

## Utilization of Energy-Providing Substrates in the Isolated Working Rat Heart

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1. An improved perfusion system for the isolated rat heart is described. It is based on the isolated working heart of Neely, Liebermeister, Battersby & Morgan (1967) (*Am. J. Physiol.* **212**, 804–814) and allows the measurement of metabolic rates and cardiac performance at a near-physiological workload. The main improvements concern better oxygenation of the perfusion medium and greater versatility of the apparatus. Near-physiological performance (cardiac output and aortic pressure) was maintained for nearly 2 h as compared with 30 min or less in the preparations of earlier work. 2. The rates of energy release ( $O_2$  uptake and substrate utilization) were 40–100% higher than those obtained by previous investigators, who used hearts at subphysiological workloads. 3. Values are given for the rates of utilization of glucose, lactate, oleate, acetate and ketone bodies, for  $O_2$  consumption and for the relative contributions of various fuels to the energy supply of the heart. Glucose can be replaced to a large extent by lactate, oleate or acetate, but not by ketone bodies. 4. Apart from quantitative differences there were also major qualitative differences between the present and previous preparations. Thus insulin was not required for maximal rates of glucose consumption at near-physiological, in contrast with subphysiological, workloads when glucose was the sole added substrate. When glucose oxidation was suppressed by the addition of other oxidizable substrates (lactate, acetate or acetoacetate), insulin increased the contribution of glucose as fuel for cardiac energy production at high workload. 5. In view of the major effects of workload on cardiac metabolism, experimentation on hearts performing subphysiologically or unphysiologically is of limited value to the situation *in vivo*.

The main factor controlling the utilization of substrate by the heart is its workload, i.e. the volume of fluid to be pumped against the impedance of the vascular bed. In most previous studies of metabolism by the isolated heart the workload was far below physiological levels. In the Langendorff (1895) preparation the aortic pressure chosen is often very low and cardiac output cannot be measured. In the 'working heart' preparation introduced by Neely *et al.* (1967a) the workload, during the period when performance was stable, was only half of that observed in the intact animal. In addition, in this preparation cardiac performance was only constant when the perfusate was frequently exchanged.

In the present work an improved perfusion technique has been developed and used to determine rates of substrate utilization under steady-state conditions simulating those in the intact animal. Rates

of myocardial  $O_2$  consumption and utilization of glucose, lactate and fatty acids were up to twice as high as those reported in previous studies. In contrast with all earlier work we found that insulin has no effect on the rate of glucose utilization at high workload when glucose is the sole added substrate. Insulin enhances glucose utilization when glucose is present together with other substrates.

### Materials and Methods

#### Animals

Male COBS Wistar rats (Charles River U.K. Ltd., Margate, Kent, U.K.) weighing 300–400 g were fed *ad libitum* on a standard Oxoid laboratory diet (Oxoid Ltd., London S.E.1, U.K.).

#### Reagents

Crystalline bovine insulin (glucagon-free, 23.6 i.u./

mg; code PJ 4609) was obtained from Lilly Laboratoires, Indianapolis, IN, U.S.A., and oleic acid (99% pure) from Fluka Chemische Werke, Buchs SG, Switzerland. A stock solution of oleic acid (0.1M) was neutralized with NaOH. Lactic acid was prepared by the method of Krebs (1961)

and neutralized with NaOH. Acetoacetate was prepared by the method of Krebs & Eggleston (1945). [ $1\text{-}^{14}\text{C}$ ]Oleate and [ $2\text{-}^{14}\text{C}$ ]-acetate were purchased from the Radiochemical Centre, Amersham, Bucks., U.K. The oleate/albumin solution was prepared as described by Krebs *et al.* (1974). Purified enzymes

