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The effects of insulin on glucose uptake and lactate release in perfused working rat heart preparations

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The effects of insulin on glucose uptake and lactate release in the perfused working rat heart have been investigated in three types of preparation: (i) a control low-workload preparation; (ii) an increased-pressure-workload preparation, simulating conditions of aortic pressure encountered *in vivo*; (iii) an increased-volume-workload preparation, where pumping work done is approximately the same as (ii) but coronary flow is restricted because of the decreased aortic pressure. Insulin stimulated glucose uptake and lactate release in preparations (i) and (ii), but failed to do so in preparation (iii). It was considered possible that preparation (iii) was hypoxic, thus necessitating a maximal stimulation of glucose uptake. This was confirmed by improving cardiac oxygenation by addition of stroma-free haemoglobin to the perfusate in preparation (iii). Under these conditions in the absence of insulin, glucose uptake and lactate release were decreased compared with perfusions in the absence of haemoglobin. Insulin stimulation of both processes was restored. We conclude that the failure of other workers to observe insulin effects on glucose uptake and lactate release under physiological workloads [preparation (ii)] may be a consequence of intracellular hypoxia in their preparations.

The Langendorff (1895) '*in-vitro*' rat heart preparation perfused with Krebs & Henseleit (1932) saline is frequently used in the investigation of cardiac metabolism. This preparation is retrogradely perfused through the coronary circulation and does not do work against a pressure load. In working rat heart preparations (Neely *et al.*, 1967*a*), the heart works externally against a frequently sub-physiological pressure load. In both these preparations, glucose uptake and lactate output are stimulated by insulin (Morgan *et al.*, 1961; Neely *et al.*, 1967*b*; see also Taegtmeyer *et al.*, 1980). However, it has been recently reported that in working rat heart preparations subjected to physiological workloads, glucose uptake and lactate output are insensitive to insulin (Taegtmeyer *et al.*, 1980). These findings throw doubt on the importance of insulin in the stimulation of cardiac glucose uptake *in vivo*. It could be supposed that under physiological conditions, glucose transport is operating at its maximal rate. However, Taegtmeyer *et al.* (1980) observed that at physiological workloads *in vitro*, hearts still produced lactate in the absence of insulin. One possible explanation of this is that the heart is slightly hypoxic and this causes insulin insensitivity rather than the physiological workload *per se*. In view of the importance of the finding of Taegtmeyer *et al.* (1980), we re-investigated the insulin sensitivity of glucose transport and lactate output in the perfused working rat heart.

Experimental

Materials

Soluble insulin from ox pancreas for injection (80 units/ml; lot no. A6306) was from The Wellcome Foundation, London, U.K. Chemicals were from BDH Chemicals, Enfield, Middx, U.K. Biochemicals were from Sigma (London) Chemical Co., Poole, Dorset U.K. [6,6′(n)-^{3}H]Sucrose was from Amersham International, Amersham, Bucks., U.K. Male Sprague–Dawley-strain rats (weighing 250–300 g on arrival) were from Bantin & Kingman, Hull, Humberside, U.K. They were kept for at least 48 h before use, during which time they had free access to food and water. Stroma-free haemoglobin was prepared by the method of De Venuto *et al.* (1977) from recently expired human red blood cells from the hospital blood bank. It was dialysed into $CaCl_2$-free Krebs & Henseleit (1932) buffered saline and stored as a 10% (w/v) solution at −80°C. Haemoglobin was standardized by using a kit purchased from

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Sigma (London) Chemical Co. Methaemoglobin content was determined as described by Evelyn & Malloy (1938) and was less than 5% of the haemoglobin content. Bovine serum albumin [essentially fatty acid-free from Sigma (London) Chemical Co.] was also dialysed against 30vol. of $CaCl_2$-free Krebs & Henseleit (1932) buffered saline (buffer changed once) before use.

