## Glucose-6-Phosphate Pools in Isolated Rat Diaphragm

SHAW and STADIE[1] have postulated the existence, in isolated rat diaphragm, of two separate Embden-Meyerhof pathways different in cellular localization and response to insulin.

However, incubation with different metabolites of a reaction sequence results in shifting pool sizes, sequential uptake of the substrate and a rearrangement in the yields of the same final products. A compartmental situation can therefore be deduced from parallel reactions, in which both substrates are present alternately labelled. In this manner the distribution of label in the final products allow a comparison of the fate of glucose-6-phosphate coming from the external medium and glucose-6-phosphate derived from glucose. LANDAU and SIMS[2], in fact, incubating diaphragm with both glucose (10 m$M$) and glucose-6-phosphate (2 m$M$) alternately labelled, presented evidence which suggested that 2 pools of glucose-6-phosphate are located in muscle cells. The assumptions made by these authors did not take into account the possible limitations of cell surface permeability to the uptake of either substrate.

In the present investigation, an attempt has been made to study the role of substrate concentration on the presence of the 2 compartments. The data obtained were consistent with a metabolic compartmentalization under particular experimental conditions only.

Male Wistar rats, weighing 150–200 g, starved for 24 h, were used in all the experiments. 100 mg of diaphragm was incubated for 90 min at 37 °C in Warburg vessels, containing 2 ml of Krebs-Ringer[3] bicarbonate buffer, after equilibration with 95% $O_2$ and 5% $CO_2$. Substrates concentrations were 5.5 or 55.0 m$M$ for glucose and 6.6 or 66.0 m$M$ for glucose-6-phosphate. In the experiments with both substrates in the same flask, glucose 5.5 or 55.0 m$M$ was paired with 66.0 or 6.6 m$M$ glucose-6-phosphate respectively. In one set of experiments flasks containing both glucose and glucose-6-phosphate were paired so that in one glucose was uniformly labelled and in the other glucose-6-phosphate (approx. 20 μC/vessel). In another set of experiments either labelled glucose or labelled glucose-6-phosphate were in the medium. Diaphragms were divided so that the paired vessels had representation from the same rats. Incubations were stopped by addition of 0.2 ml of 0.4 $N$ NaOH in the central well, containing a filter paper strip, and 0.2 ml of $N$ HCl in the medium. After shaking the flasks for 30 min, the paper strip was eluted with 3 ml of water. The $^{14}CO_2$ was measured on a liquid scintillator counter. 1 ml of the washing solution was added to 10 ml of a dioxane-naphtalene mixture[4]. $^{14}C$ incorporation into glycogen was measured with the same technique after precipitation and hydrolysis to glucose in $N$ $H_2SO_4$[5]. In the experiments with either labelled glucose or labelled glucose-6-phosphate tissue extraction and paper chromatography were carried out as previously described[6]. The radiochromatograms were scanned by the automatic device developed in this laboratory[7].

The ratios of incorporation of $^{14}C$ into $CO_2$, lactate and glycogen between glucose and glucose-6-phosphate are given in Table I. When glucose concentration is 5 times higher than glucose-6-phosphate concentration[2], there is a six-fold greater incorporation of μmoles from the first substrate than from the last one. Production of radioactive glycogen was 24 times higher from glucose than from glucose-6-phosphate. To check if such observations were due to different amounts of the substrates used, because of a differential permeability, we have tested various concentrations of glucose and glucose-6-phosphate. When glucose-6-phosphate concentration was about 10 times lower than glucose concentration and below the threshold necessary for glycogen synthesis, the results for $CO_2$ and lactate remained essentially the same, while for glycogen the ratio was considerably higher. When glucose-6-phosphate concentration was about 10 times higher than glucose, small differences were found between the 2 substrates for either the catabolic or the synthetic routes. In fact, the importance of a threshold concentration of glucose-6-phosphate for incorporation of radioactive carbon into glycogen is clearly shown in Table II. Under the same conditions glucose-6-phosphate is a much better precursor of lactate than is glucose. The synthesis of glycogen and oligosaccharides from glucose was greater than that from glucose-6-phosphate independently of the concentration used, but radioactive glucose-6-phosphate is incorporated significantly into glycogen only at the

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Table I. Ratio of [U-$^{14}C$]glucose incorporation to [U-$^{14}C$]glucose-6-phosphate incorporation into $CO_2$, lactate and glycogen

| Glucose concentration (m$M$) | | | 10.0 | 55.0 | | | 5.5 | | |
|---|---|---|---|---|---|---|---|---|---|
| Glucose-6-phosphate concentration (m$M$) | | | 2.0 | 6.6 | | | 66.0 | | |
| | [U-$^{14}C$] Glucose | [U-$^{14}C$] Glc-6-P | Ratio | [U-$^{14}C$] Glucose | [U-$^{14}C$] Glc-6-P | Ratio | [U-$^{14}C$] Glucose | [U-$^{14}C$] Glc-6-P | Ratio ± 0.01 |
| $CO_2$ | 145 ± 14 | 30 ± 1 | 4.8 ± 0.1 | 153 ± 7 | 17 ± 2 | 9.0 ± 0.1 | 66 ± 3 | 88 ± 6 | 0.75 |
| Lactate | 845 ± 20 | 120 ± 10 | 7.1 ± 0.3 | 1885 ± 90 | 269 ± 17 | 7.0 ± 0.9 | 948 ± 13 | 1504 ± 132 | 0.63 |
| Glycogen | 181 ± 7 | 7.7 ± 0.8 | 24.0 ± 0.5 | 2450 ± 300 | 7 ± 1 | 350.0 ± 11.0 | 264 ± 5 | 293 ± 9 | 0.9 |