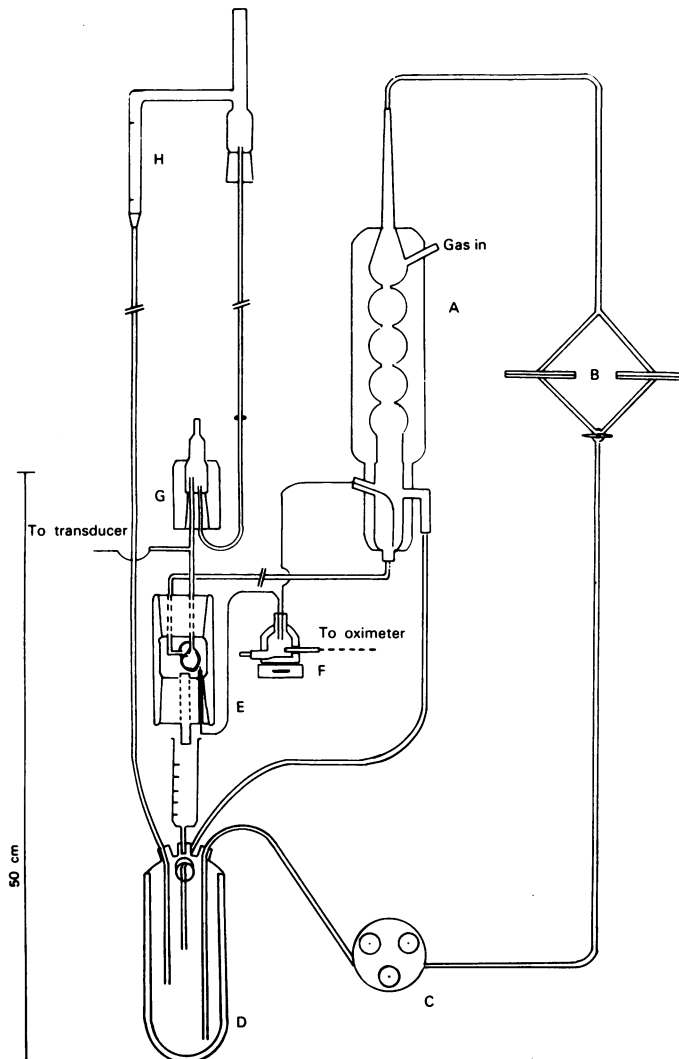


Fig. 1. *Perfusion apparatus*

The heart is mounted on a stainless-steel cannula assembly described by Neely *et al.* (1967a), which is held in place by a silicone rubber bung. The apparatus consists of eight units: a multibulb oxygenator with five bulbs of 4.2 cm diameter and a 10 ml reservoir at the bottom (A), a Millipore filter system of 5  $\mu\text{m}$  pore size and 4.5 cm diameter (B), a roller pump (C), a reservoir (D), a perfusion chamber (E), a chamber for the oxygen electrode (F), a compression chamber (G) and an aortic overflow chamber (H). Water-jacketing is provided for all glassware except for H. Perfusion medium reaches the heart directly from A via the left-atrial cannula and is expelled through the aorta. Saturation of perfusion medium at the bottom of A with  $\text{O}_2$  was between 82 and 90% (see the text). The height of A and H can be adjusted over a wide range (broken lines) which allows the workload of the heart to be varied. Preliminary retrograde perfusion is carried out via the side arm of the aortic cannula (labelled 'To transducer'). During this time the tubing leading to H is clamped with a roller clamp (two filled circles).

and coenzymes were purchased from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K.

#### *Perfusion medium*

The standard perfusion medium consisted of the bicarbonate saline of Krebs & Henseleit (1932). When oleate was present the medium contained 2% bovine serum albumin (see below) and the concentration of free  $\text{Ca}^{2+}$  was 2.5 mM.

Saline was chosen as perfusion medium because a microfilter (5  $\mu\text{m}$  pore size) has to be inserted into the perfusion circuit in order to prevent microemboli from entering the coronary capillaries and causing early cardiac failure due to ischaemia. This precluded the use of erythrocytes. Perfusion with saline represents an unphysiological situation in two respects. Firstly, the  $\text{O}_2$ -carrying capacity of saline is limited compared with blood, but this is compensated for by high rates of coronary flow. Secondly, there is an increase in tissue wet wt. by about 20%.

This made it necessary to increase the left ventricular filling pressure in order to maintain normal cardiac output at a physiological aortic pressure. These deviations from the physiological state were only of minor importance with respect to the work output of the heart.

#### *Surgical procedure*

The surgical procedure was similar to the procedure described by Neely *et al.* (1967a), except

$$\text{O}_2 \text{ consumption} = \frac{(\text{arterial O}_2 \text{ content} - \text{venous O}_2 \text{ content}) \times (\text{coronary flow rate in ml per h})}{\text{dry wt. of the heart in g}}$$

that heparin was injected into the saphenous vein 1 min before the heart was excised to avoid activation of lipoprotein lipase.

#### *Perfusion apparatus*

The perfusion apparatus was a modification of the one of Morgan *et al.* (1965) and Neely *et al.* (1967a) and is depicted in Fig. 1. The present apparatus differed from the perfusion system of Neely *et al.* (1967a) in the following major points.

(a) Perfusate reached the heart through a minimum of tubing from the bottom of a multibulb oxygenator. This allowed well-oxygenated medium ( $\text{O}_2$  saturation  $87 \pm 4\%$ ) to reach the heart without delay.

(b) The height of the aortic overflow chamber could be adjusted over a wide range. This arrangement made it possible to vary the load of the heart and to set the apparatus at physiological pressures.

A pressure head of 140 cmH<sub>2</sub>O simulated the load imposed by the systemic vascular resistance *in vivo* (Pfeffer & Frohlich, 1973).

(c) After each use the apparatus was dismantled, cleaned and stored in detergent solution or acid. This minimized bacterial contamination of the apparatus, which can be a major source of error in metabolic studies of perfused heart (Fanburg & Posner, 1969; H. Taegtmeier & R. Hems, unpublished observations). For the same reason the tubing was renewed before each use of the apparatus.

#### *O<sub>2</sub> consumption*

$\text{O}_2$  concentration was measured polarographically with a Clark type oxygen electrode (Yellow Springs Laboratories, Yellow Springs, OH, U.S.A.). The electrode was fitted into a temperature-controlled glass chamber of 2 ml capacity placed on a magnetic stirrer (model MS 16B Toyo Magnetic Ministirrer, Scientific Supplies Co., London EC1R, U.K.) and mounted next to the perfusion chamber. Oxygenated 'arterial' or desaturated 'venous' perfusion medium was aspirated without exposure to air into the glass chamber and returned to the perfusion chamber after each reading. Before and during each experiment the electrode was calibrated at 37°C against water saturated with room air (21%  $\text{O}_2$ ), aqueous dithionite (0%  $\text{O}_2$ ) and/or the gas mixture used in the experiment (95%  $\text{O}_2$ ).

