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The Rate of Amino Acid Nitrogen and Total Nitrogen Accumulation in the Fetal Lamb[1] (41198)

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Abstract. Total body water and nitrogen concentrations were determined for whole body homogenates of 18 fetal lambs. Nitrogen concentration averaged 10.5 ± 0.1 mg nitrogen/100 mg dry tissue. Amino acid represented approximately 82% of the total nitrogen and 55% of the total carbon content of the fetus. The carbon/nitrogen ratio attributable to amino acids decreased from 3.42 at 66 days to 3.06 at term. Total amino acid concentrations in the whole body homogenates were also determined for 20 amino acids. Cystine and hydroxyproline concentrations increased significantly (correl. coeff. = 0.78) and markedly with gestation. The branch chain amino acids, leucine, isoleucine, and valine, along with phenylalanine, showed a significant decline in concentration (correl. coeff. ≅ −0.79). From these rates of amino acid and nitrogen accretion the adequacy of the exogenous supply of amino acids and nitrogen to the fetus via the umbilical uptake can be assessed. Calculations of the excess supply of amino acids to the fetus above their rates of accretion are presented for the fetal lamb during late gestation.

In a growing organism the intake of calories, carbon and nitrogen required by the organism can be divided into two components: the requirements for maintenance of the organism and those required for accretion of new tissue. The latter component will vary depending upon the growth rate and the chemical composition of the new tissue. For amino acids there will be a quantity required for accretion of new protein with the excess used for catabolism and urea production. During fetal life these requirements for amino acids must be met by the umbilical uptake which represents the exogenous supply of nutrients to the fetus. Thus far, the umbilical uptake of amino acids has been measured in only one mammalian species, the fetal lamb (1). In that study we found marked differences in the quantities of individual amino acids delivered to the fetus; there was no umbilical uptake of the acidic amino acids, glutamate and aspartate, whereas the umbilical uptake of the neutral and basic amino acids was significant. The present study was carried out in order to compare the quantities required for accretion with the quantities provided to the fetus by the placenta. The study includes measurements of body water, total nitrogen, and individual amino acids in whole body homogenates of the fetal lamb at different stages of gestation. From these data the accretion rates of amino acid nitrogen and non-amino acid nitrogen in the fetus can be calculated.

Materials and Methods. Eighteen fetal sheep ranging between 66 and 145 days gestation were obtained for study by caesarian section. Following delivery, each fetus received an overdose of barbiturates and was weighed. The entire fetal carcass was ground in a large capacity industrial grinder, and then homogenized using a standard laboratory size Waring blender. Multiple 1- to 2-g samples of the homogenate were weighed and placed in hydrolysis flasks. Additional samples were taken for determination of body water. A third sampling of carcass homogenates was used for determination of nitrogen content.

Hydrolysis of whole body homogenates was performed with 6 *N* hydrochloric acid

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containing 20 μl of phenol. The details of the technique used were similar to those previously described (2). The samples were hydrolyzed under vacuum in nitrogen at 108–110° for 24 hr and then dried. Longer hydrolysis times were found to increase the loss of serine and threonine with little change in valine and isoleucine concentration. Under these conditions all of the glutamine was deaminated to glutamate and asparagine to aspartate. Thus, the glutamate and aspartate concentrations herein reported are the sum of glutamate + glutamine and aspartate + asparagine, respectively. After hydrolysis, the amino acids were dissolved in buffer at pH 2.2 and analyzed on a Jeol 6AH amino acid analyzer by techniques previously described (1).

Percent body water was calculated as the ratio of original tissue weight minus dry tissue weight to original tissue weight after dehydration of the original sample to a constant weight. Nitrogen content was determined in 12 fetuses by an independent laboratory (Hoffman Laboratories, Inc.) with both internal and external controls to insure accuracy and reproducibility of the determinations.

Results. For purposes of comparison the data obtained on the 18 fetuses were divided into three gestational age categories: Group I, eight animals from 66 to 76 days; Group II, six animals from 106 to 125 days; Group III, four animals from 135 to 145 days. Table I presents the body composition of the 18 fetuses studied in terms of their wet weights, dry weights, weight of nitrogen and amino acid nitrogen for the fetuses. The percentage of body water decreased from 89.5 ± 0.1 in Group I to 81.2 ± 0.2 in Group III ($P < 0.001$). There were no significant changes in nitrogen concentration with gestational age, the mean for all groups was 10.5 ± 0.1 g nitrogen/100 g dry weight. The amino acid nitrogen represented by the 20 amino acids analyzed constituted 82% of the total nitrogen.