Heart perfusions

The perfusion method was as described by Taegtmeyer *et al.* (1980). Hearts were removed from rats (280–330g) that had been anaesthetized by an intraperitoneal injection (60mg/kg body wt.) of freshly prepared 6% (w/v) sodium pentobarbital (Sigma Chemical Co.) in water. Heparin (500 units; 5000 units/ml) was injected via the femoral vein. The skin was removed over the chest and abdomen. A transverse abdominal incision was made and the thoracic cavity was opened. The heart and some lung tissue was removed into ice-cold Krebs & Henseleit (1932) bicarbonate-saline. After cessation of contraction, the heart was removed and cannulated via the aorta. Retrograde (Langendorff, 1895) perfusion at 37°C was commenced at 100cm of water pressure with Krebs & Henseleit (1932) bicarbonate-saline containing 5mM-glucose (and insulin, when required) equilibrated with an O_2/CO_2 (19:1) mixture. While the heart was beating on the aortic cannula, lung tissue was removed and the left pulmonary vein was cannulated. (The lungs, bronchi and trachea are useful in finding the pulmonary vein, which lies immediately distal to the division of the trachea into the bronchi.) The heart was then switched to a working preparation by opening the left pulmonary vein cannula, closing the retrograde perfusion line and opening the line to the aortic overflow. [For details of filling (left atrial) and aortic pressures, see below]. The time from initiation of retrograde perfusion to initiation of the working perfusion was about 5min and all perfusate during this time was discarded. Perfusate [120ml of Krebs & Henseleit (1932) bicarbonate saline containing 5mM-glucose] during the working perfusion was recirculated. Insulin (50m-units/ml), when present (see the Figures and Tables), was added to both retrograde and working perfusion media. $CaCl_2$ concentration was 2.54mM. Aortic flow was measured using a calibrated overflow (see Taegtmeyer *et al.*, 1980) and coronary flow from the rate of passage of perfusate from the heart chamber. The rate of flow of perfusate into the oxygenator was 150ml/min and the gas flow rate was 300ml/min (Taegtmeyer *et al.*, 1980). The apparatus used is described in full by Taegtmeyer *et al.* (1980); see also Neely *et al.* (1967*a*,*b*); Neely & Rovetto (1975).

Three perfusion conditions were studied: (i) a control perfusion, with a filling pressure of 5cm of water and an aortic pressure of 70cm of water [this preparation is identical with the low-workload preparation of Taegtmeyer *et al.* (1980)]; (ii) an increased-pressure-workload perfusion with a filling pressure of 15cm of water and an aortic pressure of 140cm of water [this preparation is identical with the high-workload preparation of Taegtmeyer *et al.* (1980)]; (iii) an increased-volume-workload preparation with a filling pressure of 15cm of water and an aortic pressure of 70cm of water. The effects of the three perfusion conditions on the coronary flow, aortic flow and cardiac output (sum of coronary and aortic flows) are shown in Table 1. In the increased-pressure-workload preparation [preparation (ii)], the work done by the heart is approximately twice that done by the control. This is because, although the cardiac output is about the same as the control, the aortic pressure is twice as high. The increased workload is primarily caused by the increased pressure element in the calculation of work done. Since coronary flow is proportional to aortic pressure under these conditions, the coronary flow is twice that of the control. In the increased-volume-workload preparation [preparation (iii)] the work done is again approximately twice that done by the control. However, in this instance the cause of the increased work is the increased volume element in the calculation of work done. This is caused by the increased filling pressure at an aortic pressure identical with that of the control. Coronary flow is identical with that of the control. Thus the work done by preparations (ii) and (iii) is similar but in preparation (ii) the pressure element is increased and in preparation (iii) the volume element is increased. It should be noted that this calculation of cardiac work is an oversimplification. Although the main work done by the heart is pressure-volume work (Frank, 1895), work is also done kinetically and in overcoming frictional forces etc. The increased-pressure-workload perfusion most closely resembles the rat heart *in vivo* in terms of mean aortic pressure and in systolic and diastolic pressures. In the preparation *in vitro*, the mean aortic pressure is equivalent to the height of the aortic overflow. In the rat *in vivo* the systolic and diastolic pressures are 172 and 123cm of water respectively with a mean aortic pressure of 140cm of water (Altman & Dittmer, 1971; Lundin *et al.*, 1981). Oxygen uptake by the increased-pressure-workload preparation also resembles the heart *in vivo* (Taegtmeyer *et al.*, 1980).