$\text{O}_2$  consumption (mmol/h per g dry wt.) was calculated by the following formula:

#### *Perfusion*

Perfusion was begun as retrograde perfusion at a pressure of 100 cmH<sub>2</sub>O and a single pass for 10 min. Left-atrial perfusion was started with 150 ml of saline recirculating in the apparatus. Substrates and 2% bovine serum albumin were added to the perfusion medium as stated in the Figures and Tables. Perfusate (1 ml) was removed every 15 min for analysis of metabolites. At the end of the perfusion the heart was removed from the cannulae, blotted after incision of both ventricles and freed of the atria. After determination of the wet weight, tissue was dried to constant weight in a drying oven. The wet wt./dry wt. ratio was 5.5.

#### *Analytical methods*

Samples of perfusion medium (1 ml) were mixed with 0.1 ml of 60% (w/v)  $\text{HClO}_4$  to destroy trace enzyme activities which may have leaked from the

heart and to remove protein. Most substrates and all metabolites were determined in neutralized samples by enzymic methods: glucose by the coupled hexokinase and glucose 6-phosphate dehydrogenase method as described by Bergmeyer *et al.* (1974); L-(−)-lactate with lactate dehydrogenase as described by Hohorst (1963); acetoacetate and D-(−)-3-hydroxybutyrate by the method of Williamson *et al.* (1962).

Oleate removal was estimated by the disappearance of [ $1-^{14}\text{C}$ ]oleate from the perfusion medium by a modification of the procedure described by Whitelaw & Williamson (1977). Acetate removal was estimated by disappearance of [ $2-^{14}\text{C}$ ]acetate from the perfusion medium. In this case a duplicate 0.4 ml sample of the perfusion medium was directly transferred to 10 ml of liquid-scintillation fluid, which consisted of (per litre) 600 ml of toluene, 400 ml of 2-methoxyethanol, 60 g of naphthalene and 5.5 g of Permablend III (Packard Instruments, Caversham, Berks., U.K.).

#### *Workload and estimation of work output of the isolated perfused heart*

It was important to quantify the work of the heart. This is complicated by the fact that a single universal description of the pump performance of the heart is not available (Elzinga & Westerhof, 1979). The definitions and calculations of the workload and the work output of the heart in the perfusion system were based on the following.

(1) The workload of the heart is determined by the impedance to left-ventricular ejection. In the present system impedance was controlled by the height of the aortic overflow chamber, by the length and diameter of the tubing simulating the aorta, and by the viscosity and density of the perfusion medium. In addition, the amount of fluid ejected into the aorta is dependent on the filling of the left ventricle with fluid. In the present system left-ventricular filling pressure was controlled by the fluid level above the left atrium (i.e. at the bottom of the oxygenator).

(2) The main component of the work output of the heart is the product of pressure and volume (Frank, 1895), which in this investigation was arrived at by multiplying cardiac output (aortic and coronary flow in ml/min) by the height of the aortic fluid column (cmH<sub>2</sub>O) and the specific gravity of the perfusion medium. The final units of hydraulic work per unit of time (hydraulic power) are therefore kg·m/min.

This is a simplified calculation of work output. In addition, the heart does kinetic work and work in overcoming inertial, frictional and turbulent forces. The calculation of these components of cardiac work is fraught with difficulties. An estimate of the magnitude of this neglected work can be obtained in the present system from the decrease in flow that occurs when the length of the tubing connecting the aorta to the aortic overflow is doubled (to 280 cm) while the height of the overflow is maintained at

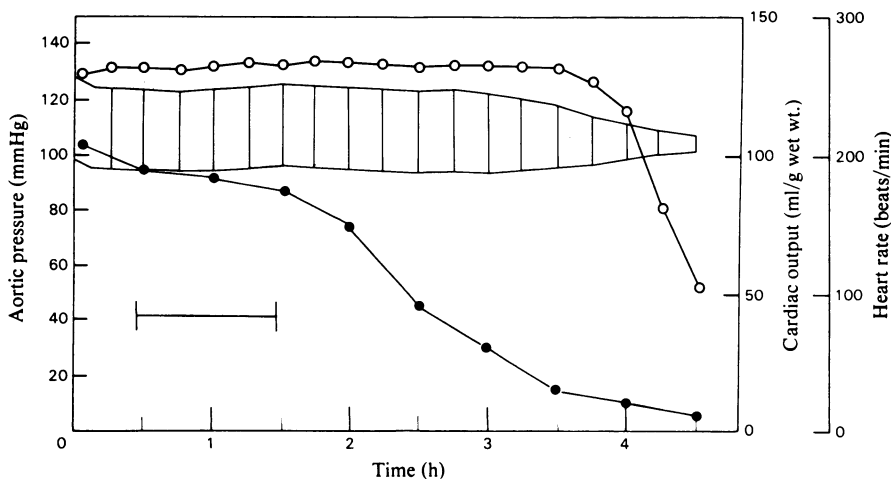


Fig. 2. *Stability of the isolated rat heart*

In four experiments hearts were perfused with 150 ml of Krebs–Henseleit bicarbonate saline containing 10 mM glucose at a filling pressure of 10 cmH<sub>2</sub>O and an aortic pressure head of 140 cmH<sub>2</sub>O. Heart rate (○), aortic pressure (bars) and cardiac output (●) were recorded over a period of 4½ h. After this time all indices of cardiac performance declined rapidly. Values are means. In subsequent experiments metabolic changes were recorded for up to 3 h, but rates of substrate removal were calculated for the period of relative physiological stability indicated by —.

140cm. The increase in resistance which this extension of the tubing imposed together with the extra mass of fluid it contained resulted in a diminution of flow of 17%. Precise estimations of the above-mentioned additional contributions to the total work output could not be made. They probably represent a constant factor, but they made it impossible to calculate the external efficiency of the heart. We took the cardiac output at a given height of the aortic overflow and at 60min of perfusion time as criterion of the physiological performance of the heart.