Results are expressed as nmoles of converted substrate/100 mg of tissue wet weight and are the mean values of 7 experiments ± S.E. mean. The ratios were calculated from paired vessels containing both glucose and glucose-6-phosphate alternately labelled.

Table II. Metabolism of glucose and glucose-6-phosphate

| Substrate concentration (m$M$) | Glucose 5.5 | 55.0 | Glucose-6-phosphate 6.6 | 66.0 |
|---|---|---|---|---|
| Glycogen and oligosaccharides | 331 ± 32 | 2440 ± 160 | 15 ± 5 | 208 ± 7 |
| Glucose and Hexose phosphate esters | 135 ± 12 | 2628 ± 163 | 29 ± 5 | 1798 ± 126 |
| Lactate | 764 ± 58 | 1852 ± 93 | 334 ± 96 | 2520 ± 46 |
| Phosphoglycerate | 96 ± 16 | 556 ± 63 | 166 ± 13 | 2522 ± 63 |

Results are expressed as nmoles of converted and unchanged substrate/100 mg of tissue wet weight. Mean values of 5 experiments ± S.E. mean are reported.

66.0 m$M$ level. The amount of radioactive carbon from glucose increases in the tissue proportionally to the external concentration, while in the case of the hexose ester an appreciable amount is found only at the higher concentration.

The differences in ratios of incorporation of $^{14}C$ into $CO_2$ and lactate with respect to glycogen, shows that glucose-6-phosphate was utilized as such, rather than rapidly hydrolyzed to glucose.

Assuming that 2 compartments coexist, it seems likely that glucose-6-phosphate would not enter each compartment with equal facility. In this case, the cellular system should be more affected by factors able to modify the equilibrium constants of the enzymes than by concentration factors. The results obtained, on the other hand, clearly show that mass effects are more important (Tables I and II) than possible regulatory actions of glucose-6-phosphate. If this action was to be limited by glucose-6-phosphate concentration in the cell, the ratios must remain unaffected by changes in glucose-6-phosphate concentration in the medium. The concentration effects could be due to a permeability factor. Glucose-6-phosphate, in fact, requires a threshold concentration[8–10] before it can be utilized. If the cells discriminate to a greater or lesser extent against the entry of substances from the external medium, this phenomenon would not require different effects, experimentally not evidenced, on the 2 compartments. At the higher concentration, instead, the 2 pools, if they exist independently of the experimental conditions, are completely mixed.

*Riassunto.* È stato studiato l'effetto della concentrazione sul metabolismo del glucosio e del glucosio-6-fosfato nel diaframma isolato di ratto. Si discute brevemente della esistenza di due pools di glucosio-6-fosfato in relazione alle diverse condizioni sperimentali.

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## Alterations in the Activity of Glyceraldehyde-3P-Dehydrogenase of the Rat Liver as a Function of Age

Alteration in the activities of several enzymes are known to occur in the tissues of aging animals[1–4]. Our earlier reports[5,6] on the lactate and malate dehydrogenases of the heart, brain, skeletal muscle and liver of rats have shown that the tissues become more aerobic during old age. The present study on glyceraldehyde-3P- dehydrogenase (gly-3P-DH) shows that the activity of Krebs cycle is increased in old age of the rat and supports the above conclusion.

The liver of 6-, 12-, 33- and 70-week-old male albino rats of Wistar strain was taken out and a 20% (w/v) homogenate of the tissue was prepared in 0.25$M$ sucrose at 0–2 °C using a Potter-Elvehjem homogenizer and teflon pestle. The oxygen consumption and the percent inhibition of oxygen consumption by iodoacetic acid (IAA; 0.125$M$) of the liver homogenate were measured manometrically using an Aminco Warburg Respirometer. The total volume of the incubation medium was 3.0 ml which included 0.9 ml of sucrose (0.25$M$), 0.4 ml of phosphate buffer (0.066$M$; pH 7.4), 0.1 ml of $MgCl_2$ (0.1$M$), 1.0 ml of homogenate, 0.5 ml of glucose (0.1$M$) and 0.1 ml of IAA (0.125$M$ final concentration). The centre well contained 20% KOH. The temperature of measurement was 37 °C and the gas phase was air. Readings were linear for 60 min after which the oxygen consumption was measured as ml $O_2$/g wet wt./h. The effect of IAA was expressed as percent inhibition of oxygen consumption.

The Table shows that the rate of oxygen consumption of the liver increases significantly with development of the rat up to 12 weeks after which it decreases significantly up to 33 weeks. Thereafter, there was no significant

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