Rates of protein synthesis and turnover in fetal life

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In 1939 Schoenheimer et al. (11) studied the fate of labeled amino acids injected in the rat and for the first time demonstrated that there is both a rapid synthesis and degradation of body proteins. Subsequent investigators have compared rates of total body protein turnover in several species and at different ages. These studies have shown a relatively higher rate of protein turnover in small mammals (5). Within a given species, protein turnover appears to be related to the age of the organism. For example, Young et al. (16) have measured total-body protein turnover in premature newborns, infants, young adults, and the elderly. Rates ranged from 17.4 ± 7.9 g·kg⁻¹·day⁻¹ for the premature newborn to 1.9 ± 0.2 g·kg⁻¹·day⁻¹ for the elderly.

The inverse relationship of protein turnover to body size and age raises the question of whether rapid protein turnover is an important component of fetal metabolism. To investigate this question, we have studied the rate of incorporation of L-[U-¹⁴C]lysine in the body proteins of eight ovine fetuses ranging from 110 to 145 days gestation. Lysine was selected because it is an essential amino acid abundant in body proteins. Furthermore, after deamination, its carbon skeleton is not reutilized for lysine resynthesis (15).

MATERIALS AND METHODS

Eight mixed-breed Western ewes with gestational ages ranging from 110 to 145 days were studied. Catheters were placed in the fetal abdominal aorta, the fetal inferior vena cava, the umbilical vein, and the maternal femoral artery by surgical techniques previously described (7, 12). The catheters were exteriorized through a subcutaneous tunnel to a plastic pouch fixed on the ewe's flank. With one exception (animal 3), all animals were standing and eating within 12-24 h after surgery. Catheters were irrigated daily with normal saline containing 30 U heparin/ml. Following surgery, the ewes were maintained on an ad libitum diet of hay, dehydrated alfalfa pellets, barley, oats, corn, and water. Experiments were performed on postsurgical day 4 or 5. By means of a calibrated infusion pump, L-[U-¹⁴C]lysine was infused at a constant rate into the fetal inferior vena cava for a period of 9-13 h. The amount of radioactivity infused was approximately 10⁶ dpm·min⁻¹·kg fetal body wt⁻¹. At various times during the infusion blood samples were withdrawn from the fetal aorta. In six of the eight animals, umbilical blood flow was measured during part of the infusion period with the antipyrine steady-state diffusion technique (8). Simultaneous samples of umbilical venous and fetal arterial blood were withdrawn in these animals for estimating net umbilical lysine uptake (fetuses 1, 2, 6, and 7) and ¹⁴CO₂ excretion rate (fetuses 5, 6, and 8) by means of the Fick principle.

At the conclusion of the infusion, the ewe was killed and the uterus was removed. The fetus, uterus, placenta, and fetal membranes were weighed and homogenized separately. Portions of each homogenate (2-3 mg) were weighed and solubilized in TS-1 tissue solubilizer (Radiation Products, Elk Grove Village, IL) for 24-36 h at 50°C. At the same time, weighed portions of the homogenized fetal carcass were transferred to hydrolysis flasks for complete acid hydrolysis. Acid hydrolysis was performed with azeotropic HCl at 110°C for 24-36 h. Additional portions of the fetal carcass were analyzed for nitrogen content (Huffman Laboratories, Wheat Ridge, CO). Among fetuses, the nitrogen content per dry weight of...
the fetal carcass was virtually constant and equal to 10.48% ± 0.13 SE.