*Statistical analysis*

Data are means  $\pm$  s.d. unless otherwise indicated. Student's *t* test was used for testing the significance of the difference of means. *P* values greater than 0.05 were considered as not significant (NS).

**Results**

*Stability of heart rate, aortic pressure and cardiac output*

When hearts were perfused with oxygenated Krebs-Henseleit bicarbonate saline containing 10mM-glucose they continued to beat for over 4h, but peak systolic pressure in the aorta declined after 3h, and cardiac output had fallen by about 20% at the end of 2h (Fig. 2). The non-parallelism between heart rate, aortic pressure and cardiac output during the later part of the perfusion makes it clear that in the isolated heart, as in the intact animal, cardiac output is the best indicator of cardiac performance. Because the performance of the heart was constant between 30 and 90min of perfusion we chose this period for the measurement of metabolic rates.

*Glucose removal*

With the heart pumping against a physiological pressure head, the rates of glucose removal and of O<sub>2</sub> consumption were constant between 15 and 120min. As expected, an increase in workload increased the rates of O<sub>2</sub> consumption and glucose utilization (Table 1). At a low workload (defined as left-ventricular filling pressure of 5cmH<sub>2</sub>O and aortic pressure of 70cmH<sub>2</sub>O), and glucose concentration of 5mM, glucose uptake was only 60% of that observed with the more physiological high workload (left-ventricular filling pressure of 15cmH<sub>2</sub>O, aortic pressure of 140cmH<sub>2</sub>O). The data on O<sub>2</sub> consumption and substrate removal show that at low workload, but not at high workload, endogenous substrate was oxidized as well as glucose. When glucose uptake at low workload was increased by insulin, the contribution of endogenous substrate was almost nil. Unexpected was the finding that insulin did not cause a significant increase in glucose utilization at high workload when glucose

Table 1. Dependence of glucose consumption on workload, addition of insulin and glucose concentration. Hearts were perfused with 150ml of bicarbonate saline at low and high workload as described in the text. Values are means  $\pm$  s.d. The *P* values are given in parentheses and refer to the effect of insulin. NS, not significant.

[Glucose] (mM)	No. of experiments	Insulin added (m-i.u./ml)	Workload	Cardiac output (ml/min per g dry wt.)	Hydraulic power (kg-m/min per g dry wt.)	Glucose removal ( $\mu$ mol/h per g dry wt.)	Lactate production ( $\mu$ mol/h per g dry wt.)	Glucose not accounted for as lactate ( $\mu$ mol/h per g dry wt.)	Glucose C not accounted for as lactate ( $\mu$ mol/h per g dry wt.)	O <sub>2</sub> consumption (mmol/h per g dry wt.)
5	6	Nil	Low	235 $\pm$ 40	0.16 $\pm$ 0.03	455 $\pm$ 95	141 $\pm$ 74	383 $\pm$ 108	2.30 $\pm$ 0.65	3.06 $\pm$ 0.32
5	6	10	Low	275 $\pm$ 45 (NS)	0.19 $\pm$ 0.02 (NS)	818 $\pm$ 167 ( <i>P</i> < 0.001)	360 $\pm$ 93 ( <i>P</i> < 0.02)	638 $\pm$ 102 ( <i>P</i> < 0.01)	3.83 $\pm$ 0.82 ( <i>P</i> < 0.05)	3.42 $\pm$ 0.20 ( <i>P</i> < 0.05)
5	4	Nil	High	303 $\pm$ 57	0.42 $\pm$ 0.04	758 $\pm$ 123	212 $\pm$ 91	652 $\pm$ 97	3.91 $\pm$ 0.58	4.28 $\pm$ 0.86
5	4	10	High	353 $\pm$ 51 (NS)	0.49 $\pm$ 0.03 (NS)	851 $\pm$ 177 (NS)	205 $\pm$ 157 (NS)	740 $\pm$ 137 (NS)	4.48 $\pm$ 1.02 (NS)	4.51 $\pm$ 0.77 (NS)
15	5	Nil	Low	220 $\pm$ 19	0.15 $\pm$ 0.02	635 $\pm$ 92	218 $\pm$ 93	526 $\pm$ 93	3.16 $\pm$ 0.55	Not estimated
15	3	10	Low	267 $\pm$ 24 (NS)	0.19 $\pm$ 0.03 (NS)	918 $\pm$ 163 ( <i>P</i> < 0.05)	482 $\pm$ 102 ( <i>P</i> < 0.02)	677 $\pm$ 147 (NS)	4.06 $\pm$ 0.89 (NS)	Not estimated
15	6	Nil	High	396 $\pm$ 43	0.55 $\pm$ 0.03	1360 $\pm$ 37	683 $\pm$ 226	1020 $\pm$ 89	6.12 $\pm$ 0.53	Not estimated
15	4	10	High	426 $\pm$ 71 (NS)	0.59 $\pm$ 0.05 (NS)	1370 $\pm$ 71 (NS)	612 $\pm$ 187 (NS)	1070 $\pm$ 138 (NS)	6.39 $\pm$ 0.83 (NS)	Not estimated

Table 2. *Utilization of L-lactate and glucose*

Hearts were perfused with 150 ml of bicarbonate saline at the high workload described in the text. To simulate the physiological situation, lactate was added together with pyruvate at a ratio of 10:1. Each value represents the mean  $\pm$  s.d. for four to six experiments. The *P* values refer to the effect of insulin; NS, not significant.

