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# Effects of Increased Heart Work on Glycolysis and Adenine Nucleotides in the Perfused Heart of Normal and Diabetic Rats

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1. In the isolated perfused rat heart, the contractile activity and the oxygen uptake were varied by altering the aortic perfusion pressure, or by the atrial perfusion technique ('working heart'). 2. The maximum increase in the contractile activity brought about an eightfold increase in the oxygen uptake. The rate of glycolytic flux rose, while tissue contents of hexose monophosphates, citrate, ATP and creatine phosphate decreased, and contents of ADP and AMP rose. 3. The changes in tissue contents of adenine nucleotides during increased heart work were time-dependent. The ATP content fell temporarily (30s and 2min) after the start of left-atrial perfusion; at 5 and 10min values were normal; and at 30 and 60min values were decreased. ADP and AMP values were increased in the first 15min, but were at control values 30 or 60min after the onset of increased heart work. 4. During increased heart work changes in the tissue contents of adenine nucleotide and of citrate appeared to play a role in altered regulation of glycolysis at the level of phosphofructokinase activity. 5. In recirculation experiments increased heart work for 30min was associated with increased entry of [$^{14}$C]glucose (11.1mM) and glycogen into glycolysis and a comparable increase in formation of products of glycolysis (lactate, pyruvate and $^{14}CO_2$). There was no major accumulation of intermediates. Glycogen was not a major fuel for respiration. 6. Increased glycolytic flux in Langendorff perfused and working hearts was obtained by the addition of insulin to the perfusion medium. The concomitant increases in the tissue values of hexose phosphates and of citrate contrasted with the decreased values of hexose monophosphates and of citrate during increased glycolytic flux obtained by increased heart work. 7. Decreased glycolytic flux in Langendorff perfused hearts was obtained by using acute alloxan-diabetic and chronic streptozotocin-diabetic rats; in the latter condition there were decreased tissue contents of hexose phosphates and of citrate. There were similar findings when working hearts from streptozotocin-diabetic rats with insulin added to the medium were compared with normal hearts. 8. The effects of insulin addition or of the chronic diabetic state could be explained in terms of an action of insulin on glucose transport. Increased heart work also acted at this site, but in addition there was evidence for altered regulation of glycolysis mediated by changes in tissue contents of adenine nucleotides or of citrate.

In the isolated perfused rat heart the uptake both of glucose and of $O_2$ from the perfusion medium can be increased severalfold when the heart performs increased mechanical work (Opie, 1965; Neely, Liebermeister, Battersby & Morgan, 1967*a*; Chain, Mansford & Opie, 1969). At the same time there is increased flow of glucose carbon through the glycolytic pathway, as shown by studies with [$^{14}$C]-glucose (Chain *et al.* 1969). Increased glucose uptake and glycolytic flow are also found when insulin is added to the perfusion medium (Fisher & Lindsay, 1956; Morgan, Randle & Regen, 1959; Morgan, Cadenas, Regen & Park, 1961*b*; Williamson, 1962), but in this situation the total $O_2$ consumption of the heart is unaltered (Fisher & Williamson, 1961). Conversely, decreased glucose uptake and glycolytic flow are found in perfused hearts from rats with induced diabetes, or when ketone bodies or free fatty acids are present as alternative non-glucose substrates (Morgan *et al.* 1961*b*; Shipp, Opie &

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Challoner, 1961; Williamson & Krebs, 1961; Randle, Newsholme & Garland, 1964; Chain *et al.* 1969).

It is therefore possible to obtain a very wide variation in the rates of glycolytic flow in this preparation either by (i) altering the amount of muscular work performed or by (ii) altering the substrate supply by the presence or absence of insulin or of substrates other than glucose. This paper examines the effects of increased heart work on the metabolism of glucose and of some intermediates in the perfused rat heart, and compares the effects of work with those of insulin or diabetes mellitus.

## MATERIALS

*Rats.* Male rats (wt. 280–320g) of the Sprague–Dawley strain were fed *ad lib.* Acute alloxan-diabetic and chronic streptozotocin-diabetic rats were obtained as described by Mansford & Opie (1968).

*Chemicals.* Unless otherwise stated, all chemicals and enzymes used in the analyses of tissue and perfusion fluid were obtained from C. F. Boehringer und Soehne G.m.b.h., Mannheim, Germany. Alloxan, glucose, sorbitol and EDTA were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. The sources of heparin, streptozotocin, [U-$^{14}$C]glucose and [$^{3}$H]sorbitol were as given by Chain *et al.* (1969). DL-$\beta$-Hydroxybutyrate came from Sigma Chemical Co., St Louis, Mo., U.S.A. Acetoacetate was prepared from ethyl acetoacetate (British Drug Houses Ltd.), which was converted into sodium acetoacetate; the sodium compound was concentrated by evaporation, neutralized and stored at −20°C. Bovine serum albumin (fraction V) was obtained from the Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K. Insulin was a glucagon-free preparation obtained from Boots Pure Drug Co., Nottingham, U.K. Adrenaline B.P. (1mg/1ml) was obtained from Macarthys Ltd., Romford, Essex, U.K.

## METHODS

*Perfusion techniques.* Rats were anaesthetized with ether and given 200 units of heparin into the femoral vein. The heart (average fresh wt. 1.0g) was rapidly removed and plunged into ice-cold perfusion medium before being mounted on an aortic cannula for isolated perfusion.

(i) Non-recirculation system. In all experiments the arrested heart was restarted by perfusion as described by Langendorff (1895). In initial experiments (Table 1) hearts were perfused for only 15min either by aortic perfusion with a hydrostatic pressure of 45, 65 or 100cm$H_2O$, or by the atrial-perfusion technique of Neely *et al.* (1967*a*), by allowing the coronary flow to run away but recirculating the aortic output. In all other experiments the initial non-recirculation perfusion of 15min preceded a recirculating perfusion; the non-recirculating perfusion was usually at 100cm$H_2O$ pressure, but hearts to be perfused in the recirculating system at 65cm$H_2O$ were also preperfused at 65cm$H_2O$ pressure.

(ii) Langendorff recirculation system. This description refers to hearts perfused by the Langendorff technique as modified by Morgan, Henderson, Regen & Park (1961*a*) to allow recirculation of about 40ml of perfusion fluid. The perfusion pressure was either 65 or 100cm$H_2O$.

(iii) Working recirculation system. This description refers to hearts perfused by the left atrium, by the technique of Neely *et al.* (1967*a*), at an atrial perfusion pressure of 20cm$H_2O$. The left ventricle spontaneously ejected 40–60ml of perfusate/min, against a hydrostatic pressure of 100cm$H_2O$. The apparatus was modified to allow a recirculation volume of only 50ml, and continuous measurements of $O_2$ partial pressure in the incoming and outgoing perfusate.

(iv) Variation in heart work. When the aortic perfusion pressure of Langendorff-perfused hearts is increased, the left-ventricular pressure increases (Opie, 1965) and the peak left-ventricular systolic pressure approximately equals the aortic perfusion pressure (Neely *et al.* 1967*a*). Increasing the aortic perfusion pressure from 45 to 65 to 100cm$H_2O$ increases left-ventricular pressure development accordingly and hence increases left-ventricular work. A further increase in left-ventricular pressure can be obtained by filling the left ventricle from the left atrium; with a left-atrial filling pressure of 20cm$H_2O$ normal hearts in the present experiments developed a peak left-ventricular pressure of about 180cm$H_2O$. Hence variations in the perfusion system allowed peak left-ventricular pressures of about 45, 65 and 100cm$H_2O$ in the Langendorff perfused hearts, and about 180cm$H_2O$ in the atrially perfused hearts. The term 'non-working' is applied to Langendorff perfused hearts, and 'working' to atrially perfused hearts performing external work. Nevertheless it must be emphasized that all contracting hearts are working (Opie, 1968) and that the work performed is related to the peak left-ventricular systolic pressure.

*Oxygen uptake.* This was obtained by passing samples of perfusate entering and leaving the heart through jacketed glass holders containing Beckmann macro-electrodes (see Chain *et al.* 1969). The calculated $O_2$ content of the perfusate was related to the coronary flow rate. Measurements were usually made at the beginning, the middle and the end of the perfusion periods; the mean of these three values was the $O_2$ uptake ($\mu$l/min per g) for that heart.

*Mechanical measurements.* Heart rate, coronary flow rate, aortic flow rate and aortic pressure were monitored as described by Chain *et al.* (1969). Judged by these criteria, and by $O_2$ uptake, our working heart preparation was stable for over 90min.

*Perfusion medium.* The perfusion medium was a Krebs–Henseleit bicarbonate buffer medium (Krebs & Henseleit, 1932) at 37°C, equilibrated with $O_2+CO_2$ (95:5). Glucose (11.1mM) was the usual substrate, unless otherwise indicated. [$^{3}$H]Sorbitol (5.5mM) was used to determine the extracellular space (Morgan *et al.* 1961*a*) and the exact volume of the perfusion medium. Insulin (2munits/ml) was added in some experiments.

*Tissue analyses.* At the end of the perfusion period hearts were freeze-clamped in aluminium tongs chilled in liquid $N_2$ (Wollenberger, Ristau & Schoffa, 1960). To obtain consistent dry-weight/wet-weight ratios and sorbitol spaces, it was essential to remove carefully (i) the frozen perfusate around the edges of the clamped heart, and (ii) the major part of the atrium. This was done by placing the heart on a block of solid $CO_2$ and gently prising

off the unwanted parts with the sharp tip of scissors chilled in liquid $N_2$. The remainder of the heart was very rapidly weighed on a torsion balance, powdered in a percussion mortar, chilled by liquid $N_2$ and deproteinized in a frozen $HClO_4$–acetone–EDTA mixture (Opie & Newsholme, 1967). However, values reported in Table 7 and those on ketone-body-perfused hearts were on hearts deproteinized in 7% (v/v) $HClO_4$ only.

