Regulation of Glucose Uptake by Muscle

3. THE EFFECTS OF INSULIN, ANOXIA, SALICYLATE AND 2:4-DINITROPHENOL ON MEMBRANE TRANSPORT AND INTRACELLULAR PHOSPHORYLATION OF GLUCOSE IN THE ISOLATED RAT HEART*

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In previous papers (Randle & Smith, 1958*a*, *b*) evidence was presented that insulin, anoxia and substances such as salicylate or 2:4-dinitrophenol, which inhibit oxidative phosphorylation, increase the uptake of glucose and D-xylose by isolated rat diaphragm by accelerating the transfer of these sugars across the muscle-cell membrane. The conclusion was drawn that the transfer process for sugars in muscle is inhibited by a substance generated during oxidative phosphorylation and that insulin activates the transfer process by interfering with the action of this substance.

The present study was undertaken to examine the effects of anoxia, salicylate and 2:4-dinitrophenol on the uptake of sugars by muscle in more detail. For this purpose the uptake of a sugar such as glucose which is phosphorylated by muscle can be considered to involve three sequential steps: (i) passage of sugar from the interior of the capillary to the cell membrane, (ii) transfer of sugar across the cell membrane, and (iii) phosphorylation of sugar within the cell (with sugars such as D-xylose or L-arabinose, which are not phosphorylated, step iii is not involved). The rate of uptake of glucose depends on the resistance to the flow of the sugar through each of these steps individually but the one which has the greatest resistance will be predominantly rate-limiting. It follows therefore that any factor which accelerates uptake must affect this step in particular. One consequence of this may be that another step becomes rate-limiting.

The isolated perfused rat heart has been used for measurement of the uptake process in these investigations. Pertinent information obtained previously with this tissue is as follows. In the heart from the normal animal passage of sugars between the capillary and the cell membrane occurs very rapidly and does not affect the rate of uptake appreciably (Morgan, Cadenas & Park, 1958). Transfer across the cell membrane, on the other hand, appears to constitute a major barrier to the flow of glucose and is rate-limiting for uptake in the absence of insulin (Park, 1955; Morgan & Park, 1958). The kinetics of the transfer process, competition between sugars for transfer and the action of inhibitors such as phlorrhizin show that this transfer step involves transport of the sugar by combination with a specific site on the cell membrane (Bronk & Fisher, 1957; Park, Reinwein, Henderson, Cadenas & Morgan, 1959). In this connexion transport could be considered as the first reaction in glucose metabolism, preceding phosphorylation by the hexokinase system. When transport is accelerated by insulin (Levine & Goldstein, 1952) the capacity for phosphorylation may be exceeded, as shown by the accumulation of free glucose inside the cell (Park & Johnson, 1955; Fisher & Lindsay, 1956). Under these conditions phosphorylation becomes the rate-limiting step for glucose uptake.

With these considerations in mind the effects of anoxia, salicylate and 2:4-dinitrophenol on the transport and phosphorylation of sugars in the perfused isolated heart have been investigated and compared with those of insulin.

EXPERIMENTAL

Procedures

Perfusion medium. Heart perfusion was carried out with a bicarbonate-buffered salt solution (Krebs & Henseleit, 1932) containing D-[^{12}C]sorbitol (0·5 mg./ml.) and D-[$^{14}C_6$]-sorbitol (0·12 μc/ml.; 6·2 mc/m-mole; The Radiochemical Centre, Amersham, Bucks). The medium was maintained at pH 7·4 at 37° by gassing with $O_2 + CO_2$ (95:5). D-Glucose, D-xylose, D-galactose or L-arabinose (T. Kerfoot and Co. Ltd.) was added at the concentration given in the tables.

Animals. Hearts were obtained from male albino Wistar rats of 200–300 g. wt., fed on a stock laboratory diet (Bruce & Parkes, 1949). Animals had free access to water at all times but food was withheld for 18–20 hr. before each experiment. The rats were injected intraperitoneally with heparin (200 units; Evans Medical Supplies Ltd.) 15–60 min. before the experiment.