[L-Lactate] (mM)	[Glucose] (mM)	Insulin added (m-i.u./ml)	Cardiac output (ml/min per g dry wt.)	L-Lactate removal ( $\mu$ mol/h per g dry wt.)	Glucose removal ( $\mu$ mol/h per g dry wt.)	Glucose C removal (mmol/h per g dry wt.)	O <sub>2</sub> consumption (mmol/h per g dry wt.)
5	—	Nil	331 $\pm$ 45	1410 $\pm$ 137	—	—	4.71 $\pm$ 0.59
10	5	Nil	369 $\pm$ 88	1220 $\pm$ 223	369 $\pm$ 88	2.21 $\pm$ 0.53	4.70 $\pm$ 0.51
10	5	10	404 $\pm$ 40	617 $\pm$ 82 ( <i>P</i> < 0.001)	646 $\pm$ 197 ( <i>P</i> < 0.05)	3.88 $\pm$ 1.18 ( <i>P</i> < 0.05)	4.98 $\pm$ 0.79 (NS)

Table 3. *Utilization of oleate and glucose*

Hearts were perfused with 150 ml of bicarbonate saline (containing 2% albumin) at the high workload described in the text. Values are means  $\pm$  s.d. for four experiments. The *P* values refer to the effect of insulin (lines 3 and 4); NS, not significant.

[Oleate] (mM)	[Glucose] (mM)	Insulin added (m-i.u./ml)	Cardiac output (ml/h per g dry wt.)	Oleate removal ( $\mu$ mol/h per g dry wt.)	Glucose removal ( $\mu$ mol/h per g dry wt.)	Lactate production ( $\mu$ mol/h per g dry wt.)	Glucose C not accounted for as lactate (mmol/h per g dry wt.)	O <sub>2</sub> consumption (mmol/h per g dry wt.)
1.0	—	Nil	110 $\pm$ 10	176 $\pm$ 33	—	< 5	—	4.94 $\pm$ 0.53
0.5	5	Nil	112 $\pm$ 31	115 $\pm$ 37	643 $\pm$ 133	546 $\pm$ 134	1.99 $\pm$ 0.77	4.36 $\pm$ 0.92
1.0	5	Nil	146 $\pm$ 58	120 $\pm$ 31	497 $\pm$ 122	360 $\pm$ 83	1.90 $\pm$ 0.51	5.00 $\pm$ 0.90
1.0	5	10	117 $\pm$ 38 (NS)	114 $\pm$ 39 (NS)	610 $\pm$ 45 (NS)	605 $\pm$ 141 ( <i>P</i> < 0.05)	1.85 $\pm$ 0.62 (NS)	4.53 $\pm$ 0.64 (NS)

was the sole substrate. Raising the glucose concentration from 5 to 15 mM had three effects (Table 1): firstly, it increased the rate of glucose consumption at low workload in the absence of insulin by 40%; secondly it increased the rate of glucose consumption at high workload in the absence of insulin by 80%; thirdly it decreased the insulin effect on glucose consumption at low workload from 80 to 45%. At high workload and 15 mM-glucose maximal rates of glucose utilization (Table 1, lines 7 and 8) were between 20 and 40% higher than the highest values reported for the rat heart during short term perfusions of up to 30 min duration (Opie *et al.*, 1971; Kobayashi & Neely, 1979).

#### Utilization of lactate

Rates of lactate removal in the working-heart preparation (Table 2) were 5 times greater than they were according to Williamson (1962) in hearts perfused by the Langendorff technique. Lactate and glucose shared in the supply of oxidizable fuel when presented simultaneously to the working heart, while glucose utilization was minimal under similar conditions in the Langendorff heart used by Williamson (1962). In contrast with the lack of effect of insulin when glucose was the sole substrate (Table

1), glucose utilization was increased and lactate utilization decreased when insulin was added at high workload. Thus, under the test conditions, insulin increases glucose removal only when glucose is present together with another substrate.

#### Utilization of oleate

Perfusion of the isolated heart with oleate at near-physiological workload resulted in: (1) rates of oleate removal that were 45–100% higher than those reported for fatty acids by other investigators (Shipp, 1964; Oram *et al.*, 1973; Neely *et al.*, 1976), and (2) depression of oleate removal by glucose when both substrates were present. Although a depression of glucose utilization by fatty acids is well known, the depression of fatty acid utilization by glucose has not been reported before.

At a given concentration of glucose the competition with oleate depended on the concentration of oleate in the perfusion medium. At 0.5 mM-oleate the rate of glucose removal decreased by only 15% whereas lactate production rose 2.5-fold (compare Table 3, line 2 with Table 1, line 3). This means that inhibition of glucose oxidation is much greater than the inhibition of glucose removal because a larger proportion of glucose is converted to lactate. Thus

Table 4. *Utilization of acetate and glucose*

Hearts were perfused with 150 ml of bicarbonate saline at the high workload described in the text. All values are means  $\pm$  S.D. for four experiments. The *P* values refer to the effect of insulin; NS, not significant.

