Urinary Free Amino Acid Excretions During Successive Pregnancies. (21023)

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(Introduced by I. M. Hoobler.)

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Investigators(1-9) have shown that there is little correlation between the quality or quantity of protein ingested and the amounts of amino acids excreted. Wallraff(10) reported that the free, rather than the total amino acids are excreted in significantly higher amounts during pregnancy. A study in this laboratory of 15 women during the reproductive cycle(11) disclosed that the excretion of specific free amino acids may be used as an index of physiological adaptation of the maternal body to the growth and development of the products of conception. In the investigation reported here, 24-hour collections of urine from one woman during 3 successive reproductive cycles were analyzed for free threonine, histidine, lysine, tryptophan, phenylalanine, isoleucine, valine, leucine, arginine, and methionine.

Experimental. The subject of study is a white American woman 5 feet, 7 inches tall and weighing approximately 150 lb, who was studied while she was 23 to 25 years old. Her blood type is B, Rh negative. Before pregnancy she had a basal metabolic rate of −23 and between the second and third pregnancies, the rate was +1. Frequent medical examinations before and throughout the study showed her to be in good physical health. For the subject, ranges of weight, blood pressure, hemoglobin, red and white blood counts during the 30-month study period are given in Table I. Estimated length of gestation and the infants' birth weights also are included. Carefully-checked dietary history and food intakes recorded for each 24-hour period in which urine samples were collected indicated that the subject's dietary habits were good. Eighteen 24-hour urine samples were collected for amino acid analysis. One sample was obtained when the woman was nulligravid; 8 samples were collected at approximately monthly intervals during her first pregnancy, the first in the second month (44 days after the last menstrual period), and a sample was procured during lactation, 47 days postpartum; 6 samples were obtained during the second reproductive cycle, one 6 months postpartum when the subject was not lactating; and during the third pregnancy, urine samples were collected in the second and fourth months. All samples were preserved with small amounts of glacial acetic acid stored at −20°C pending analysis. Microbiological methods reported previously(12) were used in determining the 10 free amino acids. The organisms used were *Lactobacillus plantarum* for leucine, isoleucine, and valine; *Leuconostoc mesenteroides* for histidine, lysine, methionine, and phenylalanine; and *Streptococcus faecalis* for arginine, threonine, and tryptophan. Total assay volume was 3 ml and growth responses were measured turbidimetrically. Amino acid values reported are averages of 2 or more assays in which determinations were run in duplicate at 5 levels. Duplicate assays which did not check with ±5% were repeated.

Results. The quantities of free amino acids excreted by the subject in the 24-hour collections of urine obtained during the 3 reproductive cycles are given in Table II.

Compared to nulligravid values for the subject, gravida I excretion of each of the 10 amino acids was augmented. Increases are evident from levels in the second month for all of the amino acids except methionine. Decreases in levels of excretion for each amino acid occurred in the fourth or fifth month except for threonine and methionine. From the sixth to the ninth months, excretion levels of most of the amino acids increased slightly or remained fairly constant. Excretion of threonine continued to increase as gestation progressed, while that of methionine fluctuated slightly from the nulligravid value.

TABLE I. Clinical Observations during Consecutive Pregnancies.

	1st pregnancy*	2nd pregnancy*	3rd pregnancy*
Wt, lb	150-173	149-165	149-157
Hemoglobin, g/100 ml	11.5 (7th mo)	14.2 (7th mo)	13.9
Red cell count	4.16(")	4.60(")	4.62
White cell count	8900 (8th mo)		8100
Blood pressure	100/60 to 128/70	100/60	110/70 to 124/60

* First pregnancy resulted in birth at term of a living male weighing 7 lb 9 oz; second pregnancy concluded by birth of a living premature male infant (7 mo), weighing 3 lb 12 oz; third pregnancy has not terminated.

When gravida II, the subject's excretions showed a pattern similar to that of her first pregnancy, but the levels of excretion of most of the amino acids were higher in the second month and lower in the third and fourth months. Of the 10 amino acids determined, only the excretion of threonine and leucine were as high during the second as during the first pregnancy. The second pregnancy terminated after 7 months. While trends of amino acid excretion were similar in the first two pregnancies, the quantities excreted in relation to duration of gestation were different.

The two samples collected and analyzed when the mother was gravida III showed increased amino acid excretions early in gestation similar to levels in the 2 preceding pregnancies.

The 10 amino acids were excreted during lactation in quantities not only smaller than during gestation, but also less than those found in the nulligravid state. Threonine, histidine, lysine, and tryptophan which during pregnancy showed the greatest increases in excretion over the nulligravid state also showed the greatest decreases in lactation.

In the postpartum urine sample obtained from the subject when not lactating, quantities of threonine, histidine, lysine, and phenylalanine were similar to those found when she was nulligravid. The other six amino acids however, were being excreted at levels lower than when she was nulligravid and approximating those found during lactation.