TABLE I. THE DATA FOR THE 18 FETUSES ARE PRESENTED, SUBDIVIDED BY GESTATIONAL AGE GROUPS

Animal No.	Gest. age (d)	Wet weight (g)	Dry weight (g)	Nitrogen weight (g)	Amino acid nitrogen (g)	Carbon/ amino acid nitrogen ratio
			66–76 days			
1	66	62.5	6.6	0.64	0.50	3.42
2	66	66.0	6.8	0.71	0.59	3.22
3	66	68.0	6.7	0.68	0.55	3.21
4	70	112	12.0	1.28	1.13	3.27
5	70	200	21.0	2.32	1.96	3.25
6	70	210	22.0	2.37	1.95	3.26
7	76	235	25.0	2.62	2.36	3.21
8	76	212	23.6	2.47	2.26	3.20
			106–120 days			
9	106	1410	254	26.6	20.7	3.17
10	108	1725	267	28.0	20.2	3.10
11	108	1885	288	30.2	22.7	3.11
12	115	1550	301	32.7	26.8	3.17
13	115	1820	322	32.7	25.9	3.08
14	120	2970	508	52.6	48.2	3.08
			135–145 days			
15	135	4770	968	102.2	84.6	3.18
16	140	5190	991	109.1	83.4	3.09
17	140	5450	970	101.6	79.5	3.06
18	145	5550	988	103.5	84.6	3.09

Note. The total amino acid nitrogen was calculated from the concentrations of the amino acids listed in Table II and their nitrogen content.

Figure 1A presents a semi-log plot of the wet and dry weights of the 18 fetuses used in this study versus gestational age. Similarly, Fig. 1B presents a semi-log plot of the total nitrogen and amino acid nitrogen versus gestational age.

Individual amino acid concentrations. Table II presents the amino acid concentrations for the individual amino acids given as the mean ± 1 SEM for each amino acid within the three gestational age groups. The concentrations are expressed as grams amino acids per 16 g nitrogen. For each amino acid a linear regression analysis was carried out of concentration versus gestational age to determine which amino acids were altered in their total concentration within the fetus as gestation progressed. Table III lists the amino acids in terms of increasing or decreasing concentration with gestation. The increase in concentration of cystine and hydroxyproline was particularly striking.

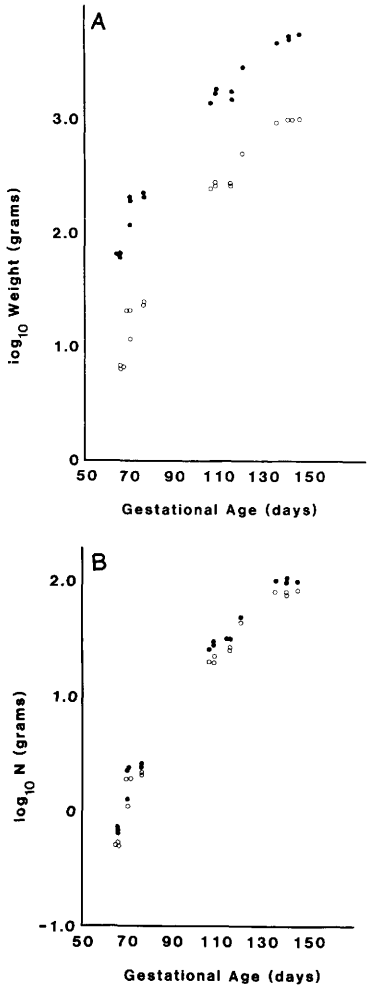

FIG. 1. (A) Presents the log of the body weight (solid circles) and dry weight (open circles) of the fetuses plotted against gestational age. (B) Presents total body nitrogen (solid circles) and amino acid nitrogen (open circles) expressed as the log versus gestational age for the fetuses studied.

