THE ACTIVITY OF THE PENTOSE PHOSPHATE PATHWAY IN ISOLATED LIVER CELLS

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SUMMARY: Isolated liver cells have been used to assess the relative contribution of the pentose phosphate pathway to glucose metabolism. The incorporation of carbon from specifically labelled glucose into $^{14}CO_2$ by isolated cells gave values (μg.atoms/g.cells/hr) of: C-1, 7.9; C-6, 1.3; C-U, 3.4. The corresponding figures for liver slices were: C-1, 2.3; C-6, 1.6; C-U, 3.0. The most striking difference was the 3.5-fold increase in the oxidation of C-1 of glucose. Isolated cells retain more than 50% of ATP and have a content of intermediates of the glycolytic pathway closely similar to freeze-clamped liver. The relative importance of the pentose phosphate pathway in isolated liver cells, approximately 16% of glucose catabolised, is consistent with the enzyme profile of liver and the reductive synthetic reactions of the tissue.

INTRODUCTION

The development of methods of preparing isolated liver cells with intact plasma membrane in high yield which possess the metabolic activities of the intact tissue has provided a useful system to study the regulation of hepatic metabolism [1-7]. In the present study isolated liver cells have been used in a re-examination of the problem of the relative contribution of the pentose phosphate pathway (PPP) to glucose metabolism using ^{14}C-labelled glucose.

It has been shown that liver slices rapidly lose ATP, and that ATP-dependent systems in the cytosol are more markedly affected than those in the mitochondria [8], thus quantitative determination of pathways of glucose metabolism is rendered extremely difficult with such a system; the isolated cell preparation, which maintains an ATP level closer to the *in vivo* level [3], overcomes certain of these problems.

In the present experiments the rate of oxidation of C-1 of glucose is more than 7-fold that of C-6, calculations from these data suggest that approximately 16% of glucose metabolism may occur by the PPP, a finding consistent with the relatively high activity of enzymes of this pathway in rat liver.

METHODS

Adult male albino rats were used. Isolated liver cells were prepared by

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enzymatic procedures using a modification of the method of Crisp & Pogson [4]. The liver was initially perfused with Ca^{2+}-free Krebs–Ringer bicarbonate medium containing 5 mg. glucose and 5 munits insulin/ml. When the perfusate was free from blood, collagenase (Sigma Type I) and hyaluronidase (Sigma Type I) were added (0.5 and 1 mg/ml medium respectively) and the perfusion continued, with recirculation, for a further 10–20 min at a flow rate of 8–10 ml/min. The cells were separated by centrifugation at 50 *g* for 2 min and were washed three times with Krebs–Ringer bicarbonate containing glucose 5 mg/ml and albumin (fatty acid free) 2 mg/ml, the final wash was with Krebs–Ringer bicarbonate containing albumin alone (2 mg/ml). The same medium was used to suspend the cells prior to determination of the flux of glucose and steady-state metabolite content as previously described [9,10]. Cell suspensions were examined microscopically for Lissamine Green dye exclusion, a high percentage (80–85%) of unstained cells was routinely observed, samples of cell suspension were used for dry weight and DNA determination.

RESULTS AND DISCUSSION

The conversion of specifically labelled glucose into $^{14}CO_2$ and ^{14}C-lipid by isolated liver cells and liver slices from normal rats are shown in Table 1. The most striking difference is the 3-fold greater rate of formation of $^{14}CO_2$ from C-1 of glucose in isolated liver cells, indicating a significantly greater contribution of the PPP, particularly when considered in relation to the decrease in $^{14}CO_2$ from C-6 of glucose and the increased incorporation of glucose into lipid. Phenazine methosulphate (PMS) caused a more marked stimulation of the PPP in liver slices than in cells and in the presence of this artificial electron acceptor there is close agreement between the rates of oxidation of C-1 of glucose in cells and slices.

The lower rate of oxidation of C-6 of glucose and of pyruvate labelled on either C-1 or C-2 could be a reflection of improved integrity of mitochondrial oxidative phosphorylation system in cells relative to slices. This receives some support from the finding that isolated cells have a higher ATP content and ATP/ADP quotient than do liver slices (see [3,8] and Table 3).

The calculation of the flow of glucose carbon through alternative routes of glucose metabolism in isolated cells is complicated by the diversity of pathways in this tissue, in particular the occurrence of an active glucuronic acid–xylulose pathway will render interpretation of specific yields of $^{14}CO_2$ and ^{14}C-lipid more difficult. If the simplifying assumption is made that the major routes of glucose catabolism are the glycolytic pathway, the tricarboxylic acid cycle and the PPP with major end products of CO_2, lipid and lactate then, as a first approximation, the formulae devised by Katz *et al.* [11,12] may be

Table 1. Conversion of specifically labelled glucose into $^{14}CO_2$ and ^{14}C-lipid by isolated liver cells and liver slices from rats.