Analytical procedures

Perfusions were usually for 90min and aortic and coronary flows were monitored throughout. Samples of perfusate (0.5ml) were removed for the measurement of glucose uptake and lactate release at 10min after the initiation of cardiac

work and subsequently at 20min intervals. Samples were immersed in a boiling-water bath for 2min and stored at −20°C. Glucose was determined by the glucose oxidase method by using a kit from Sigma (London) Chemical Co. and lactate by the method of Hohorst (1963). The perfusate volume at the end of the perfusions was determined by the addition of 1μCi of [^{3}H]sucrose and subsequent liquid-scintillation spectrometry of a volume (3ml) of perfusate with 10ml of PCS Phase Combining System (BDH Chemicals) as a fluor. Samples of perfusate containing protein were deproteinized with trichloroacetic acid [5% (w/v) final concentration] beforehand. Heart (ventricular) dry weights were determined by removing the atria and other attached tissue and subsequent drying of the ventricles to constant weight over P_2O_5 *in vacuo*. The atria etc. comprised a constant proportion (6%) of the heart dry or wet weight. The manoeuvre was carried out merely to assist removal of the heart from the cannulae. It is not meant to imply that the atria etc. do not contribute to cardiac glucose uptake or lactate output. Dry weights were corrected when appropriate for the presence of protein in the perfusate.

Statistical methods

Results are expressed as means ± S.E.M. with the numbers of observations shown in parentheses. Statistical significance ($P < 0.05$) was established by a two-tailed Student's *t*-test.

Results

Characteristics of perfused hearts

The performance values after 70min perfusion for the working hearts are shown in Table 1. Values of cardiac output for control and increased-pressure-workload conditions are comparable with values published by Taegtmeyer *et al.* (1980). Coronary flow was proportional to aortic pressure. It should be noted when comparing lines (ii) and (iii) that, although the hydraulic work done was approximately the same, coronary flow in the increased-volume-workload hearts was only about half that of the increased-pressure-workload hearts. Insulin did not cause any statistically significant change in cardiac performance. We observed a significant increase ($P < 0.001$) in the dry-weight/wet-weight ratio in hearts perfused with 1.5% (w/v) haemoglobin compared with the same preparation without haemoglobin. (A correction was made for extracellular haemoglobin present in the former perfusions.) The ratio increased from 0.1833 ± 0.0037 (8) to 0.2013 ± 0.0021 (8). Bovine serum albumin (1.5%, w/v) in the perfusate did not significantly increase the wet-weight/dry-weight ratio, which was 0.1875 ± 0.0018 (7) under these conditions. Thus perfusions with haemoglobin but not with serum albumin may

Table 1. *Performance data for the working rat heart*

Perfusion conditions and details of methods of measurement of aortic and coronary flows are described in the Experimental section. For the sake of simplicity, only data obtained after 70min of working perfusion are shown here. The cardiac output is the sum of the aortic and coronary flows. Hydraulic work done by the hearts was calculated by multiplying the cardiac output by the height of the aortic overflow above the heart [70cm for perfusion conditions (i), (iii) and (iv) and 140cm for perfusion condition (ii)].