Perfusion apparatus. Heart perfusion was carried out in an apparatus described elsewhere (Morgan, Henderson & Park, in preparation).

* Part 2: Randle & Smith (1958*b*).

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Procedure. Rats were killed by decapitation and were bled. The heart was rapidly removed and dropped into ice-cold medium. It was then attached to the perfusion cannula, washed through with about 8 ml. of medium and then placed in the perfusion chamber and perfusion begun. In all experiments, except for arabinose wash-out, perfusion involved re-circulation of about 7 ml. of medium for 15 or 30 min. At the end of perfusion the heart was detached from the cannula, dropped into ice-cold medium and the chambers were opened to facilitate rapid chilling. It was then blotted on filter paper and frozen on a planchet resting on solid carbon dioxide and kept frozen until analyses were made.

L-*Arabinose wash-out.* The heart was first allowed to accumulate L-arabinose by perfusion for 15 min. under aerobic conditions with medium containing the pentose (15 mg./ml.). Arabinose-free medium was then passed through the heart for 5 min. (without re-circulation) to remove extracellular pentose. At this point the collecting vessel was changed. Perfusion with arabinose-free medium was then continued for a further 10 min. with collection of the perfusate from the heart. During this period of perfusion the collecting vessel was changed at intervals of 1–2 min. Pentose was estimated in the collections obtained during the latter (10 min.) period of perfusion and in the heart at the end of the perfusion.

Analytical methods

D-*Glucose,* D-*xylose,* L-*arabinose or* D-[$^{14}C_6$]*sorbitol.* Analyses of heart muscle for these substances were made on extracts prepared by boiling the muscle in 4 ml. of water for 15 min. (a procedure which extracts these substances completely). The extracts were deproteinized with $Zn(OH)_2$ (Somogyi, 1945). Samples of the perfusion medium were suitably diluted and deproteinized in the same way. Glucose was determined photometrically with glucose oxidase, peroxidase and *o*-dianisidine (see Randle & Smith, 1958*a*). Xylose was estimated photometrically by the *p*-bromoaniline method (Roe & Rice, 1948) and arabinose by the orcinol method (Mejbaum, 1939). When xylose was estimated in muscle extracts or samples of incubation medium containing glucose in addition to xylose, glucose was first removed by incubation with yeast. When arabinose was estimated by the orcinol method, muscle extracts were shaken with moist ion-exchange resins (Dowex 50, H^+ form and Duolite A-4, OH^- form) to decrease the blank. These resins did not remove any pentose from the extract. For assay of D-[$^{14}C_6$]sorbitol, samples were pipetted on to aluminium planchets containing a disk of lens tissue. After the addition of a drop of a saturated aqueous solution of polyvinyl alcohol, the samples were dried under an infrared lamp and counted beneath a thin end-window Geiger–Müller tube. At least 1000 counts were recorded for each sample. Since the weight of material added in the sample was less than 0·25 mg./cm.^{2}, no correction was applied to these estimates of radioactivity.

Total water was determined by weighing the muscle before and after freeze-drying.

Calculations

Glucose uptake. This was calculated as mg. of glucose disappearing from the perfusion medium/g. of wet muscle/hr. of perfusion.

Total muscle water, glucose, pentose or sorbitol spaces. These spaces, expressed as ml./100 g. of wet muscle, were calculated from formulae given by Randle & Smith (1958*b*).

Arabinose wash-out. The exit of arabinose from the muscle cells was calculated as the percentage of initial intracellular arabinose washed out by the perfusion medium per minute. The initial intracellular arabinose was calculated by summing the arabinose removed during perfusion and the residual arabinose in the heart at the end of perfusion. The rate of exit (μg. of arabinose/g. of wet heart/min.) was also calculated as a function of the intracellular concentration. The latter was calculated by dividing the pentose content of the heart at each time period by the intracellular fluid volume (total water minus sorbitol space).