	LIVER CELLS		LIVER SLICES	
	µg.atoms of glucose or pyruvate carbon/g.wet wt./hr./37°			
	$^{14}CO_2$	^{14}C-lipid	$^{14}CO_2$	^{14}C-lipid
[1-^{14}C]glucose	6.90±0.63	0.91±0.12	2.35±0.25	0.49±0.06
[1-^{14}C]glucose + PMS	7.90±1.5	0.44±0.12	8.36±0.94	0.20±0.03
[6-^{14}C]glucose	0.97±0.06	1.47±0.22	1.55±0.10	1.22±0.17
[U-^{14}C]glucose	2.75±0.32	1.04±0.29	3.00±0.28	0.48±0.62
C1/C6	7.1	0.62	1.5	0.40
[1-^{14}C]pyruvate	14.6 ±1.5	0.46±0.04	61.2 ±3.7	0.28±0.07
[2-^{14}C]pyruvate	5.97±0.9	1.02±0.16	18.8 ±1.2	1.81±0.26

The incubation medium was Ca^{2+}-free Krebs—Ringer bicarbonate, gas phase O_2:CO_2, 95:5. Substrates were present at 20mM concentration with 0.2µCi ^{14}C-labelled glucose or pyruvate, phenazine methosulphate (PMS) 0.1mM final concentration. Incubation time 1 hr. The total glucose utilised by isolated cells, taken as the sum of incorporation of [U-^{14}C]glucose into CO_2, lipid and lactate was 16.6±1.7 µmoles/g wet weight cells/hr. Each result is the mean±SEM of not less than six observations. The DNA content of the isolated cells was 2.28±0.21 mg/g wet weight; the dry weight, 12%.

used to assess the relative contribution of the PPP. These calculations are given in Table 2. Four methods, involving different assumptions, gave good agreement of a 16% contribution of the PPP.

The content of phosphorylated intermediates of isolated liver cells and freeze-clamped liver are shown in Table 3. The steady-state level of ATP in cells was approximately 50% of that of freeze-clamped liver on a wet weight basis, the ATP/ADP quotient was considerably higher and, perhaps correlated with this, the calculated redox state of free NAD^+/NADH of the mitochondrial compartment was also high, approximating more closely to values found in rats given a high carbohydrate diet. There was good agreement among the values for the free NAD^+/NADH quotient of the cytosol and freeze-clamped liver. The total adenine nucleotides found in cells in the present experiments is somewhat higher than that reported by Hommes *et al.* [3], the values are 27 and 19 mµmoles per 10^6 cells respectively. The ATP/ADP quotient is higher than Hommes *et al.* [3] and more nearly approximates to the *in vivo* zero time value given by Faupel *et al.* [13].

In isolated liver cells those intermediates preceding the GAP dehydrogenase reaction are higher, and those following this reaction are, in general, lower, than normal rat liver and the profile again more closely approximates to

Table 2. Flow of glucose carbon in alternative pathways of glucose metabolism in isolated liver cells

Method of calculation	% Contribution of pentose phosphate pathway
(a) $PPP = \frac{S}{3 - 2S} \times 100$, where $S = \frac{G1CO_2 - G6CO_2}{1 - G6CO_2}$	17%
(b) $PPP = \frac{S'}{3 - 2S'} \times 100$, where $S' = G1CO_2 - [G6CO_2 \cdot \gamma]$	17%
(c) $PPP = \frac{1 - \gamma}{1 + 2\gamma} \times 100$, where $\gamma = \frac{[1\text{-}^{14}C]\text{glucose into lipid}}{[6\text{-}^{14}C]\text{glucose into lipid}}$	17%
(d) $PPP = \frac{[1\text{-}^{14}C]\text{glucose into lipid}}{[6\text{-}^{14}C]\text{glucose into lipid}}$, from plot of Katz & Wood	13%

Method of calculation: (a), (b) and (c) are those of Katz *et al.* [11], (d) Katz & Wood [12]. GCO_2 is the specific yield of CO_2.

that found in liver following administration of a high carbohydrate diet (Table 3), a comparison which may be more appropriate since the cells are isolated in the presence of glucose and insulin and are presented with a higher glucose concentration in the final incubation medium. The apparent control at GAP dehydrogenase may be related to the high ATP/ADP quotient of the isolated cells [14].

The content of intermediates of the PPP of isolated cells incubated with glucose was similar to that of freeze-clamped liver [10].

The postulate that the PPP accounts for a substantial proportion of the glucose catabolised in isolated liver cells, and that cells may provide a better index of the extent of this pathway than previous estimates using liver slices, has been examined (as shown in Fig.1), in relation to the known enzyme profile of liver, the requirement of hydrogen for lipogenesis and the constant proportionality of these systems in a range of tissues [10,15-19].

