Glucose Utilization by the Working Rat Heart[1] (34338)

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Previous investigations have studied the interrelation between carbohydrate utilization and contraction in isolated hearts perfused with the Langendorff technique (1, 2). Few studies have addressed themselves to the problem of carbohydrate utilization in the rat heart performing measured external work (2, 3), and to our knowledge the carbohydrate cost of calculated work has not been reported.

Isolated hearts perform adequately in the presence of either glucose, free fatty acids, triglycerides, or shorter chain substrates (4–6). However, it has been suggested that when isolated hearts ultimately deteriorate, this may be due to exhaustion of substrate availability (7).

In the present investigation we explored the cardiac performance of the isolated working rat heart in the presence and absence of glucose in order to determine the effect of glucose availability on the magnitude and duration of work output. The cardiac glycogen reserve at the point of performance deterioration was determined and the carbohydrate cost of work was estimated.

Methods. Hearts from 24-hr fasted male albino rats were perfused in a working rat heart apparatus similar to that reported by Neely *et al.* (8). The constant atrial perfusing pressure was 10 cm of buffer. While the heart was being mounted, it was preperfused in a retrograde fashion with modified Krebs–Henseleit solution containing 16 m*M* K^+ and 5 m*M* glucose (9). This high K^+ arrest, which abolishes all spontaneous contractions permits preservation of normal high energy phosphate stores (9). After 10 min of retrograde perfusion the cannula to the left atrium was opened and normal antegrade perfusion with K^+ at 6 m*M* was begun. This restored stable mechanical function within 30 sec. The heart was paced at a constant rate of 320 beats/min through a platinum wire impinging upon the right atrium. The pacing stimulus was 4-*v* amplitude and 4 msec duration. Cardiac output, coronary flow, left ventricular pressure (LVP) were measured as described by Neely *et al.* (8), and LVP was recorded on an Electronics for Medicine photographic recorder. The left ventricular pressure was differentiated by a R/C differentiating channel in order to monitor the rate of rise of left ventricular pressure (*dp/dt*). For antegrade perfusion the Krebs–Henseleit buffer containing 6 m*M* K^+, 3.1 m*M* Ca^{2+}, 0.5 m*M* Na_2 EDTA, and 0 or 5 m*M* glucose. All solutions were gassed with 95% O_2, 5% CO_2 by bubbling in the main reservoir and additionally by employing a gas lift mixing tube while the fluid was pumped up to the perfusion reservoir. This maintained an influent oxygen tension in excess of 550 mm Hg and the pH close to 7.4. Both retrograde and antegrade perfusion was carried out at 37°. The apparatus contained 150 ml of perfusion medium.

Antegrade perfusion was continued until the first sign of mechanical deterioration was detected. The experiment was stopped as soon as a decline in peak left ventricular systolic pressure, maximal *dp/dt* or cardiac output occurred. Thus as regards deterioration each heart served as its own control. The ventricles were blotted and weighed. A portion of the ventricles was removed for determining dry weight and the remainder was