After neutralization of the supernatant fluid, ADP and AMP were measured immediately by the method of Adam (1963). ATP and creatine phosphate were measured the next morning (after storage of the tissue extract below −20°C overnight) by the method of Lamprecht & Trautschold (1963) for ATP; creatine phosphate was then determined in the same cuvette by addition of ADP and excess of a solution of creatine phosphokinase (5mg of enzyme/ml of bovine serum albumin, 100mg/100ml of water). $P_i$ was measured the same evening (Table 1) or the next morning (Table 5) by a modification of the method of Berenblum & Chain (1938). The methods used for the determination of the other metabolites were: lactate, malate, glycerol 3-phosphate, dihydroxyacetone phosphate and pyruvate by the methods of Hohorst, Kreutz & Bücher (1959); glucose, glucose 6-phosphate and fructose 6-phosphate by the use of glucose 6-phosphate dehydrogenase (Hohorst, 1963); fructose 1,6-diphosphate by the method of Bücher & Hohorst (1963); citrate by the citrate lyase method of Moellering & Gruber (1966). A separate portion of powdered extract was used for glycogen assay by the method of Good, Kramer & Somogyi (1933). Pyruvate, dihydroxyacetone phosphate and fructose 1,6-diphosphate were measured immediately after the neutralization procedure, and the other compounds within a few days after storage at −20°C.

*Alternative assay method for hexose monophosphates.* In experiments in which assay of hexose phosphates but not of tissue glucose was required, hexose monophosphates could be assayed by conversion into fructose 1,6-diphosphate and then further conversion into triose phosphates and glycerol 3-phosphate. The assay system was: tris–HCl buffer, pH 8.0, 50mM; ATP, 0.1mM; $MgCl_2$, 5mM; cysteine hydrochloride, 3mM; bovine serum albumin, 3mg/100ml; NADH, 0.25mM. Subsequently cysteine and albumin were omitted. Fructose 1,6-diphosphate was determined by addition of aldolase to a cuvette containing the above assay medium and excess of triose phosphate isomerase and glycerol 3-phosphate dehydrogenase; then fructose 6-phosphate was determined by addition of excess (5$\mu$l of 10mg/ml) of fructose 6-phosphate kinase (phosphofructokinase); and glucose 6-phosphate was then assayed by addition of phosphoglucose isomerase. The advantages of this assay are (i) combined assay of glucose 6-phosphate, fructose 6-phosphate and fructose 1,6-diphosphate is possible in one cuvette; (ii) because of the action of aldolase, two molecules of NADH are converted for each molecule of hexose monophosphate in the assay cuvette, thereby doubling the sensitivity of the hexose monophosphate assay; (iii) the use of expensive enzymes and coenzymes required for glucose 6-phosphate assay by the glucose 6-phosphate dehydrogenase method is avoided.

*Chromatographic analysis of radioactive metabolites.* Powdered frozen tissue was suspended in 60% (v/v) ethanol, the residue was resuspended, the combined supernatants were evaporated to a small volume and chromatograms were run in a descending solvent before detection by an automatic radioactive chromatogram scanner with computerized quantitative determination (see Chain *et al.* 1969).

*Perfusion-fluid analyses.* Methods for the determination of glucose, lactate, pyruvate, [$^{14}$C]glucose, [$^{14}$C]lactate and [$^{3}$H]sorbitol were as described elsewhere (Opie, Shipp, Evans & Leboeuf, 1962; Chain *et al.* 1969; Opie & Mansford, 1971). Production of $^{14}CO_2$ was measured as described by Chain *et al.* (1969). Acetoacetate and $\beta$-hydroxybutyrate were measured by the method of Williamson, Mellanby & Krebs (1962).

*Measurement of glycolytic flux rate.* The glycolytic flux in recirculation experiments was assessed in two ways. First, the rate of entry of $C_6$ units into glycolysis was the sum of the glucose uptake and the net glycogen change; the latter was taken to be the difference between the glycogen value at the end of the perfusion and the mean glycogen value on another series of hearts analysed at the start of the recirculation perfusion. Secondly, the rate of exit from the glycolytic pathway was assessed by the sum of $^{14}CO_2$ formation from [$^{14}$C]glucose and rates of formation of pyruvate and lactate in the perfusion medium.

*Expression of results.* A portion of the powdered frozen tissue was dried to constant weight. The weight of the frozen tissue was corrected by the appropriate factor to express all results in terms of fresh weight (dry-weight/fresh-weight ratio 20%). In accordance with the proposals of Hohorst *et al.* (1959) the metabolite content is taken as the amount of that metabolite/g of fresh tissue, in contrast with the metabolite concentration which is the molarity of that metabolite as a solution in the water of a given cell compartment. Net glycogen changes in the heart tissue, and perfusate values of lactate and pyruvate, were expressed as $\mu$mol of glucose equivalent/perfusion period per g fresh wt.

## RESULTS

*Effect of increasing contractile activity on the oxygen uptake, glycolytic intermediates and adenine nucleotides* (*Table* 1). A wide range of contractile activity of the heart was induced in a non-recirculation system by (1) a preparation that was virtually non-contracting, obtained by a high-$K^+$ medium ($K^+$ concentration 12mequiv./l) and a low perfusion pressure; (2) increasing systolic-pressure development, associated with increased aortic perfusion pressure; (3) external work associated with atrial perfusion. The heart rate increased as did the coronary flow rate. Heart work, although not measured, must have increased (Neely *et al.* 1967*a*). The coronary-venous-effluent $O_2$ partial pressure was relatively constant; hence the $O_2$ uptake increased by about the same amount as did the coronary flow, which is a characteristic of the isolated perfused rat heart (Opie, 1965). During increasing heart work there were decreased tissue contents of hexose monophosphates, glycogen and creatine phosphate. Lactate output rose. On com-

Table 1. *Relation between increasing heart work and oxygen uptake, and tissue contents of hexose phosphates, citrate, glycogen and high-energy phosphates and related compounds in hearts perfused for 15 min*

Increasing heart work was induced by increasing the aortic perfusion pressure (45, 65 or 100 cm$H_2O$), thereby leading to increased coronary flow rate and increased left-ventricular work (Opie, 1965). When atrial perfusion was commenced, the peak systolic pressure increased further and there was a physiological cardiac output ('working' system). Decreased heart work was achieved by a low perfusion pressure and a high-$K^+$ (12 mequiv./l) medium. Hearts were perfused in the non-recirculation system with Krebs–Henseleit bicarbonate buffer, with glucose (5.5 mM) as substrate. Hearts were freeze-clamped after 15 min perfusion. A dash in this and subsequent tables indicates absence of results. $K'$(ad), apparent equilibrium constant of adenylate kinase reaction calculated from ATP, ADP and AMP contents. Mean values ± S.E.M. are given for the numbers of hearts indicated in parentheses. Values in this and subsequent tables are expressed in terms of fresh weight (dry wt. × 5).

| Perfusion conditions | Heart rate (beats/min) | Coronary flow rate (ml/min) | $O_2$ uptake ($\mu$l/min per g) | Intracellular glucose | Glucose 6-phosphate content (nmol/g) | Fructose 6-phosphate content (nmol/g) | Fructose 1,6-diphosphate content (nmol/g) | Citrate content (nmol/g) | Glycogen content ($\mu$mol of glucose equiv./g) | Tissue lactate/pyruvate content ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| (1) High-$K^+$ medium, perfusion pressure 45 cm$H_2O$ | 8±4* (18) | 4.2±0.2* (11) | 38±1* (10) | None detected | 166±12 (9) | 44±5† (9) | 59±5 (9) | 521±24* (8) | — | 28±2* (4) |
| (2) Perfusion pressure 45 cm$H_2O$ (normal $K^+$) | 123±27* (6) | 3.1±0.1* (6) | 34±4* (10) | None detected | 209±15† (10) | 41±5 (10) | 133±20* (10) | — | — | 38±5* (5) |
| (3) Perfusion pressure 65 cm$H_2O$ (normal $K^+$) | 206±5 (11) | 8.3±0.4 (11) | 88±4 (15) | None detected | 140±12 (12) | 33±3 (11) | 69±7 (14) | 143±14 (11) | 22.2±2.5 (7) | 19±2 (13) |
| (4) Perfusion pressure 100 cm$H_2O$ (normal $K^+$) | 248±28 (4) | 13.3±0.6* (8) | 130±6 (8) | None detected | 120±9 (8) | 26±3 (8) | 83±8 (8) | 131±23 (4) | — | — |
| (5) Working hearts, atrial perfusion pressure 20 cm$H_2O$ (normal $K^+$) | 307±10* (11) | 24.0±2.1* (11) | 298±11* (10) | None detected | 102±6* (11) | 21±1* (11) | 91±7† (11) | 110±5* (23) | 11.9±0.8* (4) | 10±2* (7) |

| | ATP content ($\mu$mol/g) | ADP content ($\mu$mol/g) | AMP content ($\mu$mol/g) | Creatine phosphate content ($\mu$mol/g) | $P_i$ content ($\mu$mol/g) | $K'$(ad) | ATP/ADP content ratio | ATP/AMP content ratio | Lactate output ($\mu$mol/min per g) |
|---|---|---|---|---|---|---|---|---|---|
| (1) High-$K^+$ medium perfusion pressure 45 cm$H_2O$ | 5.17±0.08 (8) | 1.05±0.04 (7) | 0.107±0.005 (8) | 9.73±0.21† (8) | 3.84±0.17 (8) | 0.51±0.02† (7) | 5.0±0.2 (7) | 49±2 (8) | 0.18±0.02† (4) |
| (3) Perfusion pressure 65 cm$H_2O$ (normal $K^+$) | 5.33±0.22 (6) | 1.09±0.05 (6) | 0.094±0.006 (6) | 8.57±0.50 (6) | 3.89±0.32 (6) | 0.43±0.03 (6) | 5.0±0.3 (6) | 51±9 (6) | 0.96±0.20 (4) |
| (5) Working hearts, atrial perfusion pressure 20 cm$H_2O$ (normal $K^+$) | 4.49±0.17* (8) | 1.27±0.04* (8) | 0.169±0.008* (8) | 4.97±0.26* (8) | 5.88±0.20* (8) | 0.48±0.05 (8) | 3.6±0.2* (8) | 27±2* (8) | 2.37±0.21* (4) |