[Acetate] (mM)	[Glucose] (mM)	Insulin added (m-i.u./ml)	Cardiac output (ml/min per g dry wt.)	Acetate removal ( $\mu$ mol/h per g dry wt.)	Glucose removal ( $\mu$ mol/h per g dry wt.)	Lactate production ( $\mu$ mol/h per g dry wt.)	Glucose C not accounted for as lactate (mmol/h per g dry wt.)	O <sub>2</sub> consumption (mmol/h per g dry wt.)
10	—	Nil	290 $\pm$ 41	1677 $\pm$ 414	—	<5	—	4.28 $\pm$ 0.35
10	5	Nil	333 $\pm$ 58	1391 $\pm$ 174	251 $\pm$ 86	183 $\pm$ 32	0.96 $\pm$ 0.10	4.69 $\pm$ 0.26
10	5	10	272 $\pm$ 29 (NS)	1095 $\pm$ 41 ( <i>P</i> < 0.05)	528 $\pm$ 87 ( <i>P</i> < 0.01)	380 $\pm$ 93 ( <i>P</i> < 0.01)	2.03 $\pm$ 0.24 ( <i>P</i> < 0.001)	4.26 $\pm$ 0.44 (NS)

oleate diverts glucose from oxidation to glycolysis. At 1 mM-oleate glucose consumption was further decreased, but glucose still contributed 41% of the substrates for oxidation. At 2 mM-oleate hearts began to fibrillate and ceased to function. In the presence of oleate more glucose was diverted to lactate. A cursory glance at the mean values presented in Table 3 suggests that insulin increases glucose utilization in the presence of oleate, but statistical analysis shows that this is not the case.

Since free fatty acids are toxic (Opie, 1970), they have to be provided in the form of a fatty acid-albumin complex. Addition of albumin is liable to cause frothing in the perfusion system of Neely *et al.* (1967a), but this did not occur in the present perfusion apparatus. Irrespective of the presence of a fatty acid, the stability of the preparation was of shorter duration with 2% albumin (Pentex fraction V; Miles Laboratories; lot no. 287, purified with charcoal) than during perfusions with saline alone. With 2% albumin cardiac output had fallen to 35% of control values at 60 min (Table 3). The reasons for this decline are not clear. In spite of the fall in cardiac output the aortic pressure was maintained and the rates of oleate and glucose removal were constant between 30 and 90 min of perfusion.

#### *Utilization of acetate*

Williamson (1964) showed that acetate is readily utilized when present as the sole substrate in hearts perfused by the Langendorff technique. In the working heart, rates of acetate utilization were more than twice as high as in Williamson's (1964) preparation. When acetate and glucose were added together acetate caused a 70% decrease in glucose utilization (Table 4). This is explained by the fact that acetate increases the concentrations of (a) acetyl-CoA (Randle *et al.*, 1970), which inhibits pyruvate dehydrogenase (Garland & Randle, 1964a), and (b) citrate (Williamson, 1965), which inhibits phosphofructokinase (Garland *et al.*, 1963).

A new observation is the doubling of the rate of glucose utilization by insulin in the presence of glucose and acetate at near-physiological workload of the heart (Table 4, line 3). The contribution of glucose to the fuel of respiration rose from 20 to 40%. Thus, under the test conditions, insulin is more effective in the presence of acetate than in the presence of oleate in promoting glucose utilization. Insulin also raised lactate formation from glucose, but the ratio of glucose used to lactate formed remained the same with or without insulin (Table 4).

#### *Utilization of ketone bodies*

As shown by Williamson & Krebs (1961), ketone bodies can serve as the sole fuel for respiration of the Langendorff heart preparation. Unlike the utilization of glucose, lactate, acetate or oleate, which were higher in the working heart than in hearts performing subphysiologically, the rates of oxidation of acetoacetate, 3-hydroxybutyrate and a mixture of acetoacetate and 3-hydroxybutyrate did not increase with an increase in workload of the heart (compare Table 5 with data given by Williamson & Krebs, 1961), and the oxidation of ketone bodies did not maintain cardiac function under the test conditions (Fig. 3). When ketone bodies were present together with glucose, cardiac performance was the same as in the presence of glucose alone (Table 5 and Fig. 3), although the rate of glucose utilization was less than under the conditions shown in Table 1, line 3. In other words, acetoacetate replaced some of the glucose as fuel for respiration. Since utilization of acetoacetate remained unchanged while 3-hydroxybutyrate formation increased in the presence of glucose, acetoacetate behaved differently from lactate, acetate and oleate in the competition with glucose. When insulin was added, acetoacetate removal decreased and the contribution of glucose to the fuel of respiration increased from 43 to 78%. As is the case with lactate and acetate, insulin favours utilization of glucose as competing fuel for oxidation.