The concentration of [14C]lysine in whole plasma and fetal carcass homogenate was assayed enzymatically using L-lysine decarboxylase type II (EC 4.1.1.19) (Sigma Chemical, St. Louis, MO). This enzyme has good substrate specificity and is catalytically inactive against D-lysine, O-(2-aminoethyl)-DL-serine, L-ornithine, and L-α,γ-diamino-n-butyric acid (13). In addition, the acetyl, ε-acetyl, or ε-carbobenzoxy derivatives of L-lysine are reported not to be substrates for this enzyme (13). Because the sheep has a relative abundance of ε-N-methyllysine, we have documented that this analogue is also not an inhibitor of the enzyme. The use of an enzymatic procedure was made necessary by the finding that a group of proteins that are rapidly labeled by [14C]lysine remained in the supernatant after deproteinization. Following precipitation of the plasma proteins with 10% sulfosalicylic acid, two radioactive fractions could be identified in the supernatant using a Sephadex G-75 column (Fig. 1). The first fraction with low optical density at 230 nm but high radioactivity appeared with the void volume of the column. This first peak contained several acid-soluble proteins or protein aggregates because analysis by acrylamide-gel electrophoresis demonstrated three major protein bands. The second peak was eluted with the salt fraction of the column and contained free lysine. The enzymatic assay of [14C]lysine was carried out in specially constructed diffusion vessels. Optimum conditions for decarboxylation were used as specified by Soda and Moriguchi (13). Typically, 0.2 ml of whole plasma was added to 0.5 ml of 0.1 M sodium acetate buffer (pH 5.8) in the reaction vessel. An initial 30-min incubation at 37°C in the uncapped diffusion vessel was shown to release the 14CO2 originally present in the sample. Following this, the vessels were capped and L-lysine decarboxylase was added through the needle orifice. A further incubation at 37°C for 60 min was shown to be sufficient for complete decarboxylation of free lysine in the plasma. The KOH trap was then removed and placed immediately into PCS scintillation fluid. Standards of L-[U-14C]lysine were used to test complete decarboxylation in each experimental group.

Lysine concentrations in plasma, whole blood, and carcass hydrolysate were measured with a JEOL 6AH amino acid analyzer using an internal standard of norleucine to correct for dilution and recovery errors (7). The diffusion vessels were also used for measuring 14CO2 in umbilical venous and fetal arterial blood. Blood samples of approximately 0.5 ml were withdrawn into heparinized glass syringes and incubated in the capped vessels for 24 h at 50°C in the presence of 0.5 M KOH. Measurements of radioactivity were performed in a liquid-scintillation counter. The method of channels ratio for quench correction was used in the analysis of the KOH solutions. Carcass radioactivity was corrected for quenching by the method of serial dilutions.

RESULTS

At the end of infusions lasting from 560 to 775 min, recoveries of the infused radioactivity in the fetal body ranged from 47.1 to 69.1%. The mean values for the distribution of 14C throughout the fetal and maternal compartments are illustrated in Fig. 2. An average of 61% remained in the fetus, 3% was located in the fetal amniotic and allantoic fluids and membranes, 10% was located in the placental cotyledons, approximately 9% could be accounted for by fetal excretion of 14CO2, and the remaining 17% was assumed to have been transferred to the maternal compartment either as lysine or as a metabolic product other than CO2. Virtually all the radioactivity recovered in the fetal carcass after acid hydrolysis was [14C]lysine and of this, greater than 90% was in the bound form.

In a single animal, at the completion of the infusion, the fetus was delivered rapidly and biopsies were taken from muscle, liver, and kidney and promptly frozen in liquid nitrogen. The total time from termination of infusion to freezing the last biopsy sample was less than 5 min. The frozen tissues were homogenized in cold 6%
perchloric acid and triplicate aliquots analyzed for total radioactivity and for free [¹⁴C]lysine using the lysine decarboxylase method. The results for the three tissue sites are presented in Table 1.

Preliminary experiments (Fig. 3) showed that whole plasma radioactivity increased throughout the infusion, whereas the concentration of free radioactive lysine reached a plateau at approximately 2 h. On the basis of this information, the mean free [¹⁴C]lysine in plasma samples collected 2 or more hours after the infusion was used to calculate the specific activity of free lysine in the plateau period (Fₗ₈ₓₘ₃). In all but one fetus, the concentration of free radioactive lysine in the sampling period varied within ±10% of the mean and did not show a systematic trend to change with time. In fetus 7 the variability was ±20%. At the end of the experiment, the specific activity of lysine in the protein of the whole-body homogenate (Pₗₐₐ) was 2.2 to 8.9% of the steady-state specific activity of free plasma lysine (Fₗ₃ₘ₃) (see Table 1). In each fetus, the Pₗₐₐ/Fₗ₃ₘ₃ ratio was used to calculate an approximate fractional rate constant, Kₛ, for the unidirectional flux of lysine into fetal protein synthesis, according to the equation

\[ Kₛ = -t^{-1} \ln \left[ 1 - \left( \frac{Pₗₐₐ + Fₗ₃ₘ₃}{Fₗ₃ₘ₃} \right) \right] \]  (1)

where t is the duration of the infusion.