* $P<0.01$; † $P<0.05$; for other differences from hearts perfused at 65 cm$H_2O$, $P>0.05$.

parison of working hearts with hearts perfused at 65 $cmH_2O$, the values of $P_i$, ADP and AMP were increased; the values for ATP and for the ATP/ADP and ATP/AMP content ratios decreased. These changes did not occur on comparison of high-$K^+$ hearts with the hearts perfused at 65 $cmH_2O$, but the citrate content was much higher in the high-$K^+$ hearts.

In view of the low contractile activity, the high fructose 1,6-diphosphate content and the high lactate/pyruvate content ratio in hearts perfused at 45 $cmH_2O$ with a normal-$K^+$ buffer, hypoxia was suspected, and 65 $cmH_2O$ was chosen as the lowest desirable perfusion pressure and hence the control situation to give the lowest left-ventricular workload in beating hearts in subsequent experiments.

In 32 hearts perfused at 65 $cmH_2O$ pressure, tissue values of hexose phosphates were closely similar whether assayed by the glucose 6-phosphate dehydrogenase procedure or by the fructose 6-phosphate kinase procedure (see the Methods section). Similar values for $O_2$ uptake and for hexose phosphates were obtained in 14 hearts when Nembutal anaesthesia (15mg intraperitoneally) was used instead of ether; the latter was found more convenient and used in all subsequent experiments.

*Effects of increased heart work on the contribution of glucose uptake and glycogen breakdown to respiration and on glycolytic flux* (*Tables* 2 *and* 3). Studies in a non-recirculation system did not permit assessment of the contribution of glucose uptake to respiration. Further studies were therefore carried

Table 2. *Effect of heart work on oxygen uptake and contribution of glucose uptake and glycogen breakdown to respiration in perfused rat heart*

All hearts were perfused for 30 min (unless otherwise stated) in the recirculation system. Langendorff hearts were perfused via the aorta at 65 or 100 $cmH_2O$ hydrostatic pressure and developed corresponding peak left-ventricular systolic pressures; working hearts were perfused by the atrium and developed peak left-ventricular systolic pressure of about 180 $cmH_2O$. The initial perfusate contained glucose (11.1 mM) and (in group 6) acetoacetate (3.7 mM) and L-$\beta$-hydroxybutyrate (2.0 mM). The '+ insulin' groups indicate insulin (2 munits/ml) added to the perfusate. Results are given as $\mu$l of $O_2$/min per g fresh wt. (mean ± S.E.M., for the numbers of hearts indicated in parentheses). Glucose contribution to respiration is calculated as the percentage of respiration accounted for by $^{14}CO_2$ formation from 11.1 mM-[U-$^{14}$C]glucose (see Chain *et al.* 1969). However, for hearts perfused with glucose for 60 min or with glucose and ketone bodies the rate of entry into glycolysis was assumed to be (glucose uptake + glycogen breakdown) and the rate of oxidation was (entry into glycolysis − formation of lactate, pyruvate and glycogen).

| Perfusion conditions | $O_2$ uptake ($\mu$l/min per g) | Glucose contribution to respiration (%) | Maximum glycogen contribution to respiration (%) |
|---|---|---|---|
| (1) Normal rat hearts | | | |
| Langendorff, 65 $cmH_2O$ | 72±16 (7) | 41±4 (7) | 0 |
| Langendorff, 100 $cmH_2O$ | 157±19 (7) | 38±4 (7) | 5 |
| Work, 30 min | 343±14 (30) | 46±3 (10) | 7 |
| Work, 60 min | 334±28 (17) | 83±11 (5) | 5 |
| (2) Normal rat hearts + insulin | | | |
| Langendorff, 100 $cmH_2O$ | 146±48 (6) | 69±6 (6) | 0 |
| Work | 373±20 (7) | 71±5 (12) | 0 |
| (3) Hearts from chronically streptozotocin-diabetic rats | | | |
| Langendorff, 100 $cmH_2O$ | 144±7 (12) | 20±8 (8) | 0 |
| Work | 338±21 (20) | 44±5 (12) | 1 |
| (4) Hearts from chronically streptozotocin-diabetic rats + insulin | | | |
| Langendorff, 100 $cmH_2O$ | 136±6 (7) | 26±4 (7) | 0 |
| Work | 338±23 (11) | 60±4 (7) | 1 |
| (5) Hearts from acute alloxan-diabetic hearts | | | |
| Langendorff, 100 $cmH_2O$ | 143±14 (4) | 6±3 (4) | 25 |
| Work | 374±23 (5) | 8±1 (5) | 16 |
| Work + insulin | 435±57 (8) | 25±6 (6) | 1 |
| (6) Normal rat hearts + ketone bodies in perfusate | | | |
| Langendorff, 65 $cmH_2O$ | 114±2 (3) | 0 (11) | 0 |
| Work, 30 min | 310±6 (4) | 20±10 (11) | 8 |
| Work, 60 min | 296 (2) | 27±7 (7) | 1 |

Table 3. *Comparison of rates of entry of glucose and glycogen into glycolysis with rates of formation of products of glycolytic flux in non-working or working perfused hearts from normal or diabetic rats*

Glucose (11.1 mM) was initial substrate and all perfusions were for 30 min except for 15 min working hearts or when ketone bodies were added (acetoacetate, 3.7 mM; L-β-hydroxybutyrate, 2.0 mM; perfusions for 60 min). Units are μmol of glucose equivalent/30 min per g fresh wt. except for 15 min and 60 min hearts. Hearts were pre-perfused for 15 min in a non-recirculation system at 65 cm$H_2O$ hydrostatic pressure or at 100 cm$H_2O$ (Langendorff 100 cm$H_2O$ hearts and working hearts). 'Langendorff' indicates hearts perfused through the aorta at the hydrostatic pressure indicated; 'working' indicates hearts perfused through the left atrium at 20 cm$H_2O$ hydrostatic pressure; '+insulin' indicates insulin (2 munits/ml) added to the perfusate; 'chronically streptozotocin-diabetic' indicates diabetic rats 7 days after treatment with streptozotocin; 'acutely alloxan-diabetic' indicates diabetic rats 48 h after treatment with alloxan; a dash indicates absence of data. Mean values ± S.E.M. are given for the numbers of hearts indicated in parentheses.

