Glucose Metabolism by Human Placental Villi

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1. Glucose phosphorylation rates of about $1 \mu\text{mole/g./min.}$ have been measured at room temperature in homogenates of human placental chorionic villi, and these rates are relatively constant throughout gestation. 2. This reaction has an apparent K_m for glucose of $3 \times 10^{-5} \text{M}$ both in early and term placenta. 3. Human foetal membranes, the amnion and chorion, also phosphorylate glucose at a rate about equal to that of the placenta. 4. On incubation of intact bits of villus tissue from 8–12-week or full-term placenta with labelled pyruvate, followed by paper chromatography of the tissue extract, the following distribution of label was observed: residual pyruvate, 40–60%; lactate, 30–50%; glucose, 6%; fructose, 7%; sorbitol, 0.6%. 5. The concept of the placenta acting as a foetal liver during early pregnancy is inconsistent with the observation that glucose production by this organ persists up to term.

In addition to its usual functions, the idea that the human placenta may act as an auxiliary foetal liver was first postulated by Claude Bernard (see Hagerman, 1964; Villee, 1960) on the basis of the rich glycogen deposits that he found in this tissue. More recently, this theory has been restated and evidence has been presented for glucogenesis from pyruvate and glycogen in early, but not term, human placenta (Villee, 1953). On this experimental basis, the presence of a placental D-glucose 6-phosphate phosphohydrolase (EC 3.1.3.9) has been presumed (Hagerman, 1964), and a major role has been ascribed to this organ in the regulation of the foetal blood sugar in early pregnancy.

Recent studies on the phosphorylation of glucose in rat liver have revealed a unique hepatic ATP-glucose 6-phosphotransferase (glucokinase; EC 2.7.1.2), which, because of its unusually high K_m for glucose, is thought to play a regulatory role in maintaining the blood sugar concentration (DiPietro, Sharma & Weinhouse, 1962). In view of the hepatic-like properties ascribed to the placenta, we have investigated the system that phosphorylates glucose in both early and term organs with particular reference to this enzyme.

Also included in the present paper are the results of a chromatographic study of the metabolites of [^{14}C]pyruvate in intact villus tissue from full-term and 8–12-week human placenta.

MATERIALS AND METHODS

Reagents. D-Glucose 6-phosphate, D-glucose 6-phosphate dehydrogenase and NADP were purchased from Boehringer

(Mannheim) Corporation, New York, N.Y., U.S.A.; D-glucose and D-fructose were from Pfanstiehl Laboratories, Waukegan, Ill., U.S.A.; sodium [^{14}C]pyruvate ($3 \mu\text{C}/\mu\text{mole}$) was from New England Nuclear Corp., Boston, Mass., U.S.A.

Preparation of tissue extracts for hexokinase assay. Placentas delivered at term were immediately transferred to ice-cold sodium tris-maleate buffer, pH 7.0 (75 mM), and subsequently perfused with this buffer through the umbilical vein or one of the umbilical arteries until blanching occurred on the maternal side. The blanched areas were dissected out taking care to exclude gross connective tissue, maternal decidua and the chorionic plate. In this way, relatively blood-free samples of chorionic villi were obtained together with the inevitable amount of microvasculature and connective-tissue stroma associated with these structures. After blotting and weighing, the samples were homogenized in glass with 2 ml. of buffer/g. of tissue. The homogenate was centrifuged at 700g and the supernatant retained for enzyme assay. Early placental tissue was obtained by curettage from therapeutic abortions, performed 7–12 weeks from the last menstrual period, and was separated as completely as possible from large pieces of membranes, foetus and decidual endometrium before homogenizing.

Hexokinase (EC 2.7.1.1) assay. The tissue extracts were diluted just before assay with 2 vol. of 0.25 M-sodium glycylglycine, pH 7.5, and 10 vol. of 0.15 M-NaCl to obtain a preparation containing the equivalent of 26 mg. of fresh tissue/ml. Assays were performed with 0.01 ml. of diluted extract by measuring initial rates of formation of glucose 6-phosphate from glucose with an Eppendorf fluorimeter and recorder as described by DiPietro (1964). The reaction medium contained: sodium glycylglycine, pH 7.5, 44 mM; NADP, 75 μM ; MgCl_2 , 7.5 mM; ATP, 3.1 mM; glucose, 0.1 M; glucose 6-phosphate dehydrogenase, 0.1 unit/ml. The fluorimeter was calibrated by adding a known amount

of glucose 6-phosphate to a cell containing the complete reaction medium less the glucose, and recording the total increase in fluorescence. A calibration constant of 0.18–0.20 μmole of glucose 6-phosphate/chart division was thus obtained for each sample assayed. Controls containing the complete system without added glucose occasionally displayed a slight increase in fluorescence due to endogenous glucose in the tissue extract; however, when ATP was omitted instead of glucose the reaction rate was invariably zero.