		No insulin			+Insulin (50m-units/ml)		
Perfusion condition	Flow condition	Number of observations	Flow [ml·min^{-1}. (g dry wt.)$^{-1}$]	Hydraulic work [kg·m·min^{-1}. (g dry wt.)$^{-1}$]	Number of observations	Flow [ml·min^{-1}. (g dry wt.)$^{-1}$]	Hydraulic work [kg·m·min^{-1}. (g dry wt.)$^{-1}$]
(i) Control	Aortic	6	191 ± 13		8	188 ± 11	
	Coronary		87 ± 4			76 ± 3	
	Cardiac output		278 ± 14	0.19 ± 0.01		264 ± 13	0.18 ± 0.01
(ii) Increased-pressure workload	Aortic	5	157 ± 24		5	164 ± 24	
	Coronary		153 ± 9			147 ± 23	
	Cardiac output		310 ± 27	0.43 ± 0.04		311 ± 32	0.43 ± 0.04
(iii) Increased-volume workload	Aortic	9	432 ± 39		9	378 ± 33	
	Coronary		81 ± 4			73 ± 4	
	Cardiac output		513 ± 43	0.36 ± 0.03		440 ± 34	0.31 ± 0.02
(iv) Increased-volume workload + haemoglobin (1.5%, w/v)	Aortic	4	492 ± 42		5	463 ± 19	
	Coronary		82 ± 6			74 ± 6	
	Cardiac output		573 ± 47	0.40 ± 0.03		538 ± 20	0.38 ± 0.01

decrease heart oedema (see the Discussion section), which always occurs under conditions *in vitro*.

Glucose uptake and lactate release by the perfused heart

The rates of glucose uptake under the various perfusion conditions are shown in Table 2. Glucose uptake was linear with time for at least 130min of perfusion (Fig. 1). It is noteworthy that even when the glucose concentration is decreased to about 2.2mM after 130min increased-pressure-workload perfusion (Fig. 1), linearity of uptake is maintained. Insulin significantly stimulated glucose uptake under all conditions except under the condition of increased-volume workload in the absence of haemoglobin. The stimulation of glucose uptake was between 25 and 40%. These findings differ from those of Taegtmeyer *et al.* (1980) in that insulin stimulation of glucose uptake was observed under conditions of physiological workload [line (ii)]. When coronary flow was decreased by reducing the aortic pressure but cardiac hydraulic work was relatively constant [lines (ii) and (iii)], glucose uptake in the absence of insulin remained unchanged but was not now stimulated by insulin. Addition of 1.5% (w/v) haemoglobin (Taegtmeyer, 1980) to the increased-volume-workload perfusions [line (iv)] depressed glucose uptake ($P<0.05$) compared with similar perfusions in the absence of haemoglobin [line (iii)] and furthermore restored the ability of insulin to stimulate glucose uptake.

Examination of lactate release by the hearts confirms the data above (Fig. 2). Under control or increased-pressure-workload conditions in the absence of insulin, little lactate was released (Figs. 2*a* and 2*b*). These findings differ from the findings of Taegtmeyer *et al.* (1980). Insulin significantly stimulated lactate release under both conditions. Taegtmeyer *et al.* (1980) were able to observe stimulation of lactate release by insulin only under conditions identical with our control perfusions. Under conditions when volume work was increased (Fig. 2*c*), insulin did not significantly stimulate lactate release (cf. glucose uptake). When haemoglobin (1.5%, w/v) was added to the perfusion medium, insulin stimulation of lactate release was again observed (Fig. 2*d*). Under these conditions in the absence of insulin, the hearts utilized lactate