Discussion. The excretion of individual amino acids is affected differently by the metabolic alterations in response to the increased nutritive demands of reproduction. Of the 10 amino acids studied, the excretions of threonine, histidine, lysine, and tryptophan were most affected. Fig. 1 illustrates for the 4 amino acids, the relationships at different intervals in the reproductive cycle between levels of excretion and the initial non-pregnant values. Only threonine showed an increasing ratio of excretion throughout gestation which reached maxima of 11- and 12-fold in the first and second pregnancies, respectively. Histidine, lysine and tryptophan reached maximum ratios of 5-, 4- and 3-fold increases, respectively, during the first trimester. In general, during the first pregnancy amino acids in the urine tended to decrease during the second and increase during the third trimester; in the second pregnancy which terminated prematurely, the increase occurred in the fifth and sixth months. Leucine, isoleucine, valine, methionine, phenylalanine and arginine showed patterns similar to that of lysine, but the changes in their excretion only varied about 2-fold during the entire reproductive period.

Biochemical changes and physiological adaptation of the maternal body are known to be initiated as early as the second month in gestation and are demonstrated by increased urinary excretion of the 10 free indispensable amino acids(11). The products of conception also are alleged to alter the hormonal balance(13); change the basal metabolic rate of 13% or more in excess of that conditioned by the gross increase in body weight(14); modify the distribution of plasma proteins(15); and vary the concentrations of hemoglobin, sera vit. C, vit. A, carotenoids, and alkaline phosphatase(16)—all of these changes occurring to accommodate the maternal organs and tissues to the increased nutritive needs and functions necessary for the growth of the mother and fetus(17). The maternal dietary requirements for protein, minerals and vitamins also are increased by

TABLE II. 24-Hour Urinary Excretion of Free Amino Acids in mg during the Reproductive Cycle.

Amino acid	Gravidity	Pregravid	Month of gestation 2	3	4	5	6	7	8	9	Postpartum Lactating	Non-lactating
Threonine	0	18.3										
	1		39.3	118	119	163	156	180	199	187	14.5	
	2		49.3	78.8	88.1	201	218					22.4
	3		30.9		74.2							
Histidine	0	115										
	1		291	570	627	581	548	556	527	552	68.9	
	2		349	520	415	494	572					112
	3		340		457							
Lysine	0	55.1										
	1		131	249	183	217	167	168	212	204	21.8	
	2		126	192	125	157	173					64.2
	3		165		186							
Tryptophan	0	15.1										
	1		29.7	50.3	44.7	43.4	41.5	48.6	53.5	45.2	6.1	
	2		32.4	40.1	32.2	44.9	48.2					11.8
	3		24.2		30.8							
Phenylalanine	0	10.8										
	1		16.1	17.0	22.0	21.3	19.4	18.8	21.8	16.1	4.4	
	2		19.5	18.1	12.1	14.8	19.0					5.2
	3		14.9		14.9							
Isoleucine	0	6.7										
	1		9.3	16.5	11.3	10.5	8.6	8.0	11.1	10.5	4.6	
	2		8.8	7.7	5.5	7.2	7.4					3.9
	3		6.2		5.5							
Valine	0	8.5										
	1		11.2	18.2	13.5	13.7	12.7	12.0	14.8	14.5	6.3	
	2		15.6	17.8	10.1	10.0	14.2					4.9
	3		10.6		10.4							
Leucine	0	11.1										
	1		14.5	25.8	20.0	23.4	17.5	15.6	20.7	23.0	8.3	
	2		27.8	16.4	10.1	10.4	13.6					4.8
	3		12.5		13.0							
Arginine	0	8.4										
	1		9.7	10.2	10.9	10.2	8.5	9.0	9.7	10.2	4.4	
	2		9.8	9.6	5.6		9.4					5.0
	3		8.5		9.4							
Methionine	0	3.1										
	1		2.7	3.5	3.8	3.8	3.8	3.0	3.7	2.8	1.1	
	2		3.3	1.6	1.4	2.3	3.4					1.2
	3		2.6		2.5							

needs for the metabolic and physiologic functioning of the developing fetus and for the nutritional conditioning(18), or reconditioning (19-21), of the maternal body in preparation for labor, parturition, and lactation.