Intracellular accumulations. To determine the intracellular accumulation of glucose or xylose, the glucose or xylose space was compared with the volume of extracellular fluid (determined by measuring the sorbitol space). Glucose or xylose was deemed to be present within the cell if the glucose or xylose space was greater than the sorbitol space.

RESULTS

Effect of anoxia and insulin, separately or in combination, on the uptake and accumulation of glucose

The effects of anoxia and insulin, separately or in combination, on the uptake and accumulation of glucose are shown in the upper part of Table 1. Under aerobic conditions insulin more than doubles uptake of glucose and causes accumulation of free glucose within the cardiac-muscle cells (i.e. the glucose space is greater than the volume of extracellular fluid estimated with sorbitol). Anoxia also more than doubles uptake of glucose but the sugar did not accumulate within the cells under these conditions. The combined influence of insulin and anoxia led to an even greater acceleration of glucose uptake and the level of uptake was approximately twice that attained with either insulin (under aerobic conditions) or anoxia and approximately four times that attained in the absence of insulin under aerobic conditions. Free glucose did not accumulate within muscle cells when insulin was present under anaerobic conditions, in clear contrast with the effect of the hormone under aerobic conditions.

With deprivation of oxygen the heart stopped beating within 2–3 min. The perfusion flow was, however, usually increased by about 50 % and the pressure somewhat reduced. In appearance the heart was flaccid and somewhat swollen and in keeping with this the extracellular (sorbitol) space and total water content were increased (Table 2). Neither sorbitol space nor water content was affected by insulin.

Effects of salicylate and 2:4-dinitrophenol

The effects of salicylate (5 mM) and 2:4-dinitrophenol (0·25 mM) on glucose uptake are shown in Table 1. Salicylate caused a small but nevertheless

Table 1. *Effect of insulin, anoxia, sodium salicylate, 2:4-dinitrophenol, D-galactose and phlorrhizin on the uptake of glucose and the glucose and sorbitol spaces of the perfused rat heart*

The number of hearts perfused is given in parentheses. Initial concentration of glucose was 5·5 mM.

Experiment	Period of perfusion (min.)	Insulin (0·1 unit/ml.)	Glucose uptake (mean ± S.E.M.) (mg. of glucose/g. of wet heart/hr.)	Space (mean ± S.E.M.) (ml./100 g. of wet muscle)	
				Glucose	Sorbitol
Aerobic control	0–15	0	4·9 ± 0·4 (11)	35·0 ± 2·6	36·0 ± 0·8
	0–30	0	4·3 ± 0·2 (10)	29·7 ± 2·1	35·6 ± 1·0
Aerobic	0–15	+	10·0* ± 0·6 (8)	45·8* ± 1·7	36·6 ± 1·0
Anaerobic	0–15	0	12·1* ± 0·6 (9)	37·3 ± 1·8	41·2* ± 2·3
	0–30	0	9·5* ± 0·7 (8)	32·0 ± 3·0	41·9* ± 0·8
Anaerobic	0–15	+	17·6*† ± 1·9 (8)	31·2† ± 1·3	44·0 ± 1·0
Aerobic control	0–30	0	4·3 ± 0·2 (10)	29·7 ± 2·1	35·6 ± 1·0
Aerobic control + sodium salicylate (5 mM)	0–30	0	6·9* ± 0·4 (8)	35·3 ± 2·6	43·1* ± 1·4
Aerobic control + 2:4-dinitrophenol (0·25 mM)	0–15	0	6·2* ± 0·4 (8)	64·4* ± 2·5	30·8 ± 1·4
	0–30	0	3·6 ± 0·6 (8)	—	—
Anaerobic control	0–15	0	12·1 ± 0·6 (9)	37·3 ± 1·8	41·2 ± 2·3
Anaerobic control + D-galactose (44 mM)	0–15	0	8·6* ± 0·6 (7)	38·0 ± 1·7	47·0 ± 1·8
Anaerobic control + phlorrhizin (3 mM)	0–15	0	2·7* ± 0·3 (7)	37·8 ± 1·1	44·0 ± 2·0

* $P < 0·01$ *vs.* appropriate control. † $P < 0·01$ *vs.* aerobic + insulin.

significant increase in glucose uptake. The glucose space was increased but nevertheless remained smaller than the sorbitol space. 2:4-Dinitrophenol also increased glucose uptake but only during the first 15 min. of perfusion. In the subsequent period, uptake fell below the control rate and a large quantity of free sugar was found inside the cell.