| Perfusion conditions | | | | Entry into glycolysis | | | Formation of $^{14}C$-labelled tissue intermediates (μmol of glucose equiv./g) | Products of glycolytic flux | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Condition of rats | Type of perfusion | Duration of perfusion (min) | Insulin | Glucose uptake (μmol/g) (*A*) | Net glycogen change (μmol of glucose equiv./g) (*B*) | Total entry (μmol of glucose equiv./g) (*A* − *B*) | | Output of [$^{14}C$]lactate (μmol of glucose equiv./g) | Output of lactate (μmol of glucose equiv./g) (*C*) | Output of pyruvate (μmol of glucose equiv./g) (*D*) | Formation of $^{14}CO_2$ (μmol of glucose equiv./g) (*E*) | Total (μmol of glucose equiv./g) (*C* + *D* + *E*) |
| Normal | Langendorff, 65 cm$H_2O$ | 30 | 0 | 15.4 ± 0.5 (16) | +0.7 ± 1.1 (7) | 14.7 ± 1.3 | 2.4 ± 0.3 (7) | — | 3.5 ± 0.6 (12) | 0.4 ± 0.1 (7) | 6.6 ± 0.5 (10) | 10.5 ± 0.8 |
| Normal | Langendorff, 100 cm$H_2O$ | 30 | | 22.1 ± 0.9* (19) | −1.6 ± 1.0 (7) | 23.7 ± 1.3 | 3.8 ± 0.6 (10) | 5.4 ± 0.5* (9) | 5.5 ± 0.7 (23) | 0.4 ± 0.1 (7) | 13.1 ± 1.3* (7) | 19.0 ± 1.5 |
| | | 30 | + | 45.6 ± 1.8* (6) | +8.0 ± 2.0 (6) | 37.6 ± 2.7 | 7.8 ± 0.6* (6) | 11.5 ± 1.2* (16) | 11.3 ± 0.9 (13) | 1.3 ± 0.2 (6) | 22.5 ± 1.4* (6) | 35.1 ± 1.6 |
| Normal | Working | 15 | 0 | 18.0 ± 0.7 (20) | −7.2 ± 1.0 (8) | 25.2 ± 0.6 | — | — | 3.6 ± 0.8 (20) | 0.7 ± 0.6 (6) | 12.7 ± 0.8 (20) | 17.0 ± 0.8 |
| | | 30 | 0 | 52.8 ± 1.6 (12) | −6.6 ± 0.6 (6) | 59.4 ± 1.7 | 7.4 ± 0.7 (7) | 3.8 ± 0.5* (4) | 3.6 ± 0.7 (12) | 0.4 ± 0.1 (4) | 42.8 ± 1.8 (12) | 46.8 ± 1.9 |
| | | 30 | + | 107.6 ± 3.4 (15) | +2.4 ± 0.6 (13) | 105.2 ± 4.0 | 14.5 ± 0.6 (7) | 16.8 ± 3.5* (7) | 16.0 ± 2.2 (19) | 1.5 ± 0.3 (8) | 66.7 ± 3.6* (12) | 84.2 ± 3.9 |
| Chronically streptozotocin-diabetic | Langendorff, 100 cm$H_2O$ | 30 | 0 | 11.4 ± 0.8* (12) | +2.0 ± 3.4 (12) | 9.4 ± 4.2 | 3.9 ± 0.5 (8) | — | 3.2 ± 0.7* (12) | 1.8 ± 0.2 (12) | 4.7 ± 0.8* (8) | 9.7 ± 1.1 |
| | Working | 30 | 0 | 44.2 ± 2.4* (8) | −0.6 ± 1.8 (8) | 49.8 ± 4.2 | 6.7 ± 0.5 (10) | — | 4.4 ± 1.0* (8) | 1.0 ± 0.3 (8) | 40.0 ± 2.0* (10) | 45.4 ± 2.3 |
| | | 30 | + | 81.0 ± 6.6* (11) | −0.8 ± 1.6 (11) | 81.8 ± 8.2 | 6.2 ± 0.2 (8) | — | 27.0 ± 3.4* (11) | 5.2 ± 0.6 (11) | 37.4 ± 5.0* (8) | 69.6 ± 6.0 |
| Acutely alloxan-diabetic | Langendorff, 100 cm$H_2O$ | 30 | 0 | 13.8 ± 1.6 (4) | −8.0 ± 1.6 (4) | 21.8 ± 3.2 | 4.4 ± 0.3 (4) | — | 3.7 ± 1.2 (4) | 2.8 ± 1.0 (4) | 2.0 ± 0.8 (4) | 8.5 ± 1.8 |
| | Working | 30 | 0 | 16.0 ± 1.4 (5) | −15.8 ± 0.8 (5) | 31.8 ± 2.2 | 4.8 ± 0.3 | — | 11.9 ± 1.6 (5) | 11.4 ± 1.1 (5) | 9.6 ± 1.0 (5) | 32.9 ± 2.2 |
| | | 30 | + | 63.2 ± 5.0 (8) | −1.6 ± 3.8 (8) | 64.8 ± 8.8 | 8.4 ± 0.5 (8) | — | 21.6 ± 3.6 (8) | 5.8 ± 0.9 (4) | 38.8 ± 6.0 (8) | 66.2 ± 6.9 |
| Normal | Langendorff, 65 cm$H_2O$, with added ketone bodies | 60 | 0 | 20.9 ± 5.1 (3) | −1.8 ± 3.1 (3) | 22.7 ± 6.0 | — | — | 23.6 ± 3.2 (2) | 0.7 ± 0.1 (2) | — | |
| | Working, with ketone bodies | 60 | 0 | 46.2 ± 7.6 (3) | −6.4 ± 2.6 (3) | 52.6 ± 8.1 | — | — | 24.6 ± 9.9 (3) | 2.1 ± 0.6 (3) | — | |

* Calculated from data of Chain *et al.* (1969).

out in a recirculation system, with a high glucose concentration (11.1mM) to achieve high rates of glucose uptake. The effects of increased heart work on respiration and on glycolysis were compared with the effects of the following conditions: the diabetic state (acute alloxan-diabetes or chronic streptozotocin-diabetes), the addition of insulin to the perfusate, or the presence of ketone bodies as alternative substrate.

In hearts perfused in the recirculation system with a high-$K^+$ medium, and glucose (11.1mM) as substrate at 100 $cmH_2O$ perfusion pressure, the rate of glucose uptake was less than 6 $\mu$mol/30 min per g (four hearts) and the rate of $^{14}CO_2$ formation from [$^{14}$C]glucose was 1.8±0.3 $\mu$mol of glucose equivalent/30 min per g (eight hearts). Because the glucose uptake was too low to be measured accurately, no further experiments on this were carried out. A decreased rate of glycolytic flux in these hearts was also supported by the low $O_2$ uptake and the low rate of lactate output shown in Table 1, and is in keeping with the finding by Neely, Liebermeister & Morgan (1967*b*) that over 90% of glucose uptake was regulated by mechanical activity of the isolated rat heart.

The contribution of glucose to respiration was about 40–50% during increased heart work over 30 min (Table 2). The contribution was increased to 70% by the addition of insulin, both to non-working and to working hearts, whereas the contribution was decreased by the diabetic states or by the addition of ketone bodies. In hearts from streptozotocin-diabetic rats external work increased the glucose contribution to respiration to within the normal range in the absence of insulin, and to about 85% of the normal value in the presence of insulin. Thus external work stimulated glucose uptake and oxidation in the chronic diabetic animal to restore the defect caused by the deficiency of insulin. However, in acute alloxan-diabetes external work did not restore the defect. In all hearts values for entry into glycolysis were similar to those obtained for total products of glycolytic flux, and accumulation of $^{14}$C-labelled tissue intermediates was low except in hearts with added insulin (Table 3). Output of [$^{14}$C]lactate was similar to that of unlabelled lactate. Because the rates of entry into glycolysis usually equalled the rates of formation of products of glycolysis, and, because the glycogen change was usually small in relation to the glucose uptake, it follows that (i) the major adjustments in the glycolytic flux rate in the conditions studied was achieved by altered rates of glucose uptake, and (ii) glucose oxidation was approximately equivalent to glucose uptake less lactate and pyruvate formation. An exception to this conclusion was that in hearts from alloxan-diabetic rats (in the absence of added insulin) there was appreciable breakdown of glycogen, which contributed nearly as much as did glucose uptake to the glycolytic flux. The high pyruvate/lactate content ratios in hearts from diabetic rats confirmed the findings of Garland, Newsholme & Randle (1964), and are fully discussed in another paper (Opie & Mansford, 1971).

*Tissue contents of glycolytic intermediates and of adenine nucleotides during variations of glycolytic flux in hearts perfused in the recirculation system for* 30 *or* 60 min (*Table* 4). To help elucidate the mechanisms underlying the large alterations in glycolytic flux, tissue contents of glucose, hexose phosphates, citrate and (in selected situations) adenine nucleotides were measured (Table 4). When glycolytic flux was increased by increasing the perfusion pressure (from 65 to 100 $cmH_2O$), hexose monophosphate values fell, fructose diphosphate values rose and citrate values fell; no intracellular glucose was detected. When glycolytic flux was decreased by the chronic diabetic state (in non-working hearts, perfusion pressure 100 $cmH_2O$), the tissue contents of hexose phosphates and of citrate decreased. These tissue changes were more striking in the presence of added insulin. In hearts from rats with acute diabetes (also perfused at 100 $cmH_2O$ pressure), the citrate content increased and mean values for hexose monophosphates rose (but not significantly). This contrasts with the significant increase in hexose monophosphates in similar hearts when insulin is added (Newsholme & Randle, 1964; Mansford & Opie, 1968).

In working hearts, the chronic diabetic state was associated with an unaltered glycolytic flux rate and decreased content of citrate when compared with normals. When insulin was added to the perfusion medium, the values for glycolytic flux and for the tissue contents of glucose 6-phosphate and fructose 6-phosphate and of citrate were significantly decreased (comparing chronically diabetic hearts plus insulin with normal hearts plus insulin). In working hearts from acutely diabetic rats, there was no increase in the tissue contents of citrate or of fructose 6-phosphate, although the glycolytic flux rate was decreased when compared with normal. In the presence of added insulin, hexose monophosphate contents were unchanged, that of citrate was increased and glycolytic flux was also decreased.