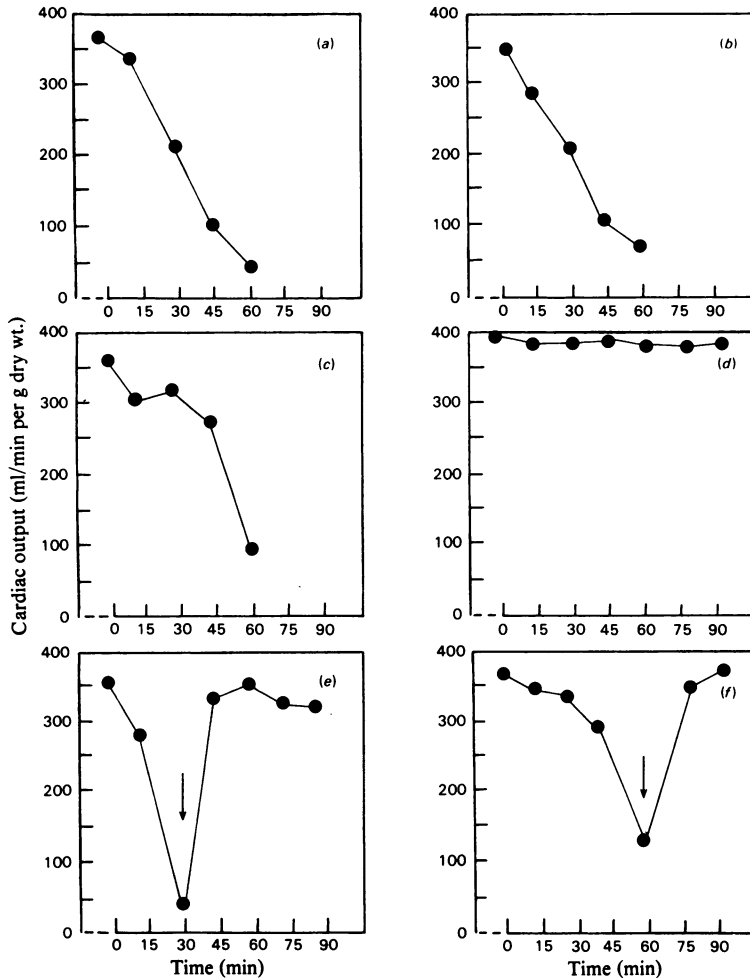


Fig. 3. Cardiac output during perfusion with ketone bodies or a mixture of ketone bodies and glucose

Hearts were perfused at high workload as defined in the text. At the beginning, the perfusion medium contained 7.5 mM-acetoacetate (a), a mixture of 2.5 mM-acetoacetate and 10 mM-DL-(–)-3-hydroxybutyrate (b), 15 mM-DL-(–)-3-hydroxybutyrate (c) and a mixture of 7.5 mM-acetoacetate and 5 mM-glucose (d). Each value is the mean for two to four experiments. In two further experiments glucose (5 mM) was added (↓) to the perfusion medium when acetoacetate (7.5 mM) or DL-(–)-3-hydroxybutyrate (15 mM) were the substrates during the initial perfusion (e and f). The addition of glucose restored cardiac output to control values.

## Discussion

### Performance of the heart in vitro

The present working-heart preparation differs from earlier preparations in two major ways: firstly, workload and work output, as measured by aortic pressure and cardiac output, were close to those in the intact animal (physiological aortic pressure and fluid output of the heart); secondly, performance of the heart was constant for nearly 2 h, whereas previous investigators using a working heart were unable to maintain this amount of hydraulic work

(0.42–0.53 kg·m/min) for this period of time (Neely *et al.*, 1967a; Opie *et al.*, 1971). In the best preparation previously described, that of Neely *et al.* (1967a), the maximal work output of the heart was 0.41 kg·m/min per g dry wt. during short-term perfusion, but the average work output during long-term perfusions was only 0.19 kg·m/min per g dry wt.

The improved performance of the heart and higher rates of substrate utilization must have been mainly due to better oxygenation of the perfusion medium. This is borne out by the following con-



**Table 5. Utilization of acetoacetate, 3-hydroxybutyrate and glucose**  
 Hearts were perfused with 150 ml of Krebs-Henseleit bicarbonate saline at the high workload described in the text. Values are means  $\pm$  s.d. for four experiments, except for values in lines 2 and 3 which are the average of two experiments. When acetoacetate, 3-hydroxybutyrate or a mixture of both substrates were the sole substrates the perfusion time was shortened to 60 min. At this time the aortic pressure had fallen to below 140 cmH<sub>2</sub>O and the aortic flow was zero. The *P* values in line 4 compare data in line 1 with data in line 4; the *P* values in line 5 compare data in line 4 with data in line 5; NS, not significant.

[Acetoacetate] (mM)	[DL-3-Hydroxybutyrate] (mM)	[Glucose] (mM)	Insulin (m-i.u./ml)	Rate ( $\mu$ mol/h per g dry wt.)				Rate (mmol/h per g dry wt.)					
				Acetoacetate removed	3-Hydroxybutyrate produced	3-Hydroxybutyrate removed	Acetoacetate produced	Glucose removed	Lactate produced	Acetoacetate C not accounted for as 3-hydroxybutyrate	Glucose C not accounted for as lactate	O <sub>2</sub> consumption	
7.5	—	—	—	645 $\pm$ 107	113 $\pm$ 28	—	132	—	—	—	—	—	4.20 $\pm$ 0.26*
—	15	—	—	284	—	699	—	—	—	Not estimated	—	—	Not estimated
2.5	10	—	—	979 $\pm$ 166 ( <i>P</i> < 0.01)	235 $\pm$ 15 ( <i>P</i> < 0.01)	300	—	—	—	Not estimated	2.18 $\pm$ 0.44	—	—
7.5	—	5	—	501 $\pm$ 31 ( <i>P</i> < 0.01)	194 $\pm$ 14 ( <i>P</i> < 0.01)	—	—	486 $\pm$ 53	—	225 $\pm$ 74	2.98 $\pm$ 0.80	2.23 $\pm$ 0.38	4.95 $\pm$ 0.39
7.5	—	5	10	—	—	—	—	824 $\pm$ 55 ( <i>P</i> < 0.01)	—	268 $\pm$ 37 (NS)	1.22 $\pm$ 0.21 ( <i>P</i> < 0.01)	4.14 $\pm$ 0.60 ( <i>P</i> < 0.01)	5.29 $\pm$ 0.68 (NS)

\* O<sub>2</sub> consumption at 15 min of perfusion.

