OXIDATION OF DL-3-PHENYLALANINE-1-^{14}C, DL-LEUCINE-1-^{14}C, AND D-GLUCOSE-1-^{14}C-6-PHOSPHATE TO $^{14}CO_2$ IN HUMAN PLACENTA

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Despite the availability of considerable biochemical information concerning the functions of phenylalanine hydroxylase, α-ketoacid oxidases, and glucose-6-phosphate dehydrogenase, the effective early diagnosis of hyperphenylalaninemia, maple syrup urine disease, and glucose-6-phosphate dehydrogenase deficiency in newborn infants awaits the development of simple and rapid techniques for screening populations for the metabolic defects attendant to these enzyme deficiency states. However, with the available biochemical methods for the determination of enzyme activities in isolated tissues, traumatic lesions may result for the newborn when liver biopsies (*1*) or large blood samples (*2*) are taken. Such difficulties require that a "noninvasive" method be used, and since these diseases are genetic disorders, it is obvious that enzyme activities in the newborn tissues will be present on the fetal side of the placenta. The present work is directed towards development of radioisotope techniques similar to those developed for early diagnosis of beri-beri (*3*), vitamin B_6 deficiency (*4*), and folic acid deficiency (*5*) which have been demonstrated recently.

The oxidation of the #1-carbon of phenylalanine, leucine, and glucose-6-phosphate to CO_2 depends upon the presence of phenylalanine hydroxylase, α-keto acid oxidases, and glucose-6-phosphate dehydrogenase, respectively. This apparent dependency suggests that systematic measurements of $^{14}CO_2$ production from phenylalanine-1-^{14}C, leucine-1-^{14}C, and glucose-1-^{14}C-6-phosphate incubated with human placental homogenates might be useful in the detection of hyperphenylalaninemia, maple syrup urine disease, and glucose-6-phosphate dehydrogenase deficiency in newborn infants without radiation danger.

In the present study, $^{14}CO_2$ production from DL-3-phenylalanine-1-^{14}C, DL-leucine-1-^{14}C, and D-glucose-1-^{14}C-6-phosphate incubated with fresh and boiled placenta was measured instantaneously and continuously using an in vitro ionization chamber method.

METHODS AND MATERIALS

Preparation of human placental homogenates. Human placental tissues obtained 1–5 hr after delivery were frozen in ice immediately. The placenta can be maintained, following delivery, by liquid nitrogen freezing for several weeks in which form the tissue may be transported from the delivery room to a distant laboratory. In each study, 3 gm of tissue were obtained from the fetal side of the placenta and washed in cold saline. The tissues were then homogenized in a "stir-R" (Tri-R Institute, Rockville Centre, N.Y.) in 30 ml of cold Gey's balanced salt solution (each 100 ml contains 700 mg NaCl, 37 mg KCl, 15 mg $Na_2H\ PO_4 \cdot H_2O$, 3 mg KH_2PO_4, 21 $MgCl_2 \cdot H_2O$, 17 mg $CaCl_2$, 100 mg glucose, and 227 mg $NaHCO_3$; Grand Island Biological Co., Grand Island, N.Y.), pH 7.55, for 20 min.

$^{14}CO_2$ production study. Details of the in vitro ionization chamber method have been described previously (*5,6*). Fresh or boiled human placental homogenates in Gey's balanced salt solution were incubated at 37°C in the presence of 10 μCi DL-3-phenylalanine-1-^{14}C (S.A.: 48.0 mCi/m*M*, Amersham/Searle), 10 μCi DL-leucine-1-^{14}C (S.A.: 55.2 mCi/m*M*, Amersham/Searle), or 1 μCi D-glucose-1-^{14}C-6-phosphate (S.A.: 2.9 mCi/m*M*, Amersham/Searle). Compressed gas with 95% O_2, 5% CO_2 was passed through the incubation chamber at a constant flow rate (100 ml/min). Continuous graphing plotting of the $^{14}CO_2$ data during 120 min was achieved by a chart recorder.

For comparison of various $^{14}CO_2$ curves, we determined T_{max} and the total fraction of the administered ^{14}C appearing as $^{14}CO_2$ within the first 120 min after incubation with a ^{14}C biochemical. These parameters have been defined previously (*5,7*).

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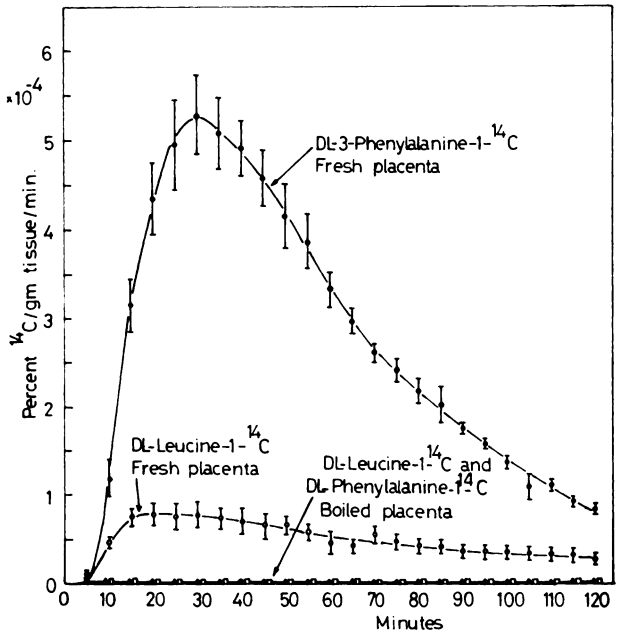

