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Comparative Sensitivity of Specific Amino Acid Ratios versus "Essential to Nonessential" Amino Acid Ratio[1,2]

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A RATIO of "nonessential-to-essential" amino acids in blood serum as an indicator of protein nutrition was proposed by Whitehead (1). The amino acids are, in this approach, separated by one-dimensional paper chromatography into groups of amino acids that are each a mixture of several compounds. The group of "essential" amino acids is formed by the branched essential amino acids, valine, leucine, and isoleucine, plus methionine, in decreasing order of concentration in normal serum. The nonessential group includes glycine, serine, glutamine, and taurine; glycine and serine are the highest in amount in normal serum. The bases for Whitehead's ratio are the changes in some of the specific components of these two groups.

There is general agreement that under the stress of restricted protein intake, the branched essential amino acids, particularly valine, decrease, while some of the nonessential, glycine for example, either drop slightly, or often even increase in concentration. Several workers have documented this point; therefore, the readers are referred to a discussion article by Arroyave (2). The present work was intended to test the hypothesis that the individual amino acid ratio, valine-to-glycine in serum, is more sensitive than a group ratio. Since serum tyrosine and cystine are known also to decrease very much in kwashiorkor their ratio with glycine was also included in the comparison.

EXPERIMENTAL

The plan of study consisted in the comparison of the ratios among groups of subjects known to differ in nutritional and dietary characteristics. Three groups of subjects were compared:

Group I. Includes three subgroups, all from Guatemala: *a*) pregnant women of the upper-income-urban level (PUIU), *b*) pregnant women of the low-income-rural level (PLIR), and *c*) nonpregnant women from the low-income-rural level (NPLIR).

The PUIU were five pregnant women taken from private obstetric clinics. Their diet provided sufficient amounts of all essential nutrients and calories to meet their recommended dietary allowances, and they showed good health and very adequate pregnancy performance, the 40 week-weight gain being 10.5 kg. The PLIR consisted of six pregnant women of the same age range as those in PUIU, from a rural village. The general characteristics of the diets of this group were a low adequacy in the intake of total calories (85%), animal protein, riboflavin (42%), and particularly vitamin A (19%). They were free of obstetrical complications but their 40-week weight gain was only 6.5 kg, which is considered inadequate. Finally the NPLIR were seven nonpregnant women from the same village as those in PLIR. Their dietary pattern was comparable to that of their pregnant women counterparts (PLIR) with the corresponding qualitative defects and similar quantitative inadequacies. All the women of *group I* were subjects of a longitudinal study on the nutritional status of pregnant women,

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[2] INCAP Publication I-508.

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carried out in a rural village in Guatemala by Beteta (3).

Group II. Consisted of two subgroups: *a*) newborn children from the five pregnant women of the upper-income-urban level and *b*) six newborn children from the six pregnant women of the low-income-rural level included in *group I.* These were all normal births apparently free of complications. The subjects of this group were borne by the mothers of *group I.*

Group III. Included: *a*) six children admitted with overt kwashiorkor, presenting the clinical biochemical characteristics that have been described elsewhere, such as edema, skin and hair lesions, and severe hypoalbuminemia; *b*) two children with typical marasmus, presenting extreme adipose tissue and muscle wasting, only moderate hypoalbuminemia, but without edema; and *c*) five children in the same age range as the malnourished ones, who had been completely recovered from previous malnutrition and were considered in a good state of health.

METHODS AND PROCEDURES

Venous blood samples were obtained from subjects in *group I,* during the morning, before lunch but not necessarily under fasting conditions. From *group II,* cord blood was examined, and the children in *group III* provided venous blood samples under fasting conditions. No anticoagulant was used; the serum was separated and frozen (—20 C) until analysis, which was carried out within 1 month in each case.

The amino acids were determined by ion-exchange resin chromatography and the ninhydrin reaction according to the method of Moore and Stein (4). The ratios of valine to glycine, cystine to glycine, and tyrosine to glycine were computed individually. Also, a group ratio was calculated to simulate Whitehead's ratio (in reverse), that is, valine + leucine + isoleucine divided by glycine + serine + alanine (VLI/GSA).

RESULTS

The comparisons among the subgroups of *group I* are presented in Table I. Both valine-to-glycine and VLI-to-GSA ratios were significantly different between the pregnant women of upper and those of low socioeconomic levels ($P < 0.01$). The cystine-to-glycine ratio gave a significant difference only at the 5% level indicating less discriminating power and the tyrosine-to-glycine did not distinguish between the pregnant groups at all. The data in Table II indicate that the "*t*" value is highest for the valine-to-glycine ratio.

When the pregnant and the nonpregnant women of low socioeconomic level were compared, no difference was found, except in their tyrosine-to-glycine ratios. A lack of difference was predicted as both had similar dietary characteristics and nutritional environment. In fact, the much higher values of tyrosine to glycine in the pregnant women seem a characteristic feature of pregnancy not related to nutritional status.

In *group II,* the analysis of cord blood gives a significant difference for valine to glycine ($P < 0.02$) and for cystine to glycine ($P < 0.05$). Neither tyrosine to glycine, nor VLI/GSA distinguish between the newborns of the upper-income-urban and those of the low-income-rural levels (Table III). Here again valine to glycine is the most sensitive as judged by the highest "*t*" value (see Table II).