Changes in adenine nucleotide contents were a decreased ATP value during external work (when compared with perfusions of non-working hearts, perfusion pressure 65 $cmH_2O$) and a decreased value of creatine phosphate. ADP and AMP values were unchanged; there was a loss of total adenine nucleotides. In diabetic hearts adenine nucleotide values were similar to those in working hearts, except that in the absence of insulin in hearts

Table 4. *Effects of diabetic states and of addition of insulin on tissue contents of glucose, hexose phosphates, citrate and high-energy phosphate compounds in non-working or working hearts perfused in a recirculation system for 30 min*

Glucose (11.1 mM) was the initial substrate and all perfusions were for 30 min except when ketone bodies were added (acetoacetate, 3.7 mM; L-β-hydroxybutyrate, 2 mM; perfusions for 60 min). Glycolytic flux values are taken from Table 3, except for values marked thus (¶) which are taken from unpublished results; values are usually the sum of lactate and pyruvate formation and of $^{14}CO_2$ formation from [$^{14}$C]glucose. Hearts were pre-perfused for 15 min in a non-recirculation system at 65 cm$H_2O$ hydrostatic pressure or at 100 cm$H_2O$ (Langendorff 100 cm$H_2O$ hearts and working hearts). 'Langendorff' indicates hearts perfused through the aorta at the hydrostatic pressure indicated; 'working' indicates hearts perfused through the left atrium at 20 cm$H_2O$ hydrostatic pressure; '+insulin' indicates insulin (2 munits/ml) added to the perfusate; 'chronically streptozotocin-diabetic' indicates diabetic rats 7 days after treatment with streptozotocin; 'acutely alloxan-diabetic' indicates diabetic rats 48 h after treatment with alloxan; a dash indicates absence of data. Mean values ± S.E.M. are given for the numbers of hearts indicated in parentheses.

| Perfusion conditions | Condition of rats | Glycolytic flux (μmol of glucose equiv./g) | Intracellular glucose content (μmol/ml) | Glucose 6-phosphate content (nmol/g) | Fructose 6-phosphate content (nmol/g) | Fructose 1,6-diphosphate content (nmol/g) | Citrate content (nmol/g) | ATP content (μmol/g) | ADP content (μmol/g) | AMP content (μmol/g) | Creatine phosphate content (μmol/g) | ATP/AMP content ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) Langendorff, 65 cm$H_2O$ | Normal | 13.5 ± 0.8‡ | None detected | 143 ± 11 (19) | 55 ± 3‡ (19) | 23 ± 2‡ (4) | 220 ± 19‡ (15) | 4.70 ± 0.41‡ (4) | 1.14 ± 0.07 (4) | 0.15 ± 0.01 (4) | 6.64 ± 0.45‡ (4) | 33 ± 5 (4) |
| (2) Langendorff, 100 cm$H_2O$ | Normal | 19.0 ± 1.5‡ | None detected | 125 ± 8 (12) | 25 ± 2‡ (10) | 65 ± 6‡ (10) | 140 ± 15‡ (7) | 3.78 (2) | 0.91 (2) | 0.41 (2) | 4.10 (2) | 27 (2) |
| | Chronically streptozotocin-diabetic | 9.7 ± 1.1* | 1.7 ± 1.8 (4) | 66 ± 7* (8) | 21 ± 1† (8) | 38 ± 6* (8) | 126 ± 19 (9) | — | — | — | — | — |
| | Acutely alloxan-diabetic | 8.5 ± 1.8* | 0.8 ± 0.8 ‖ | 134 ± 14 (4) | 48 ± 12 (4) | 77 ± 11 (4) | 307 ± 73† (4) | — | — | — | — | — |
| (3) Langendorff, 100 cm$H_2O$ + insulin | Normal | 35.1 ± 1.6 | — | 439 ± 24 (7) | 76 ± 8 (7) | 109 ± 4 (11) | 417 ± 39 (6) | — | — | — | — | — |
| | Chronically streptozotocin-diabetic | 31.2 ± 2.3¶ | — | 255 ± 14* (14) | 57 ± 5† (14) | 74 ± 9* (14) | 316 ± 24† (10) | — | — | — | — | — |
| (4) Working | Normal | 46.8 ± 1.9 | None detected | 113 ± 8 (21) | 29 ± 2 (23) | 23 ± 3 (7) | 181 ± 20 (14) | 3.56 ± 0.44‡ (7) | 1.08 ± 0.05 (11) | 0.11 ± 0.02 (7) | 3.00 ± 0.32‡ (7) | 37 ± 4 (7) |
| | Chronically streptozotocin-diabetic | 45.4 ± 2.3 | None detected | 97 ± 15 (9) | 28 ± 3 (9) | 47 ± 5* (9) | 118 ± 11* (10) | — | — | — | — | — |
| | Acutely alloxan-diabetic | 32.9 ± 2.2* | 0.4 ± 0.6 (5) | 94 ± 5† (4) | 33 ± 2 (4) | 56 ± 5* (4) | 188 ± 31 (5) | 3.07 ± 0.29 (5) | 1.56 ± 0.16* (5) | 0.37 ± 0.05* (5) | 3.00 ± 0.20 (5) | 9 ± 1* (2) |
| (5) Working, +insulin | Normal | 84.2 ± 3.9 | 3.9 ± 1.8 (6) | 304 ± 17 (14) | 68 ± 5 (15) | 37 ± 6 (6) | 433 ± 37 (14) | 3.49 ± 0.39 (6) | 0.90 ± 0.06 (6) | 0.12 ± 0.02 (6) | 3.79 ± 0.38 (6) | 39 ± 10 (6) |
| | Chronically streptozotocin-diabetic | 69.6 ± 6.0† | 4.6 ± 2.0 (4) | 210 ± 10* (10) | 47 ± 5* (9) | 66 ± 3* (9) | 215 ± 24* (14) | 3.61 ± 0.24 (10) | 1.00 ± 0.12 (10) | 0.24 ± 0.03* (10) | 4.62 ± 0.19 (9) | 18 ± 3† (10) |
| | Acutely alloxan-diabetic | 66.2 ± 6.9† | 4.9 ± 1.8 (4) | 280 ± 18 (11) | 74 ± 11 (11) | 12 ± 3* (6) | 662 ± 53* (8) | 3.02 ± 0.18 (10) | 0.78 ± 0.05 (6) | 0.14 ± 0.02 (6) | 4.18 ± 0.20 (9) | 25 ± 4 (6) |
| (6) Ketone-body perfusions (60 min) | | | | | | | | | | | | |
| Langendorff, 65 cm$H_2O$ | Normal | 22.7 ± 6.0 | None detected | 289 ± 33 (3) | 63 ± 4 (3) | 21 ± 2 (3) | 2125 ± 165 (3) | 4.26 ± 0.47 (3) | 0.99 ± 0.06 (3) | 0.16 ± 0.02 (3) | 7.43 ± 0.88 (3) | 26 ± 1 (3) |
| Working | Normal | 52.6 ± 8.1§ | None detected | 307 ± 25 (3) | 74 ± 11 (3) | 30 ± 6 (3) | 874 ± 125§ (3) | 2.80 ± 0.41§ (3) | 0.93 ± 0.12 (3) | 0.12 ± 0.02 (3) | 3.98 ± 0.43§ (3) | 24 ± 3 (3) |

* $P < 0.01$; † $P < 0.05$; for other differences from the appropriate normal control $P > 0.05$.
‡ $P < 0.05$ for comparison between the two series of normal hearts indicated.
§ $P < 0.05$ for differences between two groups of normal hearts perfused with ketone bodies.
‖ Taken from Morgan *et al.* (1961*b*).

from rats with acute alloxan-diabetes AMP and ADP increased, whereas in the presence of added insulin in hearts from rats with chronic diabetes AMP increased.

The addition of ketone bodies to the working heart decreased the glucose contribution to respiration from over 80% to 30% or less and decreased the glucose contribution in non-working hearts to zero. Increased work increased glycolytic flux in ketone-body-perfused hearts, but, in spite of very large decreases in tissue content of citrate, the hexose phosphate values were unchanged.

*Effects of acute increases in heart work on mechanical performance and contents of adenine nucleotides and glycolytic intermediates* (*Tables* 5–7). In view of the decreased ATP values after 15–60 min of heart work (Tables 1 and 4), further short-term studies (0–15 min) were required to define how quickly the ATP value fell. When heart work was acutely increased by the transition from a low workload (aortic perfusion pressure 65 $cmH_2O$) to a working perfusion, the peak systolic pressure rose within 1–2 s (Fig. 1) to reach a plateau value about threefold higher (Table 5). The coronary flow had increased at 5 s but not as much as at 15 s. Because the venous-effluent $O_2$ partial pressure is fairly constant in the perfused rat heart (Opie, 1965), the $O_2$ uptake is proportional to the coronary flow, and it can be assumed that the $O_2$ uptake increased by the same amount as the coronary flow at 5 s and at 15 s. Therefore the $O_2$ uptake at 5 s was less than at 15 s, even though the work done by the heart was already the same at 5 s as at 15 s. There was probably a short period of relatively inadequate $O_2$ delivery at the commencement of increased heart work by the atrial perfusion system.

The peak systolic pressure after 5 s of increased heart work could be varied by altering the angle of the atrial cannula relative to aortic cannula, presumably by altering the rate of atrial filling. More marked metabolic changes were found in hearts developing the highest systolic pressures, which were used in subsequent experiments ('high-pressure' hearts; Tables 5 and 6).

Changes after 5 s of increased work at the higher systolic pressure were (Table 5): increased contents of ADP and AMP, a decreased content of creatine phosphate, and decreased ATP/ADP and ATP/AMP content ratios. ATP underwent only small decreases at 30 s and 2 min of increased heart work and then recovered to the control value. There were rapid rises in AMP and ADP values at the start of heart work, but at 10 min only ADP was significantly increased. It is concluded that, during increasing duration of increased heart work, ATP undergoes a small and transient initial decrease, to recover after 5 and 10 min, and after that

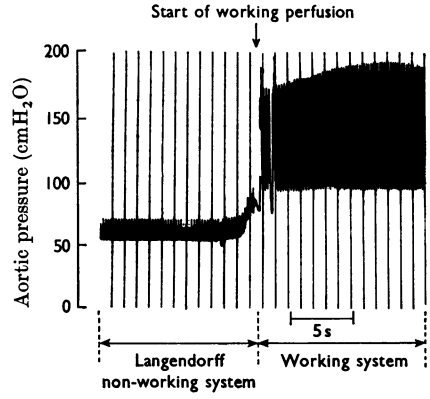


Fig. 1. Aortic-pressure tracings during the transition from the Langendorff perfused 'non-working' system to the 'working' atrial perfusion system (see the Methods section). There were considerable rises in systolic and diastolic pressures within 1 s. The brief rise in aortic pressure before the actual onset of the working perfusion is caused by the change from the non-recirculation to the recirculation system (see the Methods section).