Equation 1 is derived from the assumption that the flux is proportional to the Pₗₐₐ - Fₗ₃ₘ₃ difference and that the specific activity of lysine is equal to Fₗ₃ₘ₃ starting at time 0. Because the latter assumption is not exactly correct (see Fig. 3), a more precise calculation of the rate constant Kₛ was made, using Swick’s equation (17)

\[ \frac{Pₗₐₐ}{Fₗ₃ₘ₃} - 1 = \frac{(Kₛ \cdot e^{-Kₛt}) - (K_F \cdot e^{-K_Ft})}{K_F - Kₛ} \]  (2)

where Kₚ is the rate constant that defines the time course of Pₗₐₐ. Equation 2 was derived by Swick for the steady state in which rate of synthesis equals rate of degradation. However, the equation can be applied to a growing organism without substantial error (17). Each Kₛ was calculated by an iterative procedure using a Kₚ equal to 35 day⁻¹ (see Fig. 3) and the Kₛ value as the initial estimate. Note that the calculation of Kₛ does not require precise knowledge of Kₚ (17) because Kₚ ≫ Kₛ and that Kₛ is greater than Kₛ by only 6%.

Table 2 presents the Kₛ rate constants as well as the results of rates derived from Kₛ, namely the unidirectional flux of plasma lysine into proteins and the fetal rate of protein synthesis. The latter is a minimal rate because some of the nontracer lysine released by protein degradation is recycled intracellularly, reducing intracellular specific activity. Furthermore, the steady-state method underestimates the synthesis rate of a protein mixture in which some proteins have a short half-life in comparison to the duration of the experiment. Regression analysis of Kₛ versus fetal age (Fig. 4) shows that there is a significant negative correlation between Kₛ and age (r = 0.879, P < .01). Likewise, the two rates derived from Kₛ, namely the unidirectional flux of plasma lysine into proteins (mmol·day⁻¹·kg⁻¹) and the rate of protein synthesis (g·day⁻¹) are inversely related to age.

In three fetuses, whole blood measurements of [¹⁴CO₂] showed that the concentration of [¹⁴CO₂] in the arterial blood of the fetus was higher than in the umbilical vein and that both concentrations were higher than in the maternal blood, thus demonstrating production and excretion of [¹⁴CO₂] by the fetus.

In four animals, the net fetal uptake of lysine via the umbilical circulation was calculated as the product of the umbilical venous-arterial difference of whole blood lysine times the umbilical blood flow. The uptake (mmol·kg⁻¹·day⁻¹) ranged from 3.9 and 4.1 in fetuses 1 and 2 to 2.2 and 3.4 in fetuses 6 and 7. The mean umbilical blood flow was 226 ± 27 ml·kg⁻¹·min⁻¹.

**DISCUSSION**

Accurate knowledge of protein accretion in the fetus and of the concentration of amino acids in fetal proteins is essential to the study of two important aspects of fetal amino acid metabolism, namely a comparison of fetal amino acid utilization in net protein synthesis with other synthetic and catabolic pathways and the measurement of protein turnover rate. In the case of an essential amino acid such as lysine, the net rate of accumulation due to growth (Lᵧₛₑₓₒ) plus the rate of metabolism by routes other than protein synthesis (Lᵧₛₑₓₒ) must be equal to the exogenous entry rate via the placenta (Lᵧₑₓₒ)

\[ Lᵧₑₓₒ = Lᵧₛₑₓₒ + Lᵧₛₑₓₒ \]  (3)
FETAL PROTEIN SYNTHESIS AND TURNOVER

Lemons et al. (7) estimated the exogenous lysine entry rate into the fetus to be 2.2 mmol·kg⁻¹·day⁻¹ in a group of sheep with average fetal age 130 days. In the present study, four fetuses with average age 125 days had a mean exogenous lysine entry rate of 3.4 mmol·kg⁻¹·day⁻¹. Differences in fetal age and nutrition are likely to influence the $K_{\text{ex}}$ value, but there is no adequate information on this point. The fetal lysine requirements due to growth ($L_{\text{ysgrow}}$) can be estimated from a fetal growth curve (6) and measurements of total lysine in the fetal body (Table 2). The estimated $L_{\text{ysgrow}}$ at 125 days is 1.8 mmol·kg⁻¹·day⁻¹, i.e., less than the measured rate of entry of exogenous lysine. This implies fetal metabolism of lysine in addition to that used for protein synthesis. The present finding that a fetal lamb infused with $L-\left[U-{ }^{14}C\right]$lysine excretes $^{14}C$O₂ via the placenta demonstrates an active catabolic pathway of lysine in the ovine fetus.