The changed levels in urinary excretion of free amino acids during pregnancy is one of many manifestations of biochemical transformations that accompany physiological stress(22) and increased metabolism. Only for free threonine does increased urinary excretion persist throughout gestation, the other 9 amino acids seem to be conserved after the third month of pregnancy. The physiological economy of the maternal body during the reproductive cycle is one of intricate biochemical balance in the coordination of the physiological functions and demands of the maternal, fetal, and placental structures in sharing the nutrients available from the maternal diet. The decrease in amino acid excretion during lactation may be the result of the increased demand by the mammary glands for amino acids to synthesize protein for milk. This

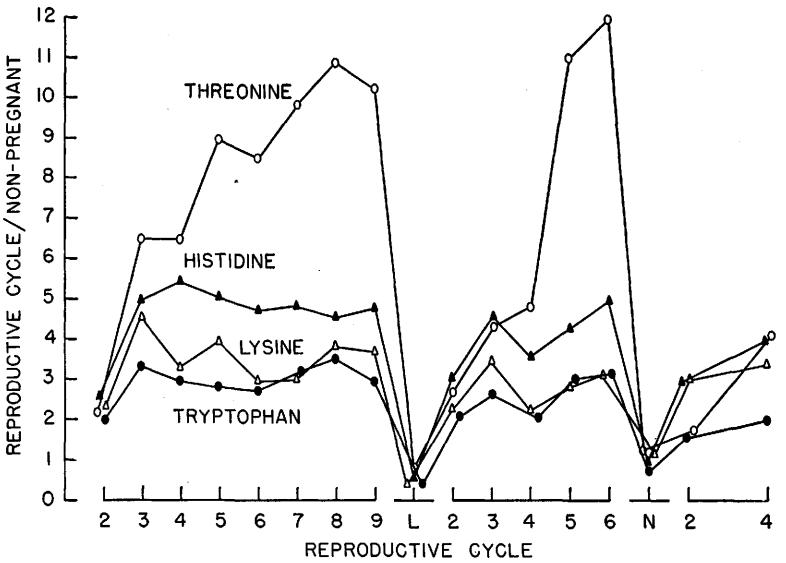

FIG. 1. Comparison of intensities of change of excretory levels of 4 amino acids based upon ratio of periods during reproductive cycle to the non-gravid state. Reproductive cycle is designated by numbers of months of gestation: L, lactating period; N, non-pregnant, postpartum gravida II period.

decrease may also be an effort on the part of the body to replace the amino acids lost during parturition.

Present methods for studying healthy mothers during pregnancy do not reveal what proportion of the maternal urinary excretion of free amino acids are contributed through the metabolic activity of the placenta, the fetus, or the mother. The growth rates of the placenta and fetus do not parallel one another(23). The metabolic activity of the embryonic protoplasmic mass increases throughout interuterine life, whereas the oxygen consumption of the placenta has been shown to decrease as early as the tenth or twelfth week of gestation(24), or as soon as the fetal organs begin to function. The organogenesis of the human fetus is completed during the first trimester of pregnancy. The early alteration in the distribution of maternal amino acid excretion during gestation therefore, coincides with a rise in maternal metabolism, an increase in active placental and embryonic protoplasmic mass, stimulated hormonal activity, and fetal kidney function.

The excretion patterns for each amino acid during each of the three reproductive cycles for this woman were similar in trend to the pattern established by the median values in the cross-sectional study of 15 women.

While amino acid patterns during pregnancy are similar to and coincide with many physiologic changes, there is need for study of more subjects to evaluate the biochemical changes in reproduction and lactation.

Summary. 1. Eighteen 24-hour urine samples collected from one woman during nulligravid, gravid and lactating periods, were analyzed for the free indispensable amino acids—leucine, isoleucine, valine, histidine, lysine, methionine, phenylalanine, arginine, threonine and tryptophan. The excretion level for each amino acid increased from the nulligravid to the gravid state, evident as early as the second month of gestation. Excretion of threonine continued to increase during gestation while methionine fluctuated slightly from the non-pregnant value. The other 8 amino acids showed a decreased excretory level during the fourth and fifth months of gestation. From the sixth to the ninth months, excretion levels of most of the amino acids increased slightly or remained fairly constant. 2. During lactation, the excretion level for each amino acid is less than

that for pregnancy or non-pregnancy. 3. Threonine, histidine, lysine and tryptophan showed the greatest changes from one phase of the reproductive cycle to another. 4. While trends of amino acid excretion were similar from one pregnancy to another, the quantities excreted in relation to duration of gestation were different.

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Received April 12, 1954. P.S.E.B.M., 1954, v86.

Production of Protective Antigens by *Pasteurella pestis* in a Synthetic Medium. (21024)

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Wright *et al.*(1) have reported the production of immunogenic substances by *Bacillus anthracis* in a synthetic medium. It is the purpose of this note to report preliminary observations of a similar nature with *Pasteurella pestis*.

Materials and methods. The avirulent strain A-12 of *P. pestis*, a single colony isolate of strain A-1122 of Jawetz and Meyer(2), was cultivated in a synthetic medium based on that developed by one of us (K.H.). The formula for the medium is given in Table I. The amino acids were dissolved in the salt solution by heating and, after cooling, the mixture was adjusted to pH 7.5 with 10 N NaOH. The medium was distributed in 234 ml amounts into 2.8 l Fernbach flasks and

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