Discussion. Determination of the accretion rate of substances in the mammalian fetus during gestation provides an important data base for determining requirements which must be met either by the placental supply of nutrients or by production within the fetus. There have been many studies in developmental biology which have described the accretion rate of nitrogen during *in utero* development and early postnatal development. Surprisingly few studies have described the accretion rate of individual amino acids during fetal or early neonatal life (3–5). To our knowledge this report presents the first description of the accretion rate of amino acids in the fetal lamb and provides the largest series of observations on amino acids concentrations in whole body homogenates. For many technical and historical reasons the nutrition and metabolism of the ovine fetus have been more thoroughly studied than in any other mammalian fetus. Specifically, the sheep is the only animal in which the umbilical uptake of amino acids has been measured (1). In the latter study an attempt was made to compare umbilical amino acid uptake with an accretion rate estimated from data reported by Blaxter on three fetal calves (3). Figure 2 now presents a comparison of the umbilical uptake with the accretion rate defined from the present study. Since there is a net excretion of glutamate from the fetal circulation of the placenta, this has been subtracted from the glutamine umbilical uptake. The accretion rates for glutamine/glutamate and for asparagine/aspartate are composites of both amino acids in each case as explained in the Methodology Section. Figure 3 illustrates the percentage of umbilical uptake ac-

TABLE II. AMINO ACID CONCENTRATIONS ± SEM IN THREE GESTATIONAL AGE GROUPS

Amino acid	66–76 days	106–120 days	130–145 days
Orn	0.14 ± .01	0.17 ± 0.03	0.12 ± 0.02
Lys	7.39 ± 0.27	6.43 ± 0.23	6.09 ± 0.02
His	2.12 ± 0.15	1.93 ± 0.12	1.95 ± 0.04
Arg	6.04 ± 0.15	6.10 ± 0.24	6.59 ± 0.11
Tau	0.43 ± 0.02	0.45 ± 0.03	0.36 ± 0.03
Asp	8.21 ± 0.25	7.21 ± 0.29	7.03 ± 0.30
Thr	4.65 ± 0.09	3.92 ± 0.12	4.00 ± 0.10
Ser	4.37 ± 0.17	4.09 ± 0.21	4.12 ± 0.22
Glu	13.72 ± 0.37	11.37 ± 0.50	11.42 ± 0.45
Gly	8.07 ± 0.30	9.18 ± 0.61	9.27 ± 0.44
Ala	6.83 ± 0.17	6.27 ± 0.28	6.18 ± 0.33
Val	5.42 ± 0.16	4.29 ± 0.12	4.33 ± 0.14
Cys	1.20 ± 0.05	1.39 ± 0.12	1.74 ± 0.13
Met	1.71 ± 0.10	1.36 ± 0.07	1.44 ± 0.13
Ileu	3.45 ± 0.09	2.72 ± 0.08	2.72 ± 0.14
Leu	8.03 ± 0.21	6.58 ± 0.17	6.51 ± 0.27
Tyr	3.47 ± 0.09	2.79 ± 0.10	2.99 ± 0.03
Phe	4.23 ± 0.08	3.45 ± 0.08	3.48 ± 0.25
Pro	8.15 ± 0.42	7.17 ± 1.06	6.99 ± 0.38
OH-Pro	1.83 ± 0.18	3.04 ± 0.37	3.30 ± 0.20
(*n*)	(8)	(6)	(4)

Concentrations expressed as g/16 g *N*.

counted for by net accretion in tissues. The basic amino acids, lysine and histidine, are delivered to the fetus in amounts which exceed accretion by only approximately 20%; similar high relative rates of accretion are found for glycine and asparagine/aspartate. In contrast the gluconeogenic amino acids alanine, serine, threonine, and glutamine/glutamate have accretion rates of approximately 35% of their rates of delivery. The other neutral amino acids are delivered also in great excess, accretion representing approximately 25 to 35% of their rates of delivery.

This report demonstrates that the 20 amino acids analyzed could account for approximately 82% of the total nitrogen in the fetal lamb. Presumably most of the remaining nitrogen represents nonprotein nitrogen in such pools as nucleic acids, porphyrins, etc. We cannot find in the literature similar estimates of the accretion rate of non-amino acid nitrogen during development. The usual method of expressing

TABLE III. AMINO ACID CONCENTRATION IN WHOLE BODY HOMOGENATES VERSUS GESTATIONAL AGE

Increased	No change	Decreased
Arginine*	Ornithine	Methionine*
Glycine*	Histidine	Aspartate–asparagine**
cysteine–cystine***	Taurine	Glutamate–glutamine**
hydroxyproline***	Serine	Tyrosine**
	Alanine	Lysine***
	Proline	Threonine***
		Valine***
		Isoleucine***
		Leucine***
		Phenylalanine***