FIG. 1. Represents composite data of rates of $^{14}CO_2$ production from DL-3-phenylalanine-1-^{14}C and DL-leucine-1-^{14}C incubated with fresh or boiled human placenta. Ordinate represents percent of incubated ^{14}C produced as $^{14}CO_2$ per gm tissue per min, and abscissa represents time in minutes after administration of ^{14}C-labeled materials. Each point represents mean of $^{14}CO_2$ production of each group of four experiments at given time, and length of vertical bar through each point represents ±1 standard error of mean.

RESULTS

Figure 1 represents composite data showing the rates of $^{14}CO_2$ production from DL-3-phenylalanine-1-^{14}C and DL-leucine-1-^{14}C incubated with fresh and boiled human placental homogenates, respectively. It is clear that there is no $^{14}CO_2$ production from ^{14}C-labeled materials incubated with boiled placenta.

Figure 2 represents composite data showing the rates of $^{14}CO_2$ production from D-glucose-1-^{14}C-6-phosphate incubated with or without $10^{-2}M$ methylene blue and boiled or fresh placental homogenates. It can be seen that there was a qualitative difference between composite data of $^{14}CO_2$ production curves obtained from fresh tissues with methylene blue and those obtained from fresh tissues without methylene blue. No $^{14}CO_2$ production curves were achieved in boiled tissues.

Table 1 summarizes T_{max} and cumulative percent ^{14}C appearing as $^{14}CO_2$ in Figs. 1 and 2. T_{max} were 31.7, 30.8, and 30.7 min in curves obtained from ^{14}C-labeled phenylalanine, leucine, and glucose-6-phosphate, respectively, in fresh placental tissues. No $^{14}CO_2$ production was detected in boiled placenta. A significantly increased $^{14}CO_2$ production ($p < 0.01$) and a prolonged T_{max} ($p < 0.01$) from D-glucose-1-^{14}C-6-phosphate were noted when incubated with $10^{-2}M$ methylene blue.

DISCUSSION

It is known that the oxidation of carboxyl-labeled phenylalanine could arise by decarboxylation either directly or after its oxidation to 3,4-dihydroxyphenylalanine. This carbon atom from the carboxyl group of phenylalanine could be lost via phenylpyruvic acid, and through the catabolism of homogentisic acid. The major route of phenylalanine metabolism is its hydroxylation to tyrosine. The enzyme responsible for this conversion, phenylalanine hydroxylase, is a mixed function oxidase requiring molecular oxygen and a tetrahydropterine cofactor. Approximately 31.7 min of T_{max} and 31.6% of the ^{14}C administered as DL-phenylalanine-1-^{14}C were obtained in a milligram of fresh placenta gassed with O_2 during the first 120 min. No $^{14}CO_2$ production was detected in boiled placenta. This result suggests the presence of phenylalanine hydroxylase activity in human placenta. The fact that the activity of this enzyme can be determined quantitatively by measuring $^{14}CO_2$ producion from ^{14}C-labeled phenylalanine suggests the possible application of this method for systematic and early detection of hyperphenylalaninemia in newborn infants. An improved method using an ionization chamber and DL-phenylalanine-^{14}C with or without a load of administered unlabeled phenylalanine has been used previously to differentiate phenylketonuric heterozygotes and homozygotes and to estimate phenylalanine hydroxylase activity in vivo (*8*).

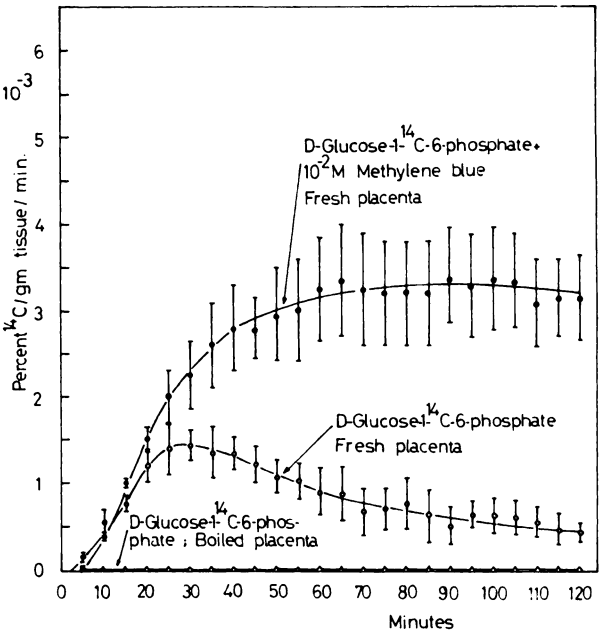

FIG. 2. Represents composite data of rates of $^{14}CO_2$ production from D-glucose-1-^{14}C-6-phosphate incubated with fresh or boiled human placenta with or with $10^{-2}M$ methylene blue. See details in Fig. 1.