Salicylate increased the sorbitol space, whereas 2:4-dinitrophenol reduced it. These different effects of salicylate and 2:4-dinitrophenol may be related to their effects on the mechanical and physical state of the tissue. When perfused with medium containing salicylate about 50% of the hearts ceased to beat after 10–15 min.; the remainder continued beating for the full 30 min. but at a slow rate (80–120/min.). There was no obvious correlation between the contractile activity and the glucose uptake. Those hearts which ceased to beat with salicylate appeared flaccid and swollen; the perfusion flow and pressure were not altered. With perfusion of 2:4-dinitrophenol, on the other hand, contraction ceased in all cases after 2–3 min. The coronary flow was maintained only by virtue of a 20–30 mm. Hg rise in perfusion pressure. The heart appeared smaller in size and was apparently in a state of contracture.

Effect of D-galactose and phlorrhizin

It appeared important, for reasons to be discussed later, to determine whether D-galactose or phlorrhizin, which inhibit the uptake of glucose under aerobic conditions (Fisher & Lindsay, 1956; Morgan & Park, 1958), exert a similar effect under anaerobic conditions. The presence of D-galactose in a molecular ratio to glucose of 8:1 reduced uptake of glucose moderately (lower part of Table 1). Phlorrhizin at a molecular ratio to glucose of 0·55:1 inhibited uptake of glucose very markedly. These effects of galactose and phlorrhizin were not associated with accumulation of intracellular free glucose.

Reversibility of the effects of anoxia

It was found that the effects of anoxia on glucose uptake could be promptly and nearly completely reversed by restoration of aerobic conditions. In Expt. 3 of Table 2 the heart was first perfused for 15 min. anaerobically; the medium was sampled and a stimulated uptake found as noted previously (cf. Expt. 2). The incubation was then continued for a second 15 min. period under aerobic conditions. The glucose uptake was restored to the same value as that observed in hearts incubated aerobically from the beginning (Expt. 1). The sorbitol space and water content were also restored to typically aerobic values, but the glucose space remained somewhat elevated. The contractile function of the heart was regained very promptly under oxygen. The muscle began to beat again within 30 sec. and within 5 min. regained a normal rate of 200–240 beats/min.

Substrate-dependence with insulin and anoxia

Expt. 4 of Table 2 shows that the extremely high rate of glucose uptake during 15 min. of perfusion under anaerobic conditions was maintained during a second 15 min. period of perfusion. On the other hand, if the heart had first been incubated anaerogically for 15 min. without glucose then uptake of glucose was impaired during a second 15 min. period of incubation under anaerobic conditions with medium containing the sugar. The sorbitol space was considerably reduced under these conditions and substantial amounts of glucose accumulated within the cell.

Effects of anoxia and insulin on the efflux of L-*arabinose*

The effect of anoxia on transport alone was studied with L-arabinose. This pentose crosses the cell membrane by the same mechanism as glucose (Morgan & Park, 1958), but unlike glucose it is not phosphorylated. It was convenient to measure the efflux of arabinose from the intracellular fluid of hearts which had accumulated a large amount of the sugar during a preliminary perfusion (see Experimental section). As discussed elsewhere (Park *et al.* 1959), efflux rates provide a more sensitive and reliable measure of transport than

Table 2. *Reversibility of effects of anoxia on glucose uptake and glucose, sorbitol and total water spaces of the perfused rat heart: effect of substrate deprivation and anaerobiosis* (15 *min.*) *on the subsequent metabolism of glucose*

Each vertical column shows a separate experimental series with the first 15 min. of perfusion in the upper part and the second 15 min. in the lower part. The number of hearts is given in parenthesis. Initial concentration of glucose in the perfusion medium was 5·5 mM.