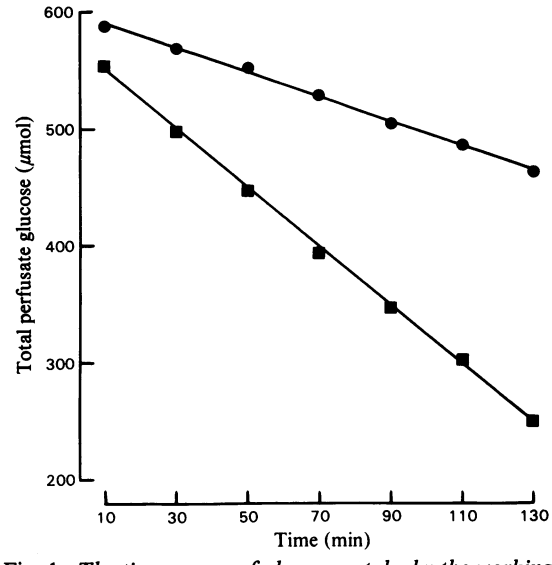

Fig. 1. *The time course of glucose uptake by the working rat heart*

The time course of glucose uptake is illustrated for two preparations showing minimum and maximum rates of glucose uptake, namely a control preparation in the absence of insulin (●) and an increased-pressure-workload preparation in the presence of 50m-units of insulin/ml (■) respectively. For the control preparation, the rate of glucose uptake is 325μmol/h per g dry wt. of heart ($r=-0.998$; heart dry wt. = 0.191g; perfusate volume = 117.0ml). For the increased-pressure-workload preparation, the rate of glucose uptake is 827μmol/h per g dry wt. of heart ($r=-0.999$; heart wt. = 0.180g; perfusate volume = 114.7ml). Rates of glucose uptake were routinely computed from the linear regression lines as shown in this figure.

Table 2. *The effects of work condition, insulin and haemoglobin on glucose uptake by the working rat heart*

Perfusion conditions are described in the Experimental section. Statistical significance: †, $P<0.05$ versus increased-volume-workload perfusion; *, $P<0.01$; **, $P<0.001$, versus the same perfusion condition in the absence of insulin.

	Glucose uptake (μmol/h per g dry wt.)		
Perfusion condition	No insulin	+ Insulin (50m-units/ml)	Stimulation by insulin (%)
Control	348 ± 14 (9)	482 ± 24 (4)**	39
Increased-pressure workload	607 ± 26 (5)	757 ± 19 (5)**	25
Increased-volume workload	594 ± 35 (9)	640 ± 23 (9)	8
Increased-volume workload + haemoglobin (1.5%, w/v)	462 ± 29 (4)†	602 ± 30 (5)*	30