siderations: at maximal rates of O<sub>2</sub> consumption and coronary flow observed in the present investigation, 60% saturation of the saline medium with O<sub>2</sub> would suffice to maintain the high work output of the heart working at physiological workload, provided that the tissue is capable of extracting all the O<sub>2</sub> from the perfusion medium. In practice, 85% saturation was required if the performance of the heart was to remain unimpaired. At this level of 'arterial' saturation the O<sub>2</sub> saturation of the 'venous' coronary effluent was between 15 and 35%, depending on cardiac work and coronary flow rates. Similar values of O<sub>2</sub> saturation in the coronary effluent were reported by Neely *et al.* (1967a), but Forster (1967) and Kessler & Schubotz (1968) have pointed out that the presence of O<sub>2</sub> in the venous effluent of perfused organs is not indicative of adequate tissue oxygenation. As the O<sub>2</sub>-carrying capacity of saline is much lower than that of whole blood (owing to the low viscosity of the saline and the high perfusion pressure in the present system), high rates of coronary flow rates are required to saturate the tissue with O<sub>2</sub>.

*Effects of workload on O<sub>2</sub> consumption and substrate utilization*

The rates of O<sub>2</sub> consumption of the working rat heart in the present experiments, as measured over a 2 h period, were considerably higher (4.2–5.1 mmol/h per g dry wt.) than those reported by Williamson & Krebs (1961) for hearts perfused by the Langendorff technique (1.7 mmol/h per g dry wt.) and those measured by Neely *et al.* (1967a) in hearts perfused at subphysiological workloads (1.8–3.3 mmol/h per g dry wt.). O<sub>2</sub> consumption in the blood-perfused rat heart of Gamble *et al.* (1970) is higher (3.8 mmol/h per g dry wt.). Since in Gamble's experiments the hearts were retrogradely perfused, this value probably still underestimates the O<sub>2</sub> consumption of the heart *in situ*. The highest values for O<sub>2</sub> consumption previously reported for the isolated heart at near-physiological workload are those of Opie *et al.* (1971) and of Neely *et al.* (1972, 1976), who found consumption rates of between 4.2 and 4.6 mmol/h per g dry wt. The O<sub>2</sub> consumption by working rat heart represents the highest rate of O<sub>2</sub> consumption recorded for any rat tissue. By comparison, maximal recorded rates of O<sub>2</sub> consumption by kidney are 2.4 mmol/h per g dry wt. (Ross & Bullock, 1976), by liver are 1.8 mmol/h per g dry wt. (Hems *et al.*, 1966) and by retina are 1.4 mmol/h per g dry wt. (Warburg *et al.*, 1924). In the present experiments constant rates of removal of lactate, acetate or oleate, when present as the sole substrates (or together with glucose), agree with the rates calculated from the O<sub>2</sub> uptake (Tables 2–4). This is also true for the part of glucose that is not converted to lactate (Table 1). In other words, the

ratio of O<sub>2</sub> used to substrate removed was of the same order as calculated for complete oxidation of the substrate.

#### *Factors regulating substrate utilization in the heart at high workload*

A striking result of the present work is the fact that addition of insulin does not increase the rate of glucose utilization at high workload when glucose is the sole substrate. Analogous observations have previously been made by Holloszy & Narahara (1965) in frog sartorius muscle. This is in contrast with the fact that insulin increases glucose uptake by rat heart perfused at a subphysiological workload (Table 1; Bleeheh & Fisher, 1954; Morgan *et al.*, 1961, 1965; Neely *et al.*, 1967*b*). When the heart is perfused with glucose and a second substrate, insulin promotes glucose utilization at high workload (Tables 2–5). Under the test conditions (hearts from normal fed animals, high workload and glucose as sole substrate) mechanical work furthers glucose utilization to the same extent as insulin.

The fact that the same rate of O<sub>2</sub> consumption at high workload was maintained by different substrates (glucose, lactate, acetate or oleate) indicates that these substances can feed electrons into the respiratory chain at a sufficient rate to saturate it. Our results are also in general agreement with the earlier observations by Neely *et al.* (1972), who found that in hearts perfused by the Langendorff technique, pressure work and not the availability of a specific substrate determined the rate of tricarboxylic acid-cycle turnover. A noteworthy observation is the increased yield of lactate from glucose in the presence of oleate or acetate when insulin is added. This means that acetate or oleate create conditions (high mitochondrial acetyl-CoA and ATP concentrations) where pyruvate dehydrogenase is inhibited but phosphofructokinase is either not inhibited or less inhibited.

#### *Utilization of ketone bodies*

Although ketone bodies can make a major contribution to the energy supply of the working heart, a second substrate such as glucose is needed to meet the energy requirements for the pump function at physiological pressure. In order to be oxidized, acetoacetate has to be converted to acetyl-CoA. Since acetyl-CoA is utilized (as the high rates of acetate utilization indicate), it is suggested that the rates of acetyl-CoA formation from acetoacetate are too low under the test conditions, presumably because the capacity of the thiolase reaction is the step limiting acetoacetate utilization (Williamson *et al.*, 1971). The limited rate of acetyl-CoA formation from acetoacetate appears to be sufficient for the heart perfused by the Langendorff technique (Williamson & Krebs, 1961), but not

for the working heart, where at physiological pressure O<sub>2</sub> consumption is 2.5 times higher. Earlier experiments by Garland & Randle (1964*b*) have shown that the acetyl-CoA concentration increases in hearts perfused with  $\beta$ -hydroxybutyrate by the Langendorff technique. This suggests that still another mechanism may be responsible for the inability of ketone bodies to support the working heart.

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