Expt. no.	1	2	3	4	5
0–15 min.	Aerobic control	Anaerobic control	Anaerobic then aerobic	Anaerobic + insulin	Anaerobic + insulin without glucose
Gas phase	O_2+CO_2	N_2+CO_2	N_2+CO_2	N_2+CO_2	N_2+CO_2
Glucose uptake (mean ± S.E.M.; mg./g./hr.)	—	12·1±0·6 (9)	11·5±1·2 (6)	17·6±1·9 (8)	No substrate
Space (mean ± S.E.M.; ml./100 g.)					
Glucose	—	37·3±1·8	—	31·2±1·3	—
Sorbitol	—	41·2±2·3	—	44·0±1·0	—
Total water	—	81·5±0·3	—	81·5±0·3	—
15–30 min.					
Gas phase	O_2+CO_2	—	O_2+CO_2	N_2+CO_2	N_2+CO_2
Glucose uptake (mean ± S.E.M.; mg./g./hr.)	4·6±0·8 (7)	—	4·7*±0·3 (6)	16·9±1·5 (6)	7·0†±0·8 (8)
Space (mean ± S.E.M.; ml./100 g.)					
Glucose	29·7±2·1	—	39·2±1·9	32·6±2·5	49·6†±2·6
Sorbitol	35·6±1·1	—	36·9*±2·0	42·7±0·8	33·7†±2·4
Total water	79·1±0·3	—	80·2*±0·4	81·2±0·5	81·0±0·4

* $P > 0·05$ *vs.* Expt. 1, lower panel. † $P < 0·01$ *vs.* Expt. 4, lower panel.

Table 3. *Effect of anoxia ± insulin on the efflux of* L-*arabinose from the perfused rat heart*

The number of hearts perfused is given in parenthesis. Aerobic *vs.* anaerobic (no insulin) $P < 0·01$ for all times; aerobic (no insulin) *vs.* aerobic (insulin) $P < 0·01$ for all times; aerobic *vs.* anaerobic (both with insulin) $P > 0·1$; anaerobic (no insulin) *vs.* anaerobic (insulin) $P < 0·01$ for all times.

Cumulative time of wash-out (min.)	Percentage of intracellular arabinose removed (mean ± S.E.M.)			
	No insulin		Insulin (0·1 unit/ml.)	
	Aerobic (5)	Anaerobic (5)	Aerobic (4)	Anaerobic (7)
1	9·6±0·8	13·2±1·1	31·5±2·0	29·7±1·1
2	18·5±1·7	26·0±1·7	50·8±2·4	48·0±1·6
3	26·7±2·6	37·2±2·2	63·1±2·3	59·4±2·0
4	33·7±3·0	46·5±2·4	70·6±2·2	67·0±2·2
6	45·0±3·3	62·0±2·8	78·8±2·2	75·4±2·2
8	53·7±3·0	73·0±3·5	83·1±2·1	80·7±1·8
10	60·2±2·6	82·7±3·5	85·6±2·0	84·4±1·6

Table 4. *Effect of glucose* (30 mM) *and phlorrhizin* (3 mM) *on the accumulation of* D-*xylose in the perfused rat heart under aerobic or anaerobic conditions*

The period of perfusion was 10 min. The number of hearts perfused is given in parentheses. Initial concentration of D-xylose in the perfusion medium was 10 mM.