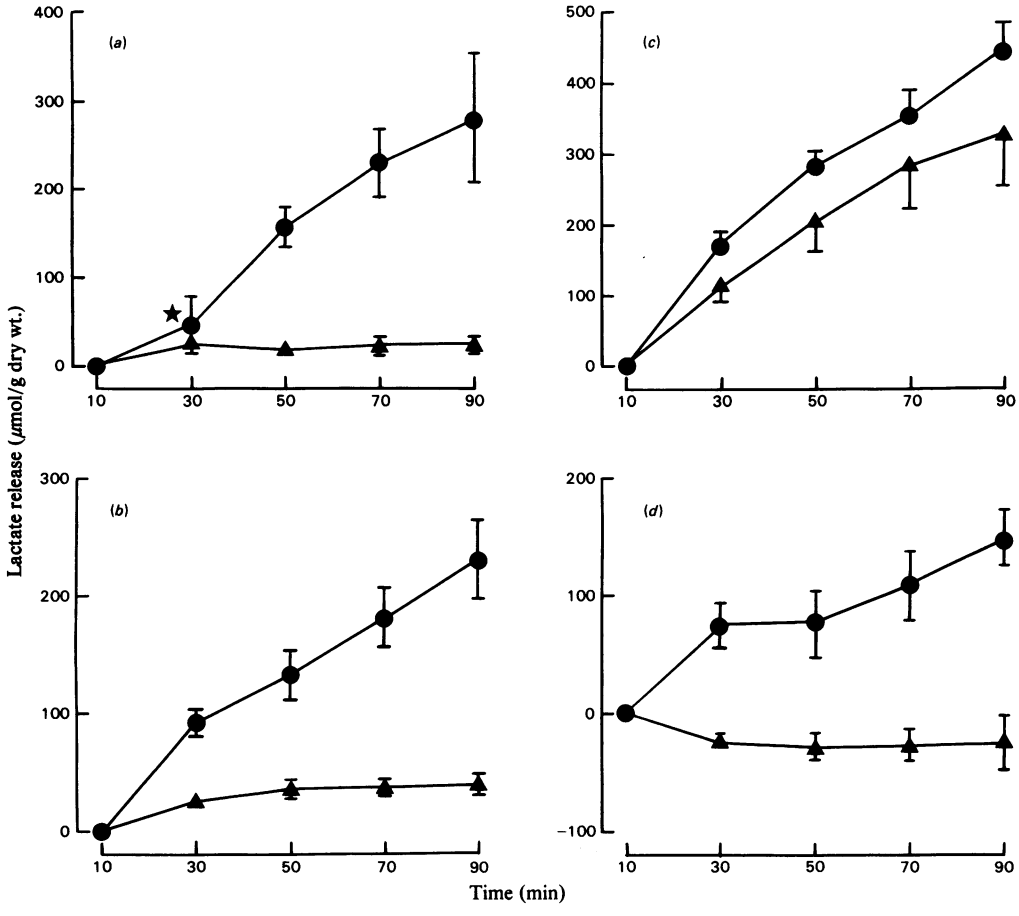

Fig. 2. *The effects of work condition, insulin and haemoglobin on lactate release by the working rat heart*
Hearts were perfused as described in the Experimental section in the presence of 50m-units of insulin/ml (●) or in its absence (▲). In (*a*) hearts were perfused as control preparations, in (*b*) as increased-pressure-workload preparations, in (*c*) as increased-volume-workload preparations and in (*d*) as increased-volume-workload preparations in the presence of 1.5% (w/v) haemoglobin. Each point represents the mean of four to nine observations ± S.E.M. (represented by the bars). In (*a*), (*b*) and (*d*) all points for perfusions in the presence of insulin are statistically significantly different for perfusions in the absence of insulin at $P < 0.05$ at least (apart from that marked with an asterisk), and more usually at $P < 0.01$ or $P < 0.001$. In (*c*) points are not significantly different.

produced over the first 10min of perfusion. Furthermore, when Figs. 2(*c*) and 2(*d*) are compared, addition of haemoglobin (1.5%, w/v) significantly decreased lactate release in the absence of insulin ($P < 0.01$ for all points) or in the presence of insulin ($P < 0.01$ for all points).

We considered the possibility that haemoglobin may be assisting oxygenation of the heart merely by reducing perfusate surface tension, thereby reducing the thickness of the perfusate film in the oxygenator (see Werner *et al.*, 1981). Hearts perfused under increased-volume-workload conditions with insulin (50m-units/ml) in the absence or presence of bovine serum albumin [1.5% (w/v)] showed identical rates of lactate release (results not shown). Thus this explanation is untenable.

Discussion

In these studies, very high added concentrations of insulin (50m-units/ml) were used. In control [preparation (i)] perfusions, we have observed maximal effects of insulin on glucose uptake and lactate release at much lower insulin concentrations (50–100μ-units/ml), which are within physiological ranges (Sugden & Smith, 1982). Such high concentrations were used here to ensure maximal effects throughout the incubation. Because of the very high insulin concentration used, we consider it probable that effects of insulin were also maximal in preparations (ii), (iii) and (iv). Results for preparation (iv) are incompatible with the possibility that the effect of haemoglobin was caused by inhibition of

insulin binding to the apparatus glassware since haemoglobin decreased lactate release in the presence of insulin compared with perfusions in the absence of haemoglobin (Figs. 2*c* and 2*d*).