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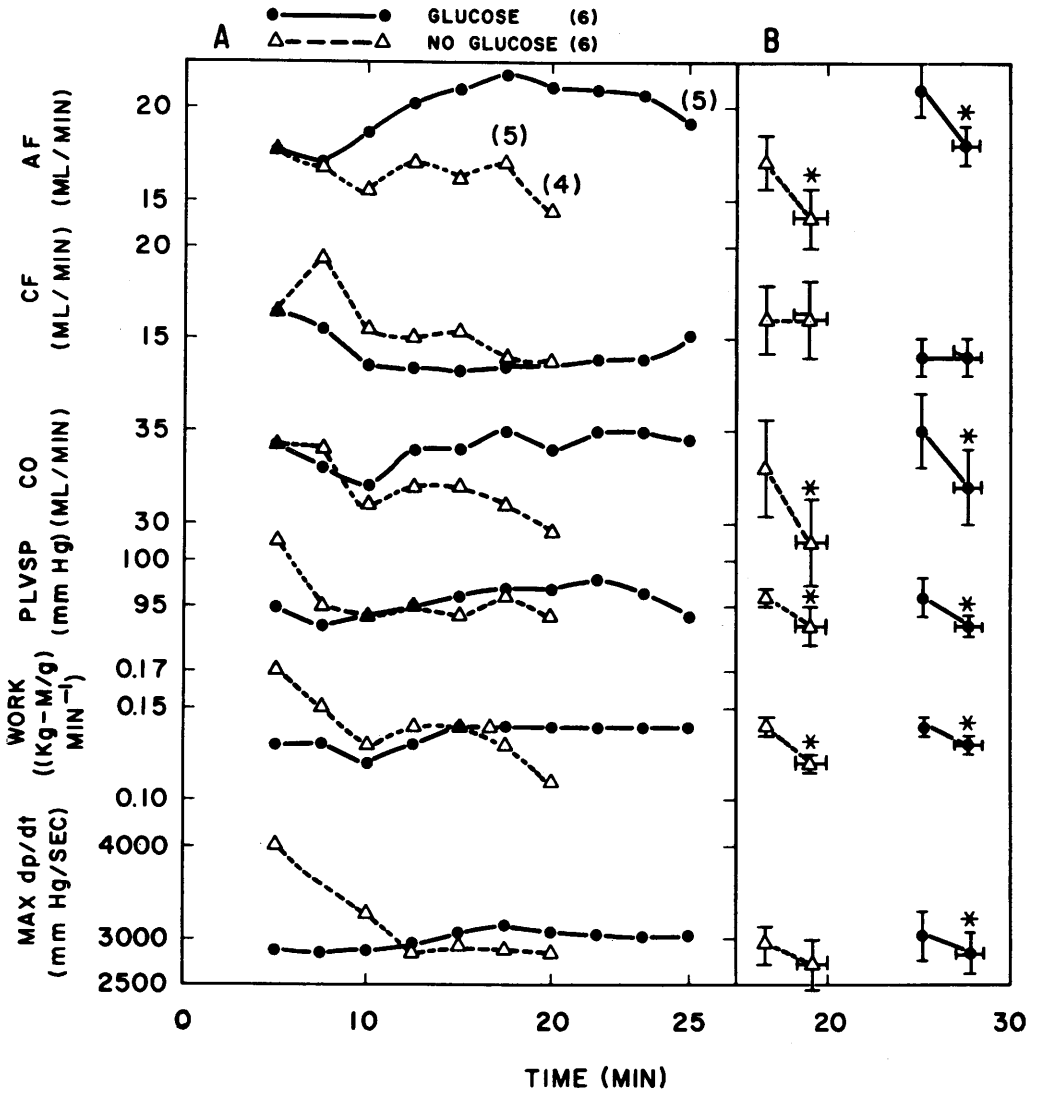

FIG. 1. Mechanical performance of the working rat heart with and without glucose: AF = aortic flow, CF = coronary flow, CO = total cardiac output, PLVSP = peak left ventricular systolic pressure. Numbers in the parentheses indicate the number of hearts at each point in time. Points without numbers represent the mean of 6 hearts. Panel A shows the time course of the variables. Because the last points only represent the most durable hearts, the deterioration is not apparent in panel A. Panel B compares the terminal values for hearts with their stable values 2.5 min earlier (mean ± SE). The asterisk signifies $p<0.05$. When represented this way, statistically significant deterioration is apparent in most variables.

used for determination of glycogen (9). The glucose content of the perfusion medium before and after perfusion was also determined (9). The left ventricular pressure curves were integrated by planimetry for the determination of systolic mean pressure, and the kinetic cardiac work was calculated as described by Neely *et al.* (8). Statistical analysis was with analysis of variance for comparison of the groups with glucose absent and present. When comparing values at the time of deterioration with those prior to deterioration, where each heart served as its own control, analysis of variance with interaction was employed.

Results. Figure 1 shows the time course of

flow, pressure, and work variables in these hearts. In panel A although coronary and aortic flow and total cardiac output appear to differ when hearts perfused with or without glucose are compared, with the small sample reported, these differences are not statistically significant. In panel B are shown the final values for each set of hearts at the time of deterioration, and these are compared with the values for the same hearts 2.5 min earlier. Although hearts ran longer when glucose was present, there are no other significant differences when groups with and without glucose are compared. However, when terminal and preterminal values are compared within a single group there is a significant decline in each variable except coronary flow in both groups and maximal *dp/dt* in the glucose absent group. Thus within the limits of the exerimental design, flow and pressure functions appear to deteriorate simultaneously.

Table I gives the metabolic data from these experiments. Although there was no statistical difference between the wet heart weights after perfusion, hearts perfused without glucose accumulated more tissue water as indicated by the lower ratio of dry to wet weight. Mean cardiac work for the stable period was calculated from the value during three 2.5-min observation periods. Total cardiac work was calculated from the sum of each 2.5-min work period. While mean cardiac work was the same in each group, total cardiac work was greater in the presence of glucose.

Initial glycogen for each group is the mean value for 6 hearts analyzed after the 10-min preperfusion period. Since the residual glycogen was the same after perfusion in both groups it is reasonable to assume that the amount of glycogen broken down was the same. Also employing the above assumption, it is clear that carbohydrate utilization was much greater when glucose was furnished and that the hexose cost of external work was also greater.