Expt.	Space (mean ± S.E.M.) (ml./100 g.) Total water	Sorbitol	Xylose	Intracellular xylose† as percentage of extracellular xylose concn. (mean ± S.E.M.)
Aerobic: no glucose (6)	79·2±0·3	36·5±1·0	62·8±0·8	62·2±1·9
Aerobic: with glucose (5)	79·6±0·3	35·2±1·4	55·0*±1·1	44·2*±2·1
Anaerobic: no glucose (6)	81·5±0·3	42·9±1·0	67·9±1·8	65·6±4·8
Anaerobic: with glucose (10)	83·3±0·2	45·9±1·3	55·1*±1·1	24·6*±2·9
Anaerobic: no glucose with phlorrhizin (4)	83·5±1·0	45·3±1·7	44·0*±1·9	0*

* $P < 0{\cdot}01$ *vs.* appropriate control.

† Calc. from $\frac{\text{xylose space} - \text{sorbitol space}}{\text{total water} - \text{sorbitol space}} \times 100$.

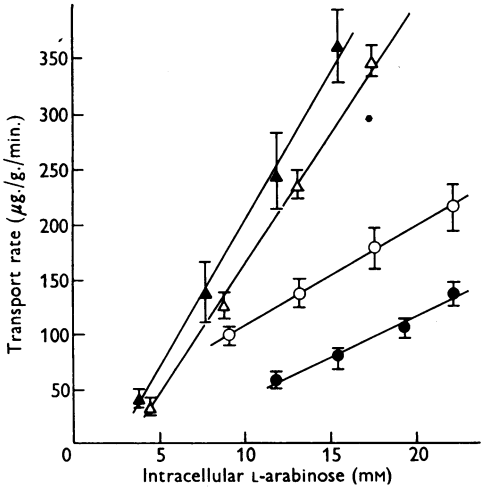

Fig. 1. Effect of anoxia on the outward transport of L-arabinose from the perfused rat heart, with and without insulin. The concentration of insulin was 0·1 unit/ml. The number of hearts are given in Table 3. Mean values are plotted, together with twice the S.E. ●, Aerobic (no insulin); ▲, aerobic (with insulin); ○, anaerobic (no insulin); △, anaerobic (with insulin).

the determination of rates of intracellular accumulation.

As shown in Table 3, anoxia caused a rise in the rate of efflux of intracellular arabinose. This effect was similar to that of insulin but of smaller magnitude. In the heart already treated with insulin, anoxia caused no further acceleration.

Since transport rates are a function of sugar concentration a more valid comparison is obtained when the efflux data are recalculated to give transport rates as a function of the intracellular pentose concentration, as shown in Fig. 1. It can be seen that transport was a linear function of concentration under all the conditions tested. By comparison with the control, anoxia caused an approximately twofold acceleration and insulin or insulin + anoxia about a fourfold acceleration.

Effect of D-*glucose and phlorrhizin on accumulation of* D-*xylose*

As shown in Table 4, D-xylose entered the cell rapidly under aerobic conditions and, after about 10 min., reached a mean concentration inside the cell which was about 60% of that in the perfusate. Comparison of these results with those reported elsewhere (Morgan *et al.* 1959) indicates that this approximates to the maximal intracellular pentose concentration. Presumably for this reason no significant increase in accumulation could be shown with anoxia, although transport of xylose was presumably accelerated as is transport of glucose and arabinose. The addition of glucose to the medium significantly depressed accumulation of xylose both aerobically and anaerobically. Phlorrhizin was a very potent inhibitor of xylose accumulation and completely prevented any measurable penetration of the cell by xylose under anaerobic conditions.

DISCUSSION

This investigation has confirmed the earlier conclusion of Randle & Smith (1958*a*, *b*) that anoxia, like insulin, increases the rate of transport of glucose across the muscle-cell membrane. On the other hand, these studies have revealed an additional effect of anoxia on the rate of phosphorylation of glucose by cardiac muscle which is not shown by insulin and which could not be detected in earlier studies with isolated diaphragm by Randle & Smith.