Net utilization rates of an essential amino acid by growth and catabolism are the most important considerations in establishing the fetal nutritional needs for that amino acid. However, from the point of view of the energy cost of fetal development, it is important to recognize that growth as reflected in net protein accretion is the resultant of two opposite rates, i.e., synthesis and degradation of tissue proteins. In the presence of a fast rate of protein degradation even a zero growth rate would require that a large amount of energy be spent in protein synthesis. Hence, there is considerable physiological significance to comparisons of fetal growth rates with fetal rates of protein synthesis.

The results of our investigation demonstrate that the infusion of $L-\left[U-{ }^{14}C\right]$lysine into a fetal vein produces a steady concentration of free radioactive lysine in fetal arterial plasma, during the 2- to 13-h period of the infusion. The simplest use of this information would be to calculate a plasma lysine turnover rate ($R$) from the infuson rate of the labeled lysine ($I$) and the specific activity of free plasma lysine at steady state ($F_{\text{max}}$), according to the equation

$$R = I + F_{\text{max}}$$

This approach, which has been used extensively in whole-body amino acid turnover studies (5, 14), would have the advantage that no analysis of radioactivity in the fetal carcass is required. However, the application of Eq. 4 to the fetal infusion of a labeled substance cannot provide a reliable estimate of its utilization by the fetus because a potentially large and variable fraction of the infused tracer may escape into the placenta and the mother. In the case of lysine this fraction is approximately 27% (see Fig. 2). Therefore we adopted a more elaborate procedure in which the specific activity of lysine was measured in both fetal plasma and carcass. This procedure allowed us to calculate the rate of incorporation of plasma lysine into fetal proteins. Given the fact that body proteins have widely different turnover rates and that there is intracellular recycling of amino acids, the calculated rate is a minimum estimate of the actual size of incorporation of lysine into proteins. Despite this limitation, the results warrant two interesting conclusions. The first is that a large fraction of protein synthesis in the fetal lamb is devoted to protein turnover, i.e., resynthesis of tissue proteins that undergo continuous degradation. This conclusion is based on a comparison of the measured fractional rate of protein synthesis, $K_{\text{s}}$, with the fractional rate of protein accretion by growth, $K_{\text{G}}$ (Fig. 4). The growth curve of fetal lambs has been studied by several investigators. There is general agreement that the percent increment of fetal weight is approximately 4%/day at 110 days gestation and then declines toward term.
plotted against natural log of body weight. Rat and man data are from Refs. 9 and 16, respectively. Fetal sheep data are from Table 1.

It is apparent that the rate of protein synthesis is 2-4 times greater than the rate of protein accretion. The second conclusion is that the fractional rate of protein synthesis declines as the fetus grows (Fig. 4). A rapid decline in relation to body size has also been observed in extrauterine life, as demonstrated by comparing the data obtained in fetal sheep with data for postnatal growth in rat (9) and man (16) (Fig. 5). Protein turnover varies in inverse relation to body weight, both within and among species. Differences in whole-body turnover rate between small and large adult mammals are related in part to differences in the relative size of the major organs (5, 10) and in part to the fact that some organs of the small animal synthesize and degrade proteins more rapidly (5). The physiological meaning of the latter finding is obscure. It should be emphasized that there is no a priori reason that the same relationship between rate of protein synthesis and body size found among adult mammals should apply also to fetal and postnatal growth. Conde and Scornik (4) have reported that the rate of protein synthesis in livers of 4-day-old mice is greater than in the adult, but Arnal et al. (2) have demonstrated in sheep that the liver and small intestine increase their fractional rates of protein synthesis as lambs increase in size. On the other hand, Arnal (1) has found the same inverse relationship between rate of protein synthesis and age reported here for the fetus in a study of skeletal muscle growth during postnatal life. Similar studies that relate rates of protein synthesis in individual organs to age and size should be carried out in the fetus.

An approximate energy cost of fetal protein synthesis can be estimated from the present data and the knowledge that the synthesis of 1 g of protein requires the expenditure of about 0.8 kcal (9). According to this estimate the average cost of protein synthesis in the 110-145 days gestational period is 11 kcal·kg⁻¹·day⁻¹, which is approximately 20% of the calories produced by fetal oxidative metabolism (3). Similar results have been obtained in extrauterine life. By comparing the rates of irreversible loss of tyrosine in rats, pigs, and man, Garlick et al. (5) have concluded that the energy cost of protein synthesis is about 17% of the metabolic rate of the whole animal.

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