TABLE 1. T_{max} AND CUMULATIVE $^{14}CO_2$ PRODUCTION/mg TISSUE DURING INITIAL 120 MIN FROM DL-3-PHENYLALANINE-1-^{14}C, DL-LEUCINE-1-^{14}C, AND D-GLUCOSE-1-^{14}C-6-PHOSPHATE INCUBATED WITH BOILED OR FRESH HUMAN PLACENTA

Category	No. of experiments	T_{max} (min ± s.e.)	Cumulative percent ^{14}C/mg, tissue during 120 min $10^2 \times$ (% ± s.e.)
DL-leucine-1-^{14}C			
Fresh placenta	4	30.875 ± 2.918	0.06065 ± 0.01163
Boiled placenta	4	—	0
DL-phenylalanine-1-^{14}C			
Fresh placenta	4	31.375 ± 1.143	0.31620 ± 0.01648
Boiled placenta	4	—	0
D-glucose-1-^{14}C-6-phosphate			
Fresh placenta	4	30.750 ± 1.108	1.00314 ± 0.25342
Fresh placenta + $10^{-2}M$ methylene blue	4	89.000 ± 4.041 ($p < 0.01$)	3.10167 ± 0.09509 ($p < 0.01$)
Boiled placenta	4	—	0

The catabolism of leucine is of importance in relation to the genetically determined metabolic disorder "maple syrup disease". Leucine is degraded to isovaleryl-CoA and CO_2 in the presence of α-keto-acid oxidases. Approximately 30.8 min of T_{max} and 6% of the ^{14}C administered as DL-leucine-1-^{14}C were measured in a milligram of fresh tissue homogenate during the first 120 min. No $^{14}CO_2$ production was detected in boiled placenta. This indicates the presence of the enzymes involved in the oxidation of leucine to CO_2 in human placenta. The fact that the activity of these enzymes can be estimated quantitatively by measuring continuously $^{14}CO_2$ production from ^{14}C-labeled leucine in human placenta suggests the use of this method for systematic detection of maple syrup urine disease at birth. It is noted that in an assay measuring liberated $^{14}CO_2$ from ^{14}C-labeled leucine and ketoisocaproic acid incubated with human leukocytes or whole blood was shown recently (*2*). However, the range of the results among the normal subjects was so wide that success with this assay in defective heterozygotes appears highly unlikely (*2*).

A deficiency of glucose-6-phosphate dehydrogenase has been implicated in the pathogenesis of some hereditary hemolytic anemias (*9*). The oxidation of carboxyl-labeled glucose-6-phosphate could arise through glucono-δ-lactone-6-phosphate and 6-phosphogluconic acid in the presence of glucose-6-phosphate dehydrogenase. Approximately 30.7 min of T_{max} and 100% of the ^{14}C administered as D-glucose-1-^{14}C-6-phosphate were obtained in a milligram of fresh placenta during the first 120 min. No $^{14}CO_2$ production was obtained in boiled placenta. This result indicates the presence of the enzyme in the placenta. A significant increase in $^{14}CO_2$ production from this ^{14}C-labeled glucose-6-phosphate in fresh placental homogenates incubated with $10^{-2}M$ methylene blue as compared with the value obtained from the tissue without methylene blue suggests that this dye stimulates the pentose phosphate pathway activity in the placenta. Similar results with glucose-1-^{14}C in human erythrocytes have been demonstrated recently (*6*). Our data suggest that studies of oxidative catabolism of D-glucose-1-^{14}C-6-phosphate in the placenta should be a useful tool for early detection of glucose-6-phosphate dehydrogenase activity in man at birth.

Our overall results suggest further that continuous measurement of $^{14}CO_2$ production from ^{14}C-labeled substrates by human placenta using an in vitro ionization chamber method should be a possible means for systematic detection of normal homozygotes, as well as heterozygotes and atypical homozygotes, of several metabolic disorders in familial studies, without any radiation danger.

SUMMARY

An ionization chamber method was used for instantaneous and continuous measurement of $^{14}CO_2$ production from DL-3-phenylalanine-1-^{14}C, DL-leucine-1-^{14}C, and D-glucose-1-^{14}C-6-phosphate in fresh human placenta. The results obtained demonstrated the presence of phenylalanine hydroxylase, α-ketoacid oxidases, and glucose-6-phosphate dehydrogenase involved in the oxidation of these labeled substrates in the placenta, respectively. This study offers an in vitro method for early detection of normal homozygotes, heterozygotes, and atypical homozygotes of hyperphenylalaninemia, maple syrup disease, and glucose-6-phosphate dehydrogenase deficiency in the newborn infants without any radiation danger.

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