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

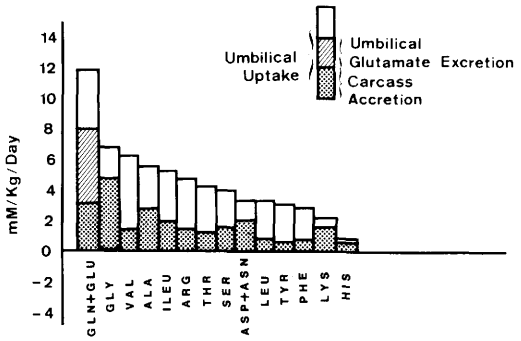

FIG. 2. The umbilical uptake of the individual amino acids expressed in μmole × kg × day is shown as the open bars taken from the study of Lemons *et al.* (1). The stippled bars represent the carcass accretion of the amino acids. For glutamine and glutamate, the open bar represents the glutamine uptake into the umbilical circulation, the cross-hatched bar represents the glutamate excretion from the umbilical circulation into the placenta and the stippled bar represents the accretion of glutamine plus glutamate in the fetal body. Thus, for all groups of amino acids, the open column represents the excess of delivery of amino acids to their rate of accretion within the fetus.

amino acid concentrations in tissues has been used in this report, namely, grams of amino acids/16 g nitrogen. This has been used in the past on the assumption that virtually all nitrogen in the tissues is within protein. This report demonstrates that an appreciable portion of total body nitrogen is non-amino acid nitrogen. Thus, it may be more appropriate to express amino acid concentrations/16 g of amino acid nitrogen or per kilogram dry fat-free weight. In the former case the values in Table II should be multiplied by 1.22. In the latter case at term when the fetal lamb has a fat concentration of 1.5 g/kg wet weight and is 81.2% water, the values in Table II should be multiplied by 7.12: ($10.48 \times 10 \times 0.92^{-1} \times 16^{-1}$) to yield grams amino acid per kilogram of fat-free dry weight.

Hydroxyproline increased markedly in concentration during gestation representing the fact that the amount of collagen in the fetus increased with age. Since hydroxyproline concentration in collagen is approximately 9.7 g/16 g nitrogen (10), collagen must represent approximately 19% of total body protein at 66–76 days and 34% at term. Collagen is also rich in glycine; thus, it is not surprising that the glycine concentration also increased with gestation. Cystine also increased markedly during gestation presumably reflecting the high sulfur concentration in wool protein.

The contribution of the 20 amino acids to total body carbon can also be calculated, at least for the gestation range between 135 and 145 days. Previously we had reported that carbon content of whole body homog-

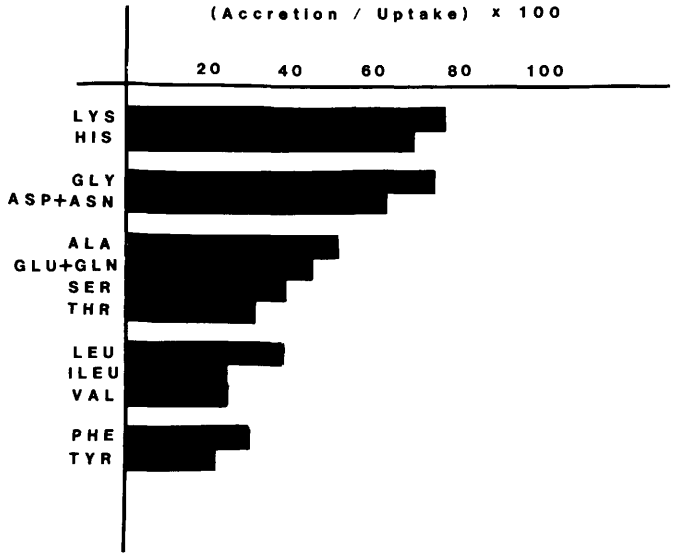

FIG. 3. Presents a comparison of accretion to umbilical uptake of amino acids. The accretion data taken from the present study and the umbilical uptake data from the previous studies (1).

enates of the fetal lamb near term (6). From these data and the several reports of the fat concentration of the fetal lamb (7–9) we have estimated that in the term fetuses amino acid carbon represented 55% of the total carbon content, fat 18% and nonfat, non-amino acid carbon 27%. It should be noted that there was a significant decrease in the composite carbon nitrogen ratio for the 20 amino acids during development decreasing from 3.42 at 66 days to 3.06 at term (Table I, last column). This change was due primarily to the decreasing concentration of amino acids with a high carbon/nitrogen ratio such as tyrosine and phenylalanine which have a carbon/nitrogen ratio of nine.

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