ATP again starts to fall in value with a decrease at 15 min and an obvious decrease at 30 or 60 min. The ATP/ADP content ratio stayed low throughout the period of increased heart work. Creatine phosphate values fell throughout the period of increased heart work.

After 5 s of increased heart work at high systolic-pressure values (Table 6), glucose 6-phosphate and fructose 6-phosphate contents fell; the increases in values of fructose 1,6-diphosphate, dihydroxyacetone phosphate and pyruvate corresponded to an increase in glycolytic flux of 27$\mu$mol of glucose equivalent/30 min per g. After 15 s of increased heart work the content of fructose 6-phosphate fell, and the increased values of fructose 1,6-diphosphate, dihydroxyacetone phosphate, pyruvate and malate corresponded to an increase in glycolytic flux of 21$\mu$mol of glucose equivalent/30 min per g. At 30 s the content of glucose 6-phosphate fell; the rise in fructose 1,6-diphosphate and lactate values corresponded to an increase in glycolytic flux of 41$\mu$mol of glucose equivalent/30 min per g. At 2 min of increased heart work, total hexose monophosphate values fell, whereas the contents of fructose 1,6-diphosphate and glycerol phosphate rose.

In the presence of added insulin (Table 7), aortic flow values in the acutely working heart were similar to those obtained without added insulin (Table 5). Changes in the values for adenine nucleotides and creatine phosphate were: decreased contents of ATP and creatine phosphate (30 s–2 min) and increased contents of AMP (30 s–1 min) and of ADP (30 s–1 min). Glycolytic flux increased,

Table 5. *Effects of duration of increased work by isolated rat heart on tissue contents of high-energy phosphates and related compounds, and on mechanical performance*

Hearts were perfused in a recirculation system with 50 ml of Krebs–Henseleit bicarbonate buffer, with glucose (11.1 mM) as initial substrate. Hearts were freeze-clamped at the times indicated after onset of atrial perfusion at 20 $cmH_2O$ hydrostatic pressure. '0s' indicates 15 min non-recirculation wash-out perfusion with the same buffer at 65 $cmH_2O$ aortic perfusion pressure (except that 100 $cmH_2O$ pressure was used for control hearts in 15 min group). $K'$(ad) is the apparent equilibrium constant of the adenylate kinase reaction calculated from ATP, ADP and AMP contents. Mean values ± S.E.M. are given for the numbers of hearts indicated in parentheses.

| Duration of increased work | ATP content (μmol/g) | ADP content (μmol/g) | AMP content (μmol/g) | Creatine phosphate content (μmol/g) | $P_i$ content (μmol/g) | $K'$(ad) | ATP/ADP content ratio | ATP/AMP content ratio | Peak systolic pressure ($cmH_2O$) | Aortic output (ml/min) | Coronary flow (ml/min) | Heart rate (beats/min) | $O_2$ uptake (μl/min per g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0s, control | 4.48±0.14 (8) | 0.85±0.02 (8) | 0.089±0.007 (8) | 7.24±0.50 (8) | 5.28±0.76 (8) | 0.56 | 5.3±0.1 (8) | 52±4 (8) | 72±1 (10) | 0 | 7.4±0.2 (15) | 280±8 (20) | — |
| 5s, low systolic pressure | 4.44±0.13 (4) | 0.95±0.03* (4) | 0.089±0.009 (4) | 6.29±0.25 (4) | 7.05±1.03 (4) | 0.44 | 4.7±0.1* (4) | 51±4 (4) | 145±6* (4) | — | — | — | — |
| 5s, high systolic pressure | 4.58±0.14 (5) | 1.13±0.05* (5) | 0.147±0.013* (5) | 4.75±0.37* (5) | 7.44±0.46 (4) | 0.53 | 4.1±0.1* (5) | 31±2* (5) | 179±3* (14) | — | 14.6±1.3* (0-5 sec) | 281±10 (12) | — |
| 15s | 4.27±0.24 (5) | 1.26±0.12* (5) | 0.192±0.017* (5) | 3.86±0.35* (5) | 5.75±0.98 (5) | 0.52 | 3.5±0.3* (5) | 23±3* (5) | 188±6* (11) | — | 20.6±0.2* (5-15s) | 315±5* (7) | — |
| 0s, control | 4.88±0.20 (8) | 0.92±0.04 (8) | 0.150±0.020 (8) | 6.12±0.36 (8) | 7.38±0.41 (4) | 0.87 | 5.4±0.2 (8) | 36±5 (8) | 72±1 (10) | 0 | 7.4±0.2 (15) | 280±8 (20) | — |
| 30s | 3.88±0.24* (8) | 1.22±0.09* (8) | 0.220±0.040 (8) | 3.56±0.24* (8) | 8.52±1.00 (8) | 0.57 | 3.6±0.3* (8) | 24±3† (3) | 186±4* (9) | 63±2* (13) | 21.2±1.8* (15-30s) | 297±9 (7) | — |
| 0s, control | 5.04±0.19 (10) | 1.07±0.08 (9) | 0.063±0.013 (9) | 9.12±0.30 (10) | 6.02±0.74 (10) | 0.29 | 5.0±0.3 (9) | 112±21 (9) | 72±1 (10) | 0 | 7.4±0.2 (15) | 280±8 (20) | — |
| 2 min | 4.03±0.38† (4) | 1.20±0.09 (4) | 0.05±0.010 (4) | 5.86±0.47* (4) | 11.28±1.54* (4) | 0.15 | 3.4±0.2* (4) | 66±8† (4) | 175±3* (10) | 57±2* (13) | 22.6±0.9* (14) | 302±6† (9) | — |
| 5 min | 5.00±0.33 (4) | 1.17±0.10 (4) | 0.082±0.017 (4) | 5.30±0.70* (4) | 6.08±0.87 (4) | 0.30 | 4.3±0.2† (4) | 68±13† (4) | 173±3* (10) | 57±3* (6) | 21.0±1.5* (5) | 298±5* (9) | — |
| 10 min | 5.01±0.27 (4) | 1.41±0.09† (4) | 0.104±0.022 (4) | 5.95±0.41* (4) | 5.58±0.46 (4) | 0.26 | 3.6±0.1* (4) | 56±9† (4) | 173±4* (5) | 59±5* (6) | 22.4±1.6* (5) | 302±17 (5) | — |
| 0s, control | 4.31±0.21 (10) | 0.95±0.04 (11) | 0.136±0.007 (11) | 5.54±0.27 (10) | — | 0.65 | 4.6±0.1 (10) | 33±3 (10) | 110±5 (10) | 0 | 11.1±1 (12) | — | 125±6 (9) |
| 15 min | 3.73±0.21 (12) | 1.10±0.04† (12) | 0.207±0.028† (12) | 3.97±0.53† (10) | — | 0.64 | 3.4±0.2* (12) | 24±5 (12) | 191±11* (14) | 38±2* (14) | 25.0±1* (21) | — | 334±13* (17) |

* $P<0.01$; † $P<0.05$; for other differences from appropriate control, $P>0.05$.

Table 6. *Effects of duration of increased work by isolated rat heart on tissue contents of hexose phosphates, dihydroxyacetone phosphate, glycerol 3-phosphate, pyruvate, lactate, glycogen, citrate and malate*

Hearts were perfused in a recirculation system with 50 ml of Krebs–Henseleit bicarbonate buffer with glucose (11.1 mM) as initial substrate. Hearts were freeze-clamped at the times indicated after onset of atrial perfusion at 20 cm$H_2O$ hydrostatic pressure. '0s' hearts were freeze-clamped after 15 min non-recirculation washout perfusion with the same buffer at 65 cm$H_2O$ aortic perfusion pressure (100 cm$H_2O$ for control hearts in the 15 min group). '5s, high pressure' indicates hearts with a mean peak systolic pressure of 179 cm$H_2O$ (see Table 5). Mean values ± S.E.M. are given for the numbers of hearts indicated in parentheses.