Pyruvate metabolism. The tissue for these experiments was collected in and washed thoroughly with buffer of the following ionic composition ($\mu\text{moles/ml.}$): phosphate, 40; chloride, 100; sodium, 80; potassium, 63; magnesium, 10; pH 6.8 at room temperature. Several small pieces of chorionic villus tissue totalling about 0.8 g. were teased from their supporting connective tissue and introduced into an open test tube containing 2 ml. of the above buffer, 0.01 M in sodium $[3\text{-}^{14}\text{C}]$ pyruvate and saturated with oxygen. A time of 2 min. was allowed for equilibration of the label with the tissue at 0° and then a 0.2 ml. zero-time sample of medium was withdrawn and mixed with 0.8 ml. of ethanol. The remainder of medium and tissue was incubated at 37° for 20 min. After incubation, the tissue was recovered, blotted and homogenized with 2 ml. of 80% ethanol, and the remaining tissue-less medium was mixed with 5 vol. of ethanol. Thus three samples were analysed in each experiment: a sample of the medium before and after incubation, and a sample of the tissue extract after incubation. The deproteinized samples were concentrated by evaporation in a stream of nitrogen at room temperature before application to the paper chromatograms.

Chromatography. Three solvent systems were used: A, a system for ascending chromatography: butan-1-ol–acetic acid–water (12:3:5, by vol.); B, another system for ascending chromatography: ethyl acetate–formic acid–water (7:2:1, by vol.); C, a system for descending chromatography: ethyl methyl ketone–acetic acid–saturated aqueous boric acid (9:1:1, by vol.). Standards labelled with ^{14}C were spotted on each chromatogram and were sometimes mixed with the sample itself when the R_F values were altered by the salt content of the sample. Solvent C was commonly run off the paper (15–25 hr.) to separate glucose, fructose and sorbitol. Whatman no. 1 chromatography paper was used throughout these studies and the chromatograms were run at 23–26°. After development, the chroma-

tograms were dried and scanned with a Nuclear–Chicago model C100B Actigraph 11, which had an efficiency of about 15–20% for ^{14}C under the conditions of these experiments.

RESULTS

Glucose phosphorylation. In Table 1 are presented average values for the glucose-phosphorylating capacity of homogenates of human full-term placental chorionic villi. A limited number of determinations on immature placentas are also included, illustrating that the hexokinase content of this organ does not change appreciably during pregnancy. The significance of the slightly lower values obtained with 7–9-week tissue is doubtful. The large variations in total hexokinase activity (last column, Table 1) is a reflection of the striking individual variation in size of these placentas, but the enzymic activity/g. remained quite constant. It is noteworthy that the foetal membranes (amnion and chorion) also possess the capacity to phosphorylate glucose, and at a rate not dissimilar to that of the placenta itself.

Several experiments were carried out in which the glucose concentration of the reaction medium was varied from 100 to 0.08 mM to determine an apparent K_m for glucose. Paired experiments with extracts of full-term and early placenta failed to reveal any differences in glucose affinity of the phosphorylating system with gestational age of the organ. Lineweaver–Burk plots of these data yielded K_m values for glucose of about 30 μM in all cases, and we have observed no indication of an hepatic-type glucokinase in either very early or term placenta.

Pyruvate metabolites in early placenta. As shown in Table 2, four major radioactive areas were detected in chromatograms of extracts of the tissue after incubation with labelled pyruvate, and these were seen in each of two solvent systems. The zero-time controls, showing just one peak with

Table 1. *Hexokinase activity in human placenta and foetal membranes*

Values and their standard deviations are given in units (μmoles of glucose 6-phosphate formed/min.) per g. of fresh tissue, per g. of dried tissue and per whole organ at 22–25°.