Discussion. In these experiments the residual glycogen content had declined to about 30% of the initial value when cardiac function deteriorated. This was true whether exogenous glucose was present or absent. This finding is consistent with the report in Langendorff perfused hearts that the onset of failure of perfused hearts occurs when there is a 60% reduction in glycogen stores, and

TABLE I. Characteristics of Working Rat Heart with and without Exogenous Glucose.[h]

Glucose	No. of hearts	Duration of perfusion (min)	Wet heart wt (mg)	Dry wt/wet wt	Cardiac work: Mean[e] (kg-m/g min^{-1})	Cardiac work: Total[f] (kg-m/g)
Present, 5 m*M*	6	27.9 ± 0.8	802 ± 24	0.21 ± 0.00	0.13 ± 0.01	3.7 ± 0.3
Absent	6	19.2 ± 0.8[a]	798 ± 22[d]	0.19 ± 0.00[c]	0.14 ± 0.01[d]	2.6 ± 0.2[b]

Glucose	Glycogen (μmole/g): Initial[g]	Glycogen (μmole/g): Residual	Utilization (μmole/g): Glycogen	Utilization (μmole/g): Glucose	Utilization (μmole/g): Total carbohydrate	Carbohydrate utilization (μmole/kg-m)
Present, 5 m*M*	143.5 ± 5.3	41.8 ± 5.6	102	299 ± 27	401	112
Absent	143.5 ± 5.3	44.4 ± 5.6[d]	99	—	99	38

[h] p values compare glucose absent and glucose present values.
[a] $p < 0.001$;
[b] $p < 0.01$;
[c] $p < 0.05$;
[d] $p > 0.1$.
[e] Mean cardiac work during 3 stable 2.5-min periods.
[f] The sum of cardiac work for each heart measured for each 2.5-min period during perfusion.
[g] Glycogen determined in 6 hearts after 10 min of preperfusion. All metabolic values are per gram of dry weight. Glycogen expressed in glucose equivalents.

that this type of failure is associated with a reduction in glycolytic flux (10). Hearts perfused without substrate until profound deterioration had occurred also showed a 60% reduction in glycogen (11). This may be different than failure caused by excessive work, where glycogen stores have been reported to be normal (12) or decreased (13). It has been reported that the work capability of skeletal muscle is related to its glycogen content, but independent of exogenous glucose (14).

More external work was performed per mole of hexose utilization when exogenous glucose was absent than when it was present, suggesting another source of substrate. Triglyceride and glycogen are the major endogenous substrate storage forms. Even if all the endogenous triglyceride were mobilized in hearts perfused without exogenous glucose this could only explain about 12% of the difference. It has been observed that when Langendorff perfused hearts are perfused with glucose or triglyceride as the sole exogenous substrate lactate accumulates in the perfusion medium (5, 15). In the absence of substrate net lactate accumulation is negligible (5). With glucose present complete oxidation of about 30% of the hexose utilized would yield equivalent amounts of ATP per unit of external work as would complete oxidation of all of the hexose when glucose was absent. In a separate group of working rat hearts, we found that with exogenous 5 m*M* glucose 50% of the hexose utilized is converted to lactate. That this high rate of lactate production is not due to hypoxia is supported by a normal mean lactate to pyruvate ratio of 5.3 and a mean oxygen tension in the myocardial effluent fluid of 152 mm Hg. Thus the differences between hearts perfused with and without glucose present probably can be in part explained by differences in the proportions of hexose metabolized to lactate as opposed to complete oxidation of hexose to CO_2. If this is true, then cardiac work is exerting more precise control over the oxidative than over the glycolytic pathway, since flux through the latter is in excess of metabolic needs when glucose is present. In Langendorff perfused hearts, the ratio of oxidative metabolism to glycolytic metabolism was markedly reduced when the heart was arrested (9), supporting this concept that the energy demands of contractile work influence the oxidative rates more than the glycolytic rates.

Summary. Rat hearts were perfused in an apparatus in which coronary flow, cardiac output, left ventricular pressure, rate of rise of left ventricular pressure (*dp/dt*) and external cardiac work could be measured. The effects of the presence and absence of exogenous glucose were compared. In the presence of glucose stable performance lasted longer than without substrate. When the heart failed cardiac output, left ventricular pressure, maximal *dp/dt* and cardiac work declined simultaneously. At the time of failure endogenous cardiac glycogen stores had declined to the same level whether or not exogenous glucose had been furnished. The hexose cost of cardiac work was greater when glucose was furnished, suggesting whatever hexose was available was more completely metabolized in the absence of exogenous substrate.

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