| Duration of increased work | Glucose 6-phosphate content (nmol/g) | Fructose 6-phosphate content (nmol/g) | Fructose 1,6-di-phosphate content (nmol/g) | Dihydroxy-acetone phosphate content (nmol/g) | Glycerol phosphate content (nmol/g) | Pyruvate content (nmol/g) | Lactate content (μmol/g) | Glycogen content (μmol of glucose equiv./g) | Citrate content (nmol/g) | Malate content (nmol/g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0s, control | 150±13 (34) | 43±3 (34) | 34±3 (22) | 39±5 (3) | 149±33 (4) | 47±6 (4) | 1.26±0.23 (4) | 25.6±3.1 (6) | 163±12 (30) | 95±9 (4) |
| 5s, high pressure | 64±7* (10) | 24±3* (10) | 54±6* (10) | 113±14* (6) | 153±29 (6) | 83±12† (6) | 1.60±0.09 (6) | 25.0±1.4 (6) | 149±22 (10) | 142±26 (14) |
| 15s | 125±13 (11) | 34±4† (8) | 74±6* (12) | 87±19† (5) | 178±34 (4) | 95±7* (5) | 1.29±0.12 (5) | — | 125±34 (8) | 170±5* (5) |
| 0s, control | 190±13 (23) | 41±3 (24) | 30±3 (8) | 20±2 (12) | 198±25 (19) | 35±3 (12) | 1.08±0.15 (13) | 23.2±1.6 (11) | 226±18 (18) | — |
| 30s | 151±23† (8) | 39±8 (7) | 53±9* (12) | 38±10 (4) | 207±25 (4) | 53±16 (7) | 2.41±0.32* (8) | 18.7±2.1 (4) | 272±34 (8) | — |
| 0s, control | 121±13 (10) | | 40±7 (9) | 80±11 (9) | 104±13 (10) | 85±6 (10) | 2.48±0.13 (10) | 20.7±0.8 (11) | 104±7 (10) | 320±75 (10) |
| 2 min | 91±10† (4) | | 64±7† (4) | 102±24 (4) | 183±35† (4) | 95±26 (4) | 3.18±0.31 (4) | 18.7±1.6 (4) | 117±14 (4) | 305±7 (4) |
| 5 min | 178±29† (4) | | 22±5† (4) | 95±14 (4) | 67±6* (4) | 60±9 (4) | 2.38±0.11 (5) | 17.8±1.6 (4) | 151±17* (4) | 743±34* (4) |
| 10 min | 160±11† (5) | | 46±8 (5) | 42±7* (5) | 49±5* (4) | 111±22 (5) | 3.16±0.27† (5) | 13.8±0.7* (5) | 178±10* (5) | 386±45 (5) |
| 0s, control | 195±21 (10) | 45±4 (10) | 61±3 (7) | 31±4 (7) | 103±13 (10) | 47±6 (10) | 1.20±0.20 (6) | 19.6±1.0 (23) | 203±19 (10) | — |
| 15 min | 119±13† (8) | 32±4† (8) | 56±8 (8) | 38±3 (5) | 202±44† (5) | 31±3† (10) | 0.90±0.11 (4) | 10.7±0.9* (13) | 163±18 (11) | — |

* $P<0.01$; † $P<0.05$; for other differences from appropriate control, $P>0.05$.

Table 7. *Effects of duration of increased work by isolated rat heart on tissue contents of hexose phosphates, trioses, citrate, adenine nucleotides, creatine phosphate and on the calculated glycolytic flux in hearts perfused with glucose* (11.1 mM) *and insulin* (2 *munits/ml*)

Hearts were perfused in a recirculation system with 50 ml of Krebs–Henseleit bicarbonate buffer, and freeze-clamped at the times indicated after onset of atrial perfusion at 20 cm$H_2O$ hydrostatic pressure. '0 s' hearts were freeze-clamped after 15 min non-recirculation wash-out perfusion with the same buffer but without insulin at 100 cm$H_2O$ aortic perfusion pressure. Tissue extracts were deproteinized in 7% (v/v) $HClO_4$ only (see the Methods section). Increase in glycolytic flux was calculated from the increase in the values of fructose 1,6-diphosphate and trioses (glycerol phosphate, dihydroxyacetone phosphate and pyruvate) from the start of increased heart work, and expressed as μmol of glucose equivalent/30 min per g for comparison with Tables 3 and 4. Mean values of two hearts are given.

| Duration of increased work | Aortic flow (ml/min) | Glucose 6-phosphate content (nmol/g) | Fructose 6-phosphate content (nmol/g) | Fructose 1,6-di-phosphate content (nmol/g) | Total triose content (nmol/g) | Citrate content (nmol/g) | ATP content (μmol/g) | ADP content (μmol/g) | AMP content (μmol/g) | Creatine phosphate content (μmol/g) | Increase in glycolytic flux (μmol of glucose equiv./30 min per g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 s, control | 0 | 229 | 52 | 89 | 358 | 458 | 3.77 | 1.07 | 0.17 | 4.92 | 0 |
| 30 s | 57 | 224 | 50 | 194 | 555 | 311 | 3.36 | 1.40 | 0.35 | 3.18 | 12.2 |
| 1 min | 60 | 270 | 62 | 211 | 492 | 457 | 3.32 | 1.19 | 0.33 | 3.70 | 5.7 |
| 2 min | 52 | 239 | 48 | 207 | 493 | 378 | 3.12 | 1.05 | 0.33 | 3.47 | 2.8 |

but tissue values of hexose monophosphates did not decrease.

*Effects of addition of adrenaline* (*Fig.* 2). The results obtained during acutely increased heart work raised the possibility that the $O_2$ supply to the working heart was limiting. The $O_2$ uptake, coronary flow and effluent $O_2$ partial pressure of hearts performing external work during atrial perfusion were measured before and after the addition of adrenaline (1.2 mM) to the perfusate. Fig. 2 shows that there was a rapid rise in coronary flow rate and $O_2$ consumption, and the effluent $O_2$ partial pressure fell by over 60 mmHg. Changes started 1 min after addition of adrenaline and were maximal by 5 min. The working heart was therefore capable of extracting more $O_2$ and increasing the coronary flow rate after the addition of adrenaline.

## DISCUSSION

*Fuel for muscular work.* The $O_2$ uptake increased more than fourfold during increased left ventricular work, but the contribution to respiration of glucose (11.1 mM) as sole exogenous substrate in 30 min recirculating perfusions stayed constant at about 40–50% (Table 1). Glycogenolysis could account for only a small proportion of the respiration of normal hearts; the remaining 50–60% of the $O_2$ uptake was therefore accounted for by another endogenous fuel, very probably triglyceride (Olson & Hoeschen, 1967; Denton & Randle, 1967; Crass, McCaskill & Shipp, 1969). The presence of additional substrates, such as ketone bodies (Table 2) or palmitate (Crass, McCaskill & Shipp, 1970), can very substantially decrease the glucose contribution to respiration. Our results do not support the contention that glucose oxidation accounts for a larger percentage of the $O_2$ uptake during increased heart work (Crass *et al.* 1969) except (1) after the addition of insulin, (2) during prolonged perfusions (exceeding 30 min; see Chain *et al.* 1969 and Table 2), and (3) in perfusions in which the glucose contribution to the respiration of the non-working heart has been suppressed by the diabetic state or the addition of ketone bodies to the perfusate. The frequently quoted value obtained by Neely *et al.* (1967*b*), showing that glucose may contribute over 80% of the $O_2$ uptake of the working heart, applies to hearts perfused for 65 min in the recirculation system with glucose at 17.7 mM initial concentration. Over 60 min increased heart work in the present system also led to substantial increases in the glucose contribution to respiration (rising from about 40 to 80%). The increased contribution of glucose to respiration during prolonged perfusion is thought to result from depletion of endogenous triglyceride (Neely *et al.* 1967*b*; Chain *et al.* 1969).

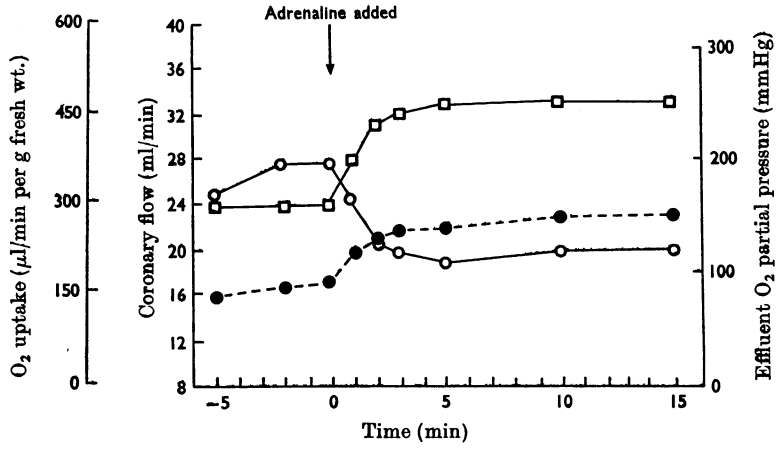


Fig. 2. Effect of adrenaline (1.2 μM) on $O_2$ uptake (□), coronary flow (●) and effluent $O_2$ partial pressure (○) in the perfused working rat heart. There was an increased $O_2$ uptake, a decreased effluent $O_2$ partial pressure and an increased coronary flow rate within 1 min of adrenaline addition.

The latter event appears to be an unphysiological situation resulting from the absence of free fatty acid from the perfusate (Crass *et al.* 1969). The contribution of palmitate (1.0 mM; bound to albumin 3 g/100 ml) as sole exogenous substrate to respiration of the working heart can also be substantial, increasing from 27% at 30 min to 75% at 60 min perfusions (calculated from data of Crass *et al.* 1970). The above findings can be explained by supposing that increased heart work (i) stimulates equally the rate of oxidation of endogenous fuel and any one exogenous fuel and (ii) does not prevent the inhibition of glucose oxidation by free fatty acids or ketone bodies found in the Langendorff 'non-working' heart (Shipp *et al.* 1961; Newsholme & Randle, 1964). However, ketone bodies in the concentration used in this study inhibited glucose oxidation less in working than in non-working hearts.