Fig.1 portrays, on a logarithmic scale and relative to PFK, the activities of G6P dehydrogenase, of fatty acid synthetase and of the extent of the PPP (as indicated by $^{14}CO_2$ formation from specifically labelled glucose) in a range of tissues with widely differing metabolic patterns (brain, uterus, liver, adipose tissue and mammary gland). There is a clear parallelism between G6P

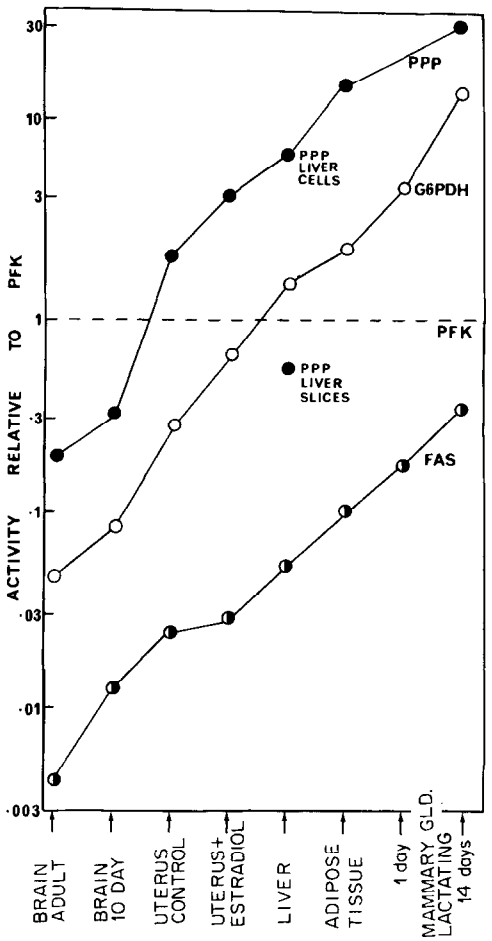

Fig.1. Correlation between G6P dehydrogenase and fatty acid synthetase and between G6P dehydrogenase and the isotopically-determined extent of the PPP in liver cells, liver slices and a range of other tissues.

Activities are given on a logarithmic scale relative to PFK on the basis of constant and specific proportionality [16,17]. Tissues with a wide range of G6P dehydrogenase (G6P DH) and fatty acid synthetase (FAS) were selected, these were adult brain, brain at 10 days after birth, immature rat uterus, before and 16 hr after treatment with estradiol [19], adult rat liver isolated cells and tissue slices, epididymal adipose tissue and rat mammary gland at the 1st and 14th days of lactation [18]. Comparative figures for the extent of PPP were derived from yields of $^{14}CO_2$ from [1-^{14}C] and [6-^{14}C]glucose and were corrected for recycling by use of [2-^{14}C]glucose [20]. The quotient $(G1CO_2 - G6CO_2)/G6CO_2$ is taken as an approximation of the PPP:glycolytic route.

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dehydrogenase and fatty acid synthetase activities in all the tissues studied. There is also a good correlation between the activity of G6P dehydrogenase and estimates of the extent of the PPP in brain, uterus, adipose tissue and mammary gland. When liver slices are used to estimate the extent of the PPP then liver

Table 3. Steady state metabolite contents of isolated liver cells and freeze-clamped liver

	LIVER CELLS	FREEZE-CLAMPED LIVER	
	Incubated with glucose	Normal	Carbohydrate diet
	mμmoles/g tissue		
ATP	1008	1920	2124
ADP	95	906	616
AMP	157	234	149
G6P	315	217	120
F6P	150	60	53
FDP	35	12	24
DHAP	39	17	46
3PG	141	264	197
2PG	24	29	23
PEP	71	61	39
αGP	457	134	247
NAD^+/NADH cytosol			
PYR:LAC K_{eq} LDH	710	728	1258
DHAP:αGP K_{eq} αGPDH	1520	2285	3333
NAD^+/NADH mitochondria			
AcAc:βOHB K_{eq} βOHBOH	35	7	21

Liver cells: Incubation medium was Ca^{2+}-free Krebs–Ringer bicarbonate, gas phase O_2:CO_2, 95:5; glucose 20mM final concentration, incubation time 1 hr at 37°. Reaction stopped by addition of $HClO_4$ and rapid cooling, metabolites estimated as previously described [9,10].

Freeze-clamped liver: Data from Greenbaum, Gumaa & McLean [10].

Liver slices: Adenine nucleotide content of rat liver slices incubated in Krebs–Ringer medium 1 hr at 37° is: ATP, 500; ADP, 350; AMP, 60 mμmoles/g. tissue (Krebs [8]).

appears to be anomalous and low. When the value for the extent of the PPP activity is calculated from isolated liver cell experiments, however, this anomaly is corrected and the new value is in good agreement with that which could be predicted from Fig.1.

Krebs [8] has shown that liver slices are particularly susceptible to loss of adenine nucleotides, while other tissues, such as brain, kidney and testis, retained a higher proportion of adenine nucleotide in incubated slice experiments. It may be suggested that the present higher estimate of the PPP in liver cells could, in part, be ascribed to the more favourable ATP content of these preparations. The contribution of the pentose phosphate pathway relative to the glycolytic route in liver now approaches more closely that found in adipose tissue and mammary gland [11,21].

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