In *group III* the differences between the averages were very large and the four ratios gave highly significant differences between kwashiorkor and well-nourished children (Table IV). In this case, tyrosine to glycine gave the largest relative difference reflecting the marked drop in serum tyrosine that occurs in the severe phase of protein–calorie malnutrition of the kwashiorkor type. Because only two cases with marasmus were studied, they are presented individually, and no attempt was made to test the significance of the differences with the other groups. These marasmus cases had ratios intermediate between the well-nourished and the kwashiorkor children.

TABLE I

Specific plasma amino acid ratios of women in relation to pregnancy and socioeconomic level

Group of women	Valine/Glycine, $\bar{x} \pm SD$	Cystine/Glycine, $\bar{x} \pm SD$	Tyrosine/Glycine, $\bar{x} \pm SD$	VLI/GSA, $\bar{x} \pm SD$
Pregnant (upper-income-urban) (5)	1.30 ± 0.32	0.52 ± 0.26	0.52 ± 0.14	0.58 ± 0.08
	$P < 0.01$	$P < 0.05$	NS	$P < 0.01$
Pregnant (low-income-rural) (6)	0.64 ± 0.13	0.21 ± 0.13	0.40 ± 0.07	0.43 ± 0.07
	NS	NS	$P < 0.01$	NS
Nonpregnant (low-income-rural) (7)	0.59 ± 0.16	0.32 ± 0.13	0.25 ± 0.10	0.42 ± 0.07

Figures in parentheses are the number of cases. NS = difference is not significant.

TABLE II

t Values between the different groups of subjects studied

Comparison	Valine/Glycine	Cystine/Glycine	Tyrosine/Glycine	VLI/GSA
Pregnant women				
Upper-income-urban versus low-income-rural	4.35	2.38	1.76	3.26
Newborn children				
Upper-income-urban versus low-income-rural	2.96	2.26	0.47	1.19

TABLE III

Specific plasma amino acid ratios of newborn children of two different socioeconomic groups

Groups	Valine/glycine, $\bar{x} \pm SD$	Cystine/glycine, $\bar{x} \pm SD$	Tyrosine/glycine, $\bar{x} \pm SD$	VLI/GSA, $\bar{x} \pm SD$
Upper-income-urban (5)	0.95 ± 0.13	0.47 ± 0.17	0.39 ± 0.06	0.55 ± 0.11
	$P < 0.02$	$P < 0.05$	NS	NS
Low-income-rural (6)	0.71 ± 0.13	0.29 ± 0.07	0.37 ± 0.08	0.48 ± 0.08

Figures in parentheses are the number of cases.

TABLE IV

Specific plasma amino acid ratios of children with kwashiorkor and marasmus

Group	*n*	Valine/glycine, $\bar{x} \pm SD$	Cystine/glycine, $\bar{x} \pm SD$	Tyrosine/glycine, $\bar{x} \pm SD$	VLI/GSA, $\bar{x} \pm SD$
Kwashiorkor	6	0.18 ± 0.06	0.07 ± 0.03	0.06 ± 0.02	0.19 ± 0.06
		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Well nourished	5	1.09 ± 0.45	0.47 ± 0.14	0.57 ± 0.15	0.70 ± 0.18
Marasmus	1	0.36	0.13	0.24	0.29
	1	0.37	0.17	0.15	0.39

DISCUSSION

The results indicate that the estimation of ratios between selected specific amino acids is more promising than that of group ratios as an index of alterations that occur as a consequence of inadequate protein intake. Of those tested, the ratio between valine and glycine is the most sensitive. Newer methods of quantitative amino acid analysis such as gas chromatography promise to make these measurements practical in terms of time and quantity of blood needed.

SUMMARY

The sensitivity of the ratios of glycine to serum valine, cystine, and tyrosine, respectively, have been studied relatively with a ratio of groups of serum amino acids constituted by valine + leucine + isoleucine in the numerator and glycine + serine + alanine in the denominator. The ratios were measured in comparable groups of subjects differing in dietary characteristics and nutritional background. The ratio of valine to glycine has a higher distinguishing (discriminating) power for moderate protein–calorie malnutrition as indicated by the statistical analysis of the data.

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REFERENCES

1. WHITEHEAD, R. G. Rapid determination of some plasma amino acids in subclinical kwashiorkor. *Lancet* 1: 250, 1964.
2. ARROYAVE, G. Biochemical signs of mild-moderate forms of protein–calorie malnutrition. In: *Mild-Moderate Forms of Protein–Calorie Malnutrition,* Symposia of the Swedish Nutrition Foundation. I, Bastad, August, 1962, edited by G. Blix. Uppsala: Almqvist & Wiksell, 1963, p. 32.
3. BETETA, C. *Embarazo y Nutrición (Estudio Longitudinal en Mujeres Embarazadas Pertenecientes al Grupo Rural de Bajo Nivel Socioeconómico de Guatemala*) (M.D. Thesis). Guatemala City: Universidad de San Carlos de Guatemala, Facultad de Ciencias Médicas, Noviembre, 1963.
4. MOORE, S., AND W. H. STEIN. Procedures for the chromatographic determination of amino acids on four per cent cross-linked sulfonated polystyrene resins. *J. Biol. Chem.* 211: 893, 1954.