KOH extractable material includes anthrone-reacting nonglycogen material.\(^2\) Russell and Bloom have concluded that the TCA soluble fraction represents metabolically active glycogen; the residual material, extractable with KOH digestion, is believed to represent bound or structural glycogen which is relatively inactive in terms of metabolic turnover.\(^5\) Most authors have accepted this latter point of view.\(^7\),\(^8\) It seemed to us that no matter which concept were correct, measurement of TCA soluble glycogen would give the information most germane to the present study. So, initially, we restricted our attention to this fraction. However, Merrick's observation that labile glycogen was converted to the more stable form (KOH extractable) under hypoxic conditions left in doubt the interpretation of our initial results.\(^8\) Therefore in subsequent studies we measured both TCA and KOH extractable glycogen. As far as we determined, the KOH fraction varies in only a random fashion during the first 2 hours of anoxia. Additional experiments carried out over a 5 to 24 hour period of anoxia have revealed eventual decay of KOH extractable glycogen after the more labile form is depleted, but none of the studies gave evidence suggesting conversion of glycogen from the TCA to the KOH fraction. Consequently the same conclusions may be drawn from the present study, regardless of whether the TCA glycogen fraction is considered alone or whether the sum of the two fractions is considered.

Our most obvious finding was a progressive time-decay of glycogen in each of the excised hearts. Thus, although one could anticipate that the rate of glycolysis during anoxia would be even greater in the beating or working heart than in the arrested heart,\(^9\) we disagree with Bloom who concluded that, in the absence of an external work load, anoxia is not associated with glycolysis.\(^10\) The cause of the glycolysis is less well documented than its occurrence. We have assumed that the disappearance of glycogen is due to its utilization by the anoxic myocardial cell as a means of providing energy for prevention of entropy. Cultures of our experimental myocardial samples have excluded bacterial growth as a possible spurious factor in the glycogen decay.
There remains at least one alternative explanation: In the presence of appropriate enzymes, glycolysis could continue independently of organized cell structure. However, myocardial cell structure apparently remains intact during relatively short periods of anoxia since, with reperfusion, the heart can resume normal function. Therefore, we have continued to assume that, at least during the early portion of the experimental period, the observed rate of glycogen decay is due to its utilization by the anoxic but intact myofibril. If this assumption is correct, the energy theoretically released as a result of glycolysis at the experimentally observed rate should approximate the minimal cell requirements under the existing circumstances. More evidence supporting our assumption could be adduced by demonstration of a good agreement between observed glycogen utilization rates and those predicted on the basis of expected energy needs. Unfortunately, quantitative information is not available as to myocardial energy needs of the arrested nonperfused heart, so the rate of glycogen utilization during anoxia cannot be confidently predicted.

As a first approximation, we estimated the required energy by equating it with that implied by the Qo2 of the arrested perfused heart.11,12 Approximation by use of the Qo2 has a disadvantage in that it may reflect the energy desirable for optimal cell function but does not necessarily indicate the minimal need sufficient to maintain structural integrity and to insure subsequent tolerance of an external work load. Furthermore, the Qo2 varies with coronary perfusion rate or pressure. Using a modified Anderson-Langendorff preparation, we have measured a Qo2 for the arrested dog heart of 1 ml./100 Gm./min. with coronary flow rates approximately one-fourth normal, in contrast to 2 ml./100 Gm./min. with flow rates 60 to 75 per cent of normal. Others also have found a decrease in myocardial oxygen consumption at relatively low coronary blood flow rates and pressures.13,14 It is not clear whether these differences reflect inadequate perfusion of a portion of the capillary bed or a reduction in individual cell oxygen consumption. If the value of 1 ml./100 Gm./min. is used for Qo2, predicted glycogen decay is in the range of 10 to 15 mg./100 Gm./min.

Only in the initial 30 min. period of the pretreated group did glycogen utilization suggest energy production closely approaching that postulated as optimal. Actually, because of the presumed availability of free intracellular glucose in these pretreated hearts,1 anaerobic energy production may have even exceeded that predicted. However, in both groups of hearts studied, glycogen utilization did not differ greatly in the first 60 min. from that predicted, the experimental ratio being on the order of 60 to 70 per cent. Since such hearts can, on being perfused, regain their functional integrity, we can surmise that this is sufficient to meet minimal cardiac requirements. On the other hand, after 60 min. of anoxia, utilization fell off so sharply it seems unlikely that cardiac needs were being met. In the control group of animals the minimal utilization might be considered a consequence of the near exhaustion of glycogen stores, but the hearts pretreated with glucose still contained substantial amounts of glycogen at a time when glycolysis was again markedly reduced. This required an alternative explanation for the observed inhibition of glycolysis; we considered the possibility that a progressive building up of lactic acid, the end product of glycolysis, might be this alternative. That is, the lactic acid was not being metabolized or removed as normally occurs, thus either preventing, by mass law effect, a faster breakdown of glycogen, or causing a decreased glycogen requirement through an adverse effect on cell metabolism. Results of the lactic acid determinations did in fact show a large accumulation of this product in the heart, the increments accounting for about 75 per cent of the glycogen utilized. Perhaps more pertinent is the finding that lactic acid content was consistently 350 to 450 mg. per cent after 2 hours of anoxia. Plot of these concentrations against
initial glycogen content indicates that they are substantially independent of the glycogen content. This observation has led us to favor the view that lactic acid primarily affects glycolysis through an adverse effect on metabolism, perhaps through lowering cell pH. That a reduced pH does occur is shown by analysis of coronary blood obtained at the onset of reperfusion following prolonged cardiac arrest. We and others have found a change from normal levels (7.2 to 7.3) to as low as 6.0.15