Gestational age of tissue (weeks)	No. of samples	Hexokinase activity		
		(units/g. wet wt.)	(units/g. dry wt.)	(units/whole organ)
40 (full term)	7	1.3 $\pm$ 0.2	9.7 $\pm$ 1.5	644 $\pm$ 155
40 (gestational diabetes)	1	1.22	10.2	622
35	1	1.32	12.0	480
24	1	1.35	11.2	181
20	1	1.16	10.5	171
7–9	2	0.7 $\pm$ 0.1	—	—
40 (foetal membranes)	2	0.85 $\pm$ 0.1	7.9	47

an R_F corresponding to pyruvate, affirm the purity of the starting material and also rule out the production of artifacts through manipulation and concentration of the samples before chromatography. In order of increasing R_F , peaks 3 and 4 were definitely identified as pyruvate and lactate respectively, peak 2 may be fructose, and peak 1 may be glucose or sorbitol or both. Chromatography of the reaction medium after incubation yielded, as might be expected, large quantities of pyruvate and lactate and only traces of the materials represented by the other two peaks.

To further separate and identify the compounds in peaks 1 and 2, these areas were eluted, combined and rechromatographed in solvent C. The results, shown in Table 3, affirm the presence of glucose, fructose and sorbitol, and in addition suggest the presence of trace quantities of what might be alanine. Pyruvate and lactate have run off this descending chromatogram, which was developed overnight, and the mobility of fructose was retarded by components of the tissue extract.

Pyruvate metabolites in term placenta. The foregoing results are representative of several experiments with different samples of early placenta in

which conditions and techniques were modified until unequivocal separations and identification were achieved. These experiments were repeated with samples from three term placentas and gave the same distribution pattern in this tissue. The data from one such representative experiment are presented in Table 4. The chromatogram was developed for 18 hr. with solvent C, which yields the best separations of the compounds in question. Pyruvate and lactate were again eluted from the paper, but the other four compounds are clearly separated. Of these four, only the identification of alanine remains uncertain, since it is present in trace amounts and does not show up at all on some chromatograms. A semi-quantitative estimation of the relative amounts of each intermediate (Table 5) reveals that the bulk of the labelled pyruvate carbon is converted into lactate, that glucose and fructose each account for 6-7%, that sorbitol accounts for less than 1%, and that a compound with an R_F similar to alanine is present in traces. Once more, no significant difference in the metabolism of pyruvate by 7-9-week and term placenta is apparent either qualitatively or quantitatively.

Table 2. *Pyruvate metabolites in 7-9-week placenta*

The R_F values are given for labelled products separated by paper chromatography from incubation medium and tissue after exposure of intact placental villi to 0.01 M-[3- 14 C]pyruvate (see the text). Data are presented for two solvent systems, and a zero-time control as well as reference standards are included for comparison.

Sample	R_F in solvent A				R_F in solvent B			
	0.185	0.24	0.50	0.74	0.10	0.26	0.70	0.86
Tissue								
Medium	0.20	0.25	0.51	0.74			0.70	0.86
Control			0.51				0.71	
Pyruvate			0.51				0.71	
Glucose	0.20				0.10			
Fructose		0.24			0.16			
Sorbitol	0.21				0.13			
Lactate				0.74				0.86
Alanine		0.37				0.35		

Table 3. *Pyruvate metabolism in 7-9-week placenta*

The labelled peaks corresponding to R_F values of 0.10 and 0.26, which were separated from the tissue sample in solvent B (see Table 2) were eluted, concentrated and rechromatographed in solvent C. The resultant distributions are presented below in terms of the distance migrated from the origin in cm. Standards, including an internal fructose standard, are included.

Sample	Distance migrated in solvent C (cm.)			
	8.9	11.1	17.8	20.8
Tissue				
Tissue + [14 C]fructose standard	8.5	11.0	17.6	20.9
Glucose	8.6			
Fructose		13.6		
Alanine			18.2	
Sorbitol				20.7

Table 4. *Pyruvate metabolism in full-term placenta*

Chromatographic separation of [$3\text{-}^{14}\text{C}$]pyruvate metabolites isolated from an extract of chorionic villi of term placenta. Pyruvate and lactate, because of their high mobility in solvent C (20 hr., descending), have run off the chromatogram. The positions of the radioactive peaks are identified in terms of their mobilities relative to glucose (R_{Glc} values).