We found that insulin stimulated glucose uptake and lactate release in the control, increased-pressure-workload and increased-volume-workload-plus-haemoglobin-perfused hearts. These findings are in contrast with the findings of Taegtmeyer *et al.* (1980), who observed stimulation by insulin of glucose uptake and lactate release under conditions akin to our control perfusions, but were unable to detect any effects of insulin in increased-pressure-workload perfusions. Since the latter preparation simulates physiological conditions *in vivo*, it could be suggested from the data of Taegtmeyer *et al.* (1980) that insulin does not affect cardiac glucose uptake *in vivo*. We suggest that the failure of Taegtmeyer *et al.* (1980) to observe effects of insulin at physiological workloads is a consequence of tissue hypoxia as suggested by their high rates (200 μmol/h per g dry wt.) of lactate release. Anoxia is known to stimulate glucose transport (Morgan *et al.*, 1959, 1961). Possibly glucose transport was operating at its maximal rate because of an increased reliance of the heart on anaerobic metabolism of glucose to lactate. The oxygen saturation of perfusate in the oxygenator is probably about 87% (Taegtmeyer *et al.*, 1980). From a knowledge of coronary flow, glucose uptake and lactate release, the oxygen consumption of the heart can be calculated, assuming (*a*) all glucose is oxidized apart from that fraction released as lactate and (*b*) no endogenous fuels are oxidized. Both these assumptions are justified (Taegtmeyer *et al.*, 1980). For the control and increased-pressure-workload perfusions, oxygen saturation in the effluent coronary perfusate should decrease to about 35% for the increased-volume-workload perfusions, oxygen saturation should decrease to about 35%; for the increased-out (Forster, 1967; Taegtmeyer *et al.*, 1980) that because of diffusion limitations, the presence of oxygen in the effluent coronary perfusate is not necessarily indicative of adequate oxygenation. Addition of haemoglobin (1.5%, w/v) approximately doubles the oxygen-carrying capacity of the perfusate at the partial pressure used and should therefore assist oxygenation. These conclusions are supported by our findings that for increased-volume-workload perfusions (*a*) high rates of glucose uptake and lactate release were observed in the absence of insulin, (*b*) these rates were not further stimulated by insulin, (*c*) addition of haemoglobin reduced the rates of glucose uptake and lactate release in the absence of insulin, (*d*) in the presence of haemoglobin, glucose uptake and lactate release were stimulated by insulin (Table 2, Fig. 2). We also find that perfusion with haemoglobin decreases cardiac oedema, which is itself indicative of anoxia (Morgan *et al.*, 1961). We further suggest that lactate release from the hearts of fed rats in the absence of insulin is indicative of intracellular hypoxia even though the effluent perfusate contains residual oxygen. It is also of interest that under conditions where there is no lactate release, there is a lower rate of glucose utilization in the increased-volume-workload preparation plus haemoglobin than in the increased-pressure-workload preparation ($P < 0.01$), although work done is the same (Tables 1 and 2). Although not the only possibility, this finding supports the suggestion that increased-pressure and -volume workloads are not equivalent, with a higher rate of oxygen consumption being shown by the former (Sarnoff *et al.*, 1958; Meerson, 1969).

Although we conclude that insulin stimulates glucose uptake in the heart under conditions *in vivo*, the stimulation was small (25–40%) compared with working skeletal muscle (see, e.g., Berger *et al.*, 1976). A second difference is that in the heart a large proportion (55–75%) of insulin-stimulated glucose uptake was released as lactate (Table 2, Fig. 2). Although it has been reported that insulin plus glucose increased the phosphocreatine/ATP concentration ratio compared with glucose alone in the perfused heart (Rannels *et al.*, 1975; Bailey *et al.*, 1982), we do not see any differences in cardiac output under such conditions (Table 1). Because cardiac glycogen synthesis (Larner, 1972) is relatively insensitive to insulin (see also Randle & Tubbs, 1979) and because synthesis *de novo* of fatty acids in the heart is minimal (Randle & Tubbs, 1979), it is possible that the most important effects of insulin in the heart are related to protein turnover (Manchester & Wool, 1963; Morgan *et al.*, 1971; Chain & Sender, 1973; Sender & Garlick, 1973; Rannels *et al.*, 1975; Sugden & Smith, 1982).

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