In interpreting our data transport of glucose is considered to be rate-limiting for glucose uptake if no free sugar accumulated intracellularly and phosphorylation of glucose is considered to be rate-limiting if free glucose was detected in the muscle

cell. Furthermore since sugars may be transported across the cell membrane in both directions (Morgan *et al.* 1958) the rate of net inward transport of glucose (inward − outward transport measured as glucose uptake) will only approximate to the true rate of inward transport if transport is the rate-limiting step in glucose uptake (i.e. if no free glucose accumulates within the cells).

In hearts perfused under aerobic conditions without addition of insulin (aerobic control) transport was the rate-limiting step in glucose uptake. Anoxia increased glucose uptake (2·5-fold over aerobic control) and transport remained rate-limiting. We conclude from this that anoxia accelerates transport of the sugar. When insulin was present under aerobic conditions glucose uptake was increased (twofold over control) but phosphorylation of glucose now became rate-limiting. On the other hand, when insulin was present under anaerobic conditions glucose uptake was increased still further (to 3·6-fold over aerobic control) but now transport and not phosphorylation was the rate-limiting step. Thus anoxia prevented phosphorylation from becoming rate-limiting in the presence of insulin and we conclude therefore that anoxia must stimulate phosphorylation of glucose in addition to accelerating transport of the sugar. In cardiac muscle, then, changes in the rate of membrane transport and in the potential rate of phosphorylation of glucose are of importance in the Pasteur effect. Moreover, these observations demonstrate that insulin is still effective under anaerobic conditions (cf. Randle & Smith, 1958*a*).

These interpretations are supported and strengthened by data obtained from measurements of the outward transport of L-arabinose. The transport of this pentose may be used as a convenient indicator of glucose transport because arabinose is not phosphorylated and studies of competitive inhibition show that both sugars are transported by the same system (Morgan & Park, 1958). Furthermore, the transport process for L-arabinose is freely reversible and subject to hormonal influences in either direction (Morgan *et al.* 1958). Anoxia approximately doubled the rate of arabinose transport whereas insulin or insulin + anoxia effected about a fourfold increase. The changes in the rate of arabinose transport induced by anoxia or by insulin + anoxia are thus comparable to the changes in the rate of glucose transport which they effect (as measured by glucose uptake). On the other hand, the effect of insulin on the rate of arabinose transport under aerobic conditions is much greater than its effect on glucose uptake. This is perhaps not surprising since free glucose accumulates in muscle cells under these conditions and outward transport of the sugar may be taking place to a significant extent. It is to be expected therefore that the effect of insulin on the rate of glucose transport under aerobic conditions will be greater than its effect on net inward transport of the sugar (see above). It seems reasonable therefore to conclude that anoxia effects about a twofold acceleration of glucose or arabinose transport and insulin about a fourfold increase. These changes are very similar to the changes in glucose uptake of isolated diaphragm induced by these factors (Randle & Smith, 1958*a*). On the other hand, a fourfold increase in glucose uptake of isolated diaphragm was seen with insulin under both aerobic and anaerobic conditions. In this tissue free glucose does not accumulate in cells when insulin is present under aerobic conditions (Randle & Smith, 1958*b*).

Although both insulin and anoxia accelerate the transport of glucose and L-arabinose in cardiac muscle the effects of the two factors acting together are not additive. This would suggest that they affect the same transport process and at some common point (cf. Randle & Smith, 1957; 1958*a*).

Our studies also show that when the heart is incubated anaerobically without glucose the tissue shows an impaired capacity to utilize glucose on subsequent incubation with the sugar. Dickens & Greville (1933) first showed, with brain slices, that glucolysis may become impaired when a tissue is incubated under anaerobic conditions without glucose. Elliott & Rosenfeld (1958) have made a similar observation with brain slices and they attribute this phenomenon to depletion of adenosine triphosphate, which is necessary for phosphorylation of glucose. An analogous phenomenon has also been observed in erythrocytes stored for long periods in the absence of metabolizable substrate. These cells fail to utilize glucose until primed by the addition of a nucleoside such as adenosine, which can apparently result in synthesis of adenosine triphosphate by a mechanism which does not involve the initial expenditure of a high-energy phosphate bond (Prankerd, 1956). Our observations with cardiac muscle are essentially similar and they may also be attributable to impaired phosphorylation of glucose owing to depletion of adenosine triphosphate. This is supported by our finding that free glucose accumulates within the cell under these conditions.