Sample	R_{Glc} values in solvent C			
	1.0	1.7	2.3	3.0
Tissue	—	—	—	—
Control	—	—	—	—
Glucose	1.0			
Fructose		1.8		
Alanine			2.2	
Sorbitol				3.0

Table 5. *Quantitative estimation of pyruvate metabolites in early and full-term placentas*

Sodium pyruvate (20 μmoles), having a ^{14}C activity of 60 μC , was incubated with 0.8 g. of tissue for 20 min. at 37° . Data from several chromatograms run in different solvents were combined, and the relative amount of each product was estimated by integration of the area under each peak recorded by the strip counter. The values are expressed in terms of percentages of initial pyruvate radioactivity.

Metabolite	Relative amount of radioactivity (%)	
	7-9-week placenta	Full-term placenta
(Pyruvate)	57	37
Lactate	29	50
Glucose	5.7	5.5
Fructose	7.5	7.0
Sorbitol	0.7	0.5
Alanine	Trace	Trace

DISCUSSION

The results of the hexokinase assays show that placental villus tissue can phosphorylate glucose at rates comparable with those of other tissues. For example, the rates are about double those found in rat liver by a similar assay method (DiPietro & Weinhouse, 1960). To our knowledge there has been only one other study of hexokinase activities in human placenta in which average values of 0.17 and 0.10 $\mu\text{mole/g./min.}$ were reported for 4-month and term organs respectively (Marazzini & Tessari, 1957). These rates are one-fifth to one-tenth of those we found. Similar low values have also been published for guinea-pig placenta (Barbieri & Pecile, 1958). The authors of both reports used the same assay method based on glucose disappearance, and presumably did not measure initial reaction rates. The present study has further shown that the hexokinase content of very early placental tissue (2 months gestation or less) is similar to or a little lower than that of the more mature tissue. The almost identical increase of reaction velocity

with glucose concentration rules out a significant contribution by a high- K_m liver-type enzyme in early or late placenta. The apparent K_m for glucose of about 10^{-5}M in both cases is about one-tenth of that reported for human term placenta by Chieffi & Mangiarotti (1963). Guinea-pig placenta contains a hexokinase having a K_m similar in magnitude to what we have found in human placenta (Walker, 1962).

In an attempt to elucidate the mechanism of glucogenesis by early placenta we have been studying placental phosphohydrolases to see whether a specific hepatic-like glucose 6-phosphatase is present at any time during the gestational period. These studies, which are still being pursued, have failed to uncover such an enzyme. More important, however, we have found that the rate of hydrolysis of glucose 6-phosphate by placental homogenates is about the same or lower in organs taken very early in pregnancy compared with those delivered spontaneously at term. Thus the notion that the human placenta acts as a foetal liver up to about 15 weeks (Haworth, 1965; Hagerman, 1964; Villee, 1953) seemed to be somewhat doubtful, since the early tissue does not possess an added capacity for glucose 6-phosphate hydrolysis relative to the term organ. This reasoning led us to re-investigate previous reports of glucose synthesis from pyruvate by early placenta (Villee, 1953), and incidentally the production of fructose by term placenta (Hagerman & Villee, 1952). Our results do indeed verify the fact that small amounts of glucose, perhaps 0.1–0.2 $\mu\text{mole/g./hr.}$, arise from exogenous labelled pyruvate in early placenta. However, although Villee and co-workers have been unable to demonstrate this reaction in term placenta (Villee, 1953; Roux & Villee, 1960), we have observed both glucogenesis and fructogenesis from pyruvate regardless of the gestational age of the tissue. This finding is therefore inconsistent with the concept of a regulatory function of early placenta on the foetal blood sugar that is gradually lost as the organ matures. Admittedly, the fact that human placenta does produce small quantities

of glucose and fructose raises certain questions that must be answered as to mechanism as well as the significance of these reactions in the overall metabolic interplay between mother and fetus. It has been suggested (Hagerman, Roux & Villee, 1959) that fructose arises from the action of a non-specific phosphatase on fructose 6-phosphate, since these workers could find no evidence of a specific placental fructose 6-phosphatase. Since the route from fructose to glucose through sorbitol is not available owing to the absence of aldose reductase (Hagerman *et al.* 1959; Hers, 1960), one might postulate a similar mechanism of glucose production involving one of the non-specific placental phosphohydrolases.

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