*Adequacy of oxygenation.* Glucose uptake, glycolysis and glycogenolysis are accelerated by both anoxia and heart work in the isolated rat heart. The question of adequacy of oxygenation of the working heart preparation is therefore raised. Arguments for adequacy of oxygenation are: (1) the $O_2$ uptake at any given peak systolic pressure is similar when the isolated rat heart is perfused with blood or with oxygenated Krebs–Henseleit buffer and this relationship is similar to that found in the blood-perfused dog heart (Gamble, Conn, Kumar, Plenge & Monroe, 1970); (2) when the isolated heart is perfused at 20 cm$H_2O$ left-atrial pressure, as in the present work, there are values for stroke volume and cardiac output (see Neely *et al.* 1967*a*) similar to those found in the rat heart *in situ* (Beznak, 1959); (3) the venous $O_2$ partial pressure of the isolated working rat heart exceeds 150 mmHg, which indicates adequate oxygenation in the absence of arteriovenous shunting (Huhmann, Niesel & Grote, 1967); (4) during the addition of adrenaline to the isolated working heart coronary flow can increase further at the same time that venous $O_2$ partial pressure falls (Fig. 2), which indicates that in the absence of added adrenaline the heart is not extracting all the $O_2$ it can from the perfusate; (5) net glycogen synthesis can be achieved in the working rat heart during perfusions with both glucose and palmitate as substrate (Crass *et al.* 1970); (6) the calculated mitochondrial free $NAD^+/NADH$ content ratio in working hearts is about 8.0 (Opie & Mansford, 1971), which is similar to the value in resting well-oxygenated rat liver (Williamson, Lund & Krebs, 1967); (7) increased heart work increases the percentage of the glucose uptake oxidized and decreases the percentage forming lactate (Table 3; Opie, 1965; Crass *et al.* 1969); (8) the perfusate lactate/pyruvate concentration ratio stays unchanged at the onset of acute heart work, whereas the heart tissue value may rise (Opie & Mansford, 1971) or fall (Table 1) depending on perfusion conditions. Although lactate production can increase markedly during increased heart work (Neely *et al.* 1967*b*), no such increase was found in the present recirculation experiments (Table 3). In view of the above arguments, lactate production by the isolated heart is held not to indicate hypoxia, but rather to result from the combination of perfusion conditions in which glucose is the sole substrate and lactate is initially absent from the perfusate.

*Effects of increased heart work on glycolysis.* The rate of membrane transport is a major limiting step in glucose uptake by both Langendorff perfused ('non-working') and by working hearts (Morgan *et al.* 1961*a*; Neely *et al.* 1967*b*). In the present experiments intracellular glucose did not accumulate in measurable amounts during increased heart work in the absence of insulin (Tables 1 and 4), as in the 'non-working' heart (Morgan *et al.* 1961*a*). Glucose transport into the heart cell can therefore be classified as a non-equilibrium reaction. During increased heart work glucose transport was stimulated by factors other than the concentration of its substrate (which fell), which allows identification of glucose transport as a regulatory process (see Newsholme, 1970). The argument applies even more forcefully to the data of Neely *et al.* (1967*b*), in which no intracellular glucose was found in the working rat heart in spite of a perfusate glucose concentration of 14–15mM. Our results show that increased heart work stimulates glucose transport in normal heart with or without added insulin, in hearts from rats with acute and chronic diabetes, and in hearts perfused with ketone bodies; in each case transport appears to be a regulatory process. The absence of appreciable glucose accumulation in working hearts shows that glucose phosphorylation is not rate-limiting in hearts from normal and diabetic rats in the absence of added insulin. In the presence of added insulin the accumulation of intracellular glucose indicates that the rate of glucose phosphorylation becomes a limiting process for glucose uptake as in the 'non-working' heart (Morgan *et al.* 1961*a*). The enzyme phosphofructokinase catalyses a non-equilibrium reaction in heart muscle (Williamson, 1965) and is regulated by factors other than its substrate concentration (Newsholme & Randle, 1961). On the basis of the properties of this enzyme a theory of metabolic control has been proposed suggesting that its activity can be regulated by adenine nucleotides, fructose 6-phosphate, fructose 1,6-diphosphate, $P_i$ and citrate (Mansour, 1963; Parmeggiani & Bowman, 1963; Newsholme & Randle, 1964; Regen, Davis, Morgan & Park, 1964; Pogson & Randle, 1966; Randle, Denton & England, 1968; Newsholme, 1970).

During increased heart work and increased glycolysis the reaction catalysed by phosphofructokinase remains non-equilibrium, and factors other than the substrate concentration appear to regulate its activity because the substrate concentration may fall. These observations show altered regulation of glycolysis at the level of phosphofructokinase activity. Increased heart work appears to adjust the rate of glycolysis by altering phosphofructokinase activity. At the same time increased rates of transport and of phosphorylation of glucose suggest that increased heart work stimulates all these steps in a co-ordinated fashion without any major accumulation of intermediates.

*Evidence for role of citrate in controlling phosphofructokinase activity during increased heart work.* The citrate content of the heart fell, at the same time that the hexose monophosphate values fell and the rate of glycolysis increased, in the following conditions: (1) during increased heart work for 15min (Table 1); (2) increasing the perfusion pressure (and hence the peak systolic pressure) in the Langendorff perfused heart from 65 to 100 $cmH_2O$; (3) during increased heart work by hearts from rats with chronic streptozotocin-diabetes with insulin added to the perfusate; (4) during increased heart work by hearts from rats with acute alloxan-diabetes.

Could the increased phosphofructokinase activity be mediated by citrate changes? The possible intracellular citrate concentration, calculated according to the principles of Newsholme & Randle (1961), fell from about 1.1mM in hearts perfused with a high-$K^+$ medium, to 0.28mM in Langendorff perfused hearts perfused at 65 $cmH_2O$ aortic pressure, to 0.21mM in working hearts (see Table 1). A citrate concentration as low as 0.1mM can substantially inhibit the activity of partially purified rat heart phosphofructokinase (Pogson & Randle, 1966) albeit at a much higher concentration of fructose 6-phosphate and a much lower concentration of ATP than normally found in the heart. A decreased citrate concentration may therefore deinhibit phosphofructokinase activity during 15min or more of increased heart work. Changes in adenine nucleotide contents may also play an important role in accelerating phosphofructokinase activity during increased heart work. However, in the transition from the non-contractile to the contractile state (comparing Langendorff perfused hearts virtually arrested by a high-$K^+$ medium with normally contracting hearts; see Table 1) there were large changes in the citrate content but not in adenine nucleotide values.

In normal hearts perfused with both glucose and ketone bodies the very high citrate content of the 'non-working' hearts was decreased by increased heart work (Table 4), but hexose monophosphates did not decrease although glycolytic flux increased. Altered regulation at the level of phosphofructokinase cannot be excluded, because an increased rate of glucose transport could obscure any decrease in the hexose monophosphate content (Bücher & Rüssman, 1964).

*Evidence for the role of adenine nucleotides in controlling phosphofructokinase activity during increased heart work.* During very acutely increased heart work (5–15s) the contents of ADP and AMP rose and the ATP/ADP and ATP/AMP content

ratios fell; ATP content fell during the period from 5s to 2min and that of $P_i$ rose at 2min. Similar changes have been held to regulate glycolysis by altering phosphofructokinase activity when the extent of contractility is altered by changing the $Ca^{2+}$ or $K^+$ concentration in the perfusate (Regen, Young, Davis, Jack & Park, 1964). The ATP/AMP content ratio may play an important part in the regulation of phosphofructokinase activity, and our results on acutely increased heart work are compatible with the suggestion that an increased intracellular AMP concentration acts as an amplification signal to magnify the consequences of very small degrees of ATP breakdown (Krebs, 1964; Newsholme, 1970). During short periods of heart work (less than 15min) accelerated phosphofructokinase activity and glycolysis could be explained by changes in adenine nucleotide contents but not by changes in citrate content.

During longer periods of heart work increased ADP and AMP contents were found at 15min but not at 30 or 60min (Tables 1, 4 and 5). The failure to find increased ADP and AMP values during prolonged perfusions may be attributed to the loss of total adenine nucleotides and to the effect of the adenylate kinase equilibrium, as found by Kübler, Grebe, Orellano, Spieckermann & Bretschneider (1968) in the isolated working dog heart. At the latter times (30 and 60min) ATP values were decreased and altered regulation at the site of phosphofructokinase could perhaps be explained by decreased inhibition of the enzyme by ATP. ATP compartmentation in the heart (Gudbjarnason, Mathes & Ravens, 1970) could allow a greater decrease in an ATP compartment available to inhibit the enzyme. Other factors altering phosphofructokinase activity during prolonged perfusions may be (i) an increased $P_i$ content, as suggested by the decrease in creatine phosphate and (ii) a decreased citrate value.

*Criticisms of the approach used.* A major criticism of studies relating overall tissue contents of metabolites to activity of any enzyme is the problem of compartmentation of metabolites. The simplest assumption is that metabolites are evenly distributed and that the mean tissue content is related to the actual concentration available to the enzyme unless there is evidence to the contrary (Hohorst *et al.* 1959; Krebs & Veech, 1970), as in the case of adenine nucleotides. A specific criticism of the conclusions concerning the measurements of the intracellular glucose concentration is the use of sorbitol as an extracellular marker; the exact distribution of sorbitol within the heart is not known, and the extracellular space is larger *in vitro* than *in vivo* (Lossnitzer, 1970). However, as the final perfusate glucose concentration in our experiments was about 9–10mM, any marked intracellular accumulation of glucose should have been detected (see Morgan *et al.* 1961*b*).

Another criticism relates to the magnitude of work load imposed on the heart to achieve the metabolic changes; further, the base-line for comparison was the Langendorff perfused 'non-working' heart, which is an unphysiological preparation. The isolated perfused rat heart is abnormal in several ways, including the rate of oedema formation and the high coronary flow rate (Opie, 1965). The decreasing value of total adenine nucleotides during 30min and 60min perfusions suggests a failing preparation. In hearts perfused with high potassium concentrations, the situation is even more unphysiological.

Thus the results presented here do not necessarily delineate patterns of regulation to be found during physiological increases of heart work *in situ*. Nevertheless we have described experimental conditions in which there is strong evidence for altered regulation of glucose transport and of phosphofructokinase activity during increased heart work.

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