Sodium salicylate and 2:4-dinitrophenol both appear to increase membrane transport of sugar in a manner similar to anaerobiosis (Randle & Smith, 1958*b*). The transport rate of hearts perfused with medium containing sodium salicylate is accelerated, as shown by the increased glucose uptake. 2:4-Dinitrophenol appears initially to have the same effect but in addition must have an inhibitory effect on phosphorylation of glucose because glucose uptake subsequently declines and free sugar accumulates within the cell.

The question can be raised whether acceleration of the entry of sugars into the cell with anaerobiosis is due simply to a non-specific breakdown of membrane integrity or to a more specific effect on the sugar-transport system. The present work appears to show that anoxia exerts a specific effect on the transport system. Thus if the effect was due to breakdown of the cell membrane it is to be expected that sorbitol would enter the cell as well as the sugars studied, and the evidence suggests that sorbitol did not enter the cell under these conditions. However, the sorbitol space did increase in the presence of salicylate and under anaerobic conditions in the presence of glucose. We believe that this was due to changes in the volume of extracellular fluid, and not to penetration of the cell by sorbitol, for the following reasons. The sortibol space did not increase progressively when the time of anaerobic incubation was prolonged from 15 to 30 min. Furthermore, restoration of aerobic conditions after anaerobic incubation led to a prompt reduction of the sorbitol space to normal and this was associated with an equivalent loss of tissue water. Moreover, the sorbitol space was actually reduced when 2:4-dinitrophenol was present or when hearts were incubated under anaerobic conditions without glucose. Further points of evidence accrue from measurements of arabinose and xylose transfer. The efflux of arabinose from muscle was increased by anoxia in the absence but not in the presence of insulin. If anoxia was accelerating transfer of arabinose by causing breakdown of the cell membrane then we would have expected it to accelerate regardless of whether the hormone was present or not. Finally D-galactose and phlorrhizin hindered glucose entry and glucose and phlorrhizin inhibited xylose entry under anaerobic conditions. The preservation of these inhibitory effects under anaerobic conditions indicates that these sugars were still entering by a specific transport process and not by a process of free diffusion.

SUMMARY

1. The mechanism by which anoxia, salicylate and 2:4-dinitrophenol stimulate glucose uptake in the perfused isolated rat heart has been investigated. The effect of these agents has been compared with that of insulin.

2. Anoxia increases glucose uptake by accelerating transport of glucose across the cell membrane and also by increasing intracellular phosphorylation of glucose. In the absence of insulin the effect of anoxia on transport is of chief importance since this is the predominantly rate-limiting step for glucose uptake. In the presence of insulin, the effect on phosphorylation is the more important because the hormone alone accelerates transport sufficiently to make phosphorylation of glucose the rate-limiting step.

3. Anoxia induces a twofold increase in glucose transport whereas insulin causes a three- to four-fold increase. The effects of these agents on transport and phosphorylation stimulate glucose uptake to the following extent: anoxia, 2·5-fold; insulin, twofold; anoxia + insulin, 3·6-fold.

4. Salicylate (5 mM) and 2:4-dinitrophenol (0·25 mM) also stimulate the transport of glucose across the cell membrane. 2:4-Dinitrophenol also inhibits intracellular phosphorylation of glucose.

5. The effects of anoxia, salicylate and 2:4-dinitrophenol appear to involve acceleration of the specific monosaccharide-transport process in the membrane and the effects are not due to a breach of membrane integrity.

6. These observations offer a partial explanation for the Pasteur effect in muscle and support the earlier suggestion of Randle & Smith (1957, 1958*a*, *b*) that membrane transport of glucose in muscle is regulated in some way by the availability of high-energy phosphate.

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