Subsequent attempts to demonstrate the deleterious effects of decreased cell pH have been partially successful. Preliminary experiments consisting of intermittent coronary perfusion of the arrested heart with a buffered plasma substitute have shown removal of large amounts of H⁺, maintenance of cardiac lactic acid at levels of 100 mg. per cent or less and re-establishment of normal sinus rhythm after as much as 90 min. of anoxia. However, reperfusion after arrest usually resulted in a progressive rise in coronary vascular resistance, in tissue edema and, in some instances, in petechial and ecchymotic hemorrhages. This complication has been recently reported by Reynolds.18 Thus the major limitations to more prolonged interruption of the coronary circulation may ultimately prove to be the susceptibility of the coronary capillaries to anoxic injury.

We are left with the following beliefs:

Cardiac glycogen content frequently approaches zero after 1 hour and usually approaches it after 2 hours of anoxia at 37 C. Pretreatment of heart with glucose and insulin increases its glycogen content very significantly and leaves the heart with considerable residual glycogen after 1 hour of anoxia. In spite of this residual glycogen, utilization does not closely approximate expected "normal" values after 60 min. At this time cardiac metabolic activity seems less governed by residual glycogen than by other factors such as lactic acid concentration. When the deleterious effect of lactic acid is minimized anoxic vascular damage becomes evident.

The requirements for prolonging the period of myocardial anoxia with subsequent functional recovery would then seem to include concomitant use of a method for increasing cardiac glycogen, a method for removing lactic acid and a method for preventing severe anoxic injury to the coronary microcirculation. Our studies give some hint of solutions for the first two problems but none for the third. Present indications are that hypothermia is a helpful adjunct toward overcoming all three.

SUMMARY

Comparative studies of the glycogen content and rate of glycolysis at 37 C. were carried out over a 2 hour period in potassium-arrested hearts excised from dogs with and without preliminary infusion of glucose and insulin. Glycogen depletion was severe in many of the untreated hearts by 60 min. Myocardial glycogen content and the initial rate of glycolysis were increased and its subsequent anoxic decay delayed by pretreatment with glucose and insulin. However, irrespective of the glycogen reserve, metabolic utilization dropped progressively during hypoxia, but particularly after 60 min. Only in the first 30 min. in the pretreated hearts did glycogen consumption suggest energy production approaching that on the basis of the QO₂ of the arrested perfused heart.

Muscle lactic acid during anoxia increased progressively with time so that about three-fourths of the glycogen decline in any given period could be accounted for by the increment in lactate recovery. It is suggested that accumulation of lactic acid and perhaps other intermediates of the glycolytic cycle, with associated pH changes, may become the critical factor with respect to continued metabolism in the arrested heart. Attempts to circumvent this difficulty led to the observation that with more prolonged cardiac arrest capillary damage becomes another limiting factor.
SUMMARIO IN INTERLINGUA

Studios comparative del contenuto de glycogeno e del rapiditate del glycolyse a 37°C esseva effectuate durante un periodo de due horas in cordes canin arrestate con kalium le quales habeva essite excidite ab animales con e sin previe infusiones de glucosa e de insulina. Le depletion de glycogeno attingeva un nivello sever intra 60 minutas in multes del non-tractate cordes. Le contenuto de glycogeno in le myocardio e le rapiditate initial del glycolyse esseva augmentate e su subsequente decadentia anoxic esseva retardate per le pretractamento con glucosa e insulina. Tamen, sin respecto al reserva de glycogeno, le utilisation metabolic de illo descendeva progressivemente durante hypoxia, specialmente post 60 minutas. Il esseva solmente durante le prime 30 minutas e solmente in le cordes pretractate con glucosa e insulina que le consumption de glycogeno pareva signalar un production de energia proxime al nivello postulate como optimal super le base del Qo2 del arrestate corde perfundite.

Le contenuto muscular de acido lactic in anoxia se augmenta progressivemente in le curso del tempore, de manera que circa tres quartos del declino de glycogeno in un periodo particolare poteva esser explicate per le augmentos del recovration de lactato. Es suggerite que le accumulation de acido lactic e forsan altere intermediaries del cyclo glycolytic insinul con associate alternationes del pH, deveni possibilmente le factor critic con respecto al continuante metabolismo in le corde arrestate. Essayos de circumvenire iste difficultate resultava un observation additional: Durante le perfusion, subsequente a un plus prolongate arresto cardiac, dannos capillari deveni apparentemente un cetere factor limitatorii.

REFERENCES

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