THE AMINO ACID COMPOSITION OF HUMAN MILK PROTEINS

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It has generally been considered that human milk is of superior nutritional value for infants as compared with cow's milk. This has been based on clinical observations since comparatively little is known of the composition of human milk and especially of its proteins. The investigation of the amino acid constituents of the cow's milk proteins has long held the attention of many workers. However, in the case of the proteins of human milk, few and very incomplete amino acid analyses have been reported (1–3).

The complete analysis of the proteins from human milk would make for a better understanding of human nutrition, since these proteins are utilized at a period of life when the rate of growth is at its maximum. Although other factors are of importance in determining the "biological value" of proteins, the amino acid composition remains the leading factor in the evaluation of this nutritional concept. The analysis of human milk proteins has been undertaken with these considerations in mind.

EXPERIMENTAL

The human milk used in this investigation represented approximately 500 collections from both white and Negro women. As the samples were collected, they were stored in a common container and kept frozen until a sufficient quantity had been accumulated. The human milk proteins were isolated and purified according to the method to be described. It was considered to be of value to analyze similar proteins at the same time to serve as a check on the results and as a basis of comparison with the human milk proteins. The proteins of cow's milk were used for this purpose. Cow casein was obtained by isoelectric precipitation. The lactoglobulin was removed from the cow's milk whey by half saturation with magnesium sulfate before the isolation of the lactalbumin (4, 5).

The moisture, ash, and nitrogen content (micro-Kjeldahl) of each protein was determined. On an ash- and moisture-free basis the nitrogen content of the proteins was found to be cow casein, 15.28 per cent, cow lactalbumin, 14.92 per cent, human casein, 14.90 per cent, human lactalbumin, 14.94 per cent. The results reported in this paper are all corrected to a moisture- and ash-free basis.

Preparation of Human Casein—5 gallons of composite breast skim milk

were warmed to 35–38° and the acidity adjusted to pH 4.5 by the slow addition of 0.1 N acetic acid with vigorous stirring. The milk was then centrifuged and the casein collected. The casein was suspended in sufficient water to make a 1 per cent solution and dissolved by the slow addition of 0.05 N NaOH to pH 6.2. After filtration, the casein was again precipitated and the procedure repeated three times more. The solution of casein was finally dialyzed under toluene against running tap water for 72 hours and then against 10 to 20 volumes of distilled water for 24 hours more.

After dialysis, the casein solution was adjusted to pH 4.5 and centrifuged. The casein was washed twice with alcohol and ether and dried over sulfuric acid *in vacuo*.

Preparation of Human Lactalbumin—The supernatant whey after the removal of the casein was adjusted to pH 5.0. It was then slowly heated to 80° in a water bath and held at this temperature for 30 to 45 minutes. The suspension of coagulated protein was allowed to cool and settle and the supernatant liquid was removed. The protein was centrifuged, suspended in water, and 0.05 N NaOH added to pH 6.5. After filtration, the protein was precipitated with acetic acid (pH 4.8 to 4.9) and centrifuged. The process was repeated three times. The solution of protein was finally dialyzed against tap and then distilled water, precipitated as before, and centrifuged. It was washed twice with alcohol and ether and dried *in vacuo* over sulfuric acid.

The lactalbumin preparation was expected to contain the other coagulable whey proteins. However, these are present in relatively insignificant amounts (6).

Amino Acid Analyses—The results of the analysis and the source of the method used for each amino acid are shown in Table I. Also shown is the per cent of the total nitrogen contributed by each amino acid to indicate the actual amount of the protein which has been accounted for. In order to check the results, there is included a comparison with the values reported by previous authors. The results, in general, are in reasonable agreement with those obtained by similar methods of analysis.

In the analyses which were determined by colorimetric methods (tyrosine, glycine, proline, cystine, arginine, phenylalanine, methionine, and tryptophane) purified synthetic amino acids dried over phosphorus pentoxide were used as standards. Those methods which depend on the formation of a volatile substance from the amino acid being tested (alanine, serine, leucine, isoleucine, threonine, and valine) were checked by testing the procedure with purified synthetic amino acids. All the color comparisons were made in a Beckman spectrophotometer and the wave-length of maximum absorption was determined, unless previously described in the literature.

TABLE I

Amino Acid Composition of Human and Cow's Milk Proteins

Amino acid (method of analysis)	Cow casein			Human casein			Cow lactalbumin			Human lactalbumin		
		Per cent of total N	Reported in literature		Per cent of total N	Reported in literature		Per cent of total N	Reported in literature		Per cent of total N	Reported in literature
	per cent		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*
Tyrosine (7)	5.5	2.8	5.2 (8), 5.8 (2), 5.0 (3)	5.5	2.9	5.5 (2), 6.1 (3)	3.5	1.8	3.4 (5), 3.6 (1)	4.5	2.3	4.5 (2), 5.2 (3)
Alanine (8)	2.3	2.4	2.7 (9), 1.5 (10)	2.0	2.1		2.6	2.8	2.4 (11)	2.5	2.6	
Glycine (12)	0.4	0.5	0.5 (12)	0	0		0	0	0 (12), 0.4 (11)	0	0	
Proline (13)	8.1	6.5	7.6 (24), 7.9 (13)	8.9	7.3		4.0	3.3	3.8 (11)	3.5	2.8	
Glutamic acid (14)	21.9	13.7	22.0 (14, 25)	20.9	13.4		13.7	8.7	12.9 (11)	12.5	8.0	
Aspartic " (14)	4.2	2.9	4.1 (9)	4.6	3.3		9.6	6.8	9.3 (11)	9.3	6.6	
Serine (15)	5.0	4.4	2.3 (9), 5.0 (15)	5.4	4.8		4.0	3.6	1.8 (11), 4.3 (15)	4.2	3.7	
Cystine (16)	0.4	0.3	0.4 (1), 0.3 (3, 8)	0.6	0.5	0.6 (1), 0.7 (3)	3.1	2.4	3.1 (8), 3.3 (1)	3.8	3.0	4.4 (1), 3.1 (3)
Arginine (17)	3.9	8.2	3.7 (1), 3.8 (17)	3.4	7.4	3.6 (1), 3.3 (3)	3.6	7.7	3.0 (8), 4.0 (1)	5.0	10.8	5.0 (1), 5.2 (3)
Phenylalanine (18)	5.5	3.1	5.8 (8), 5.0 (18)	5.8	3.3		4.5	2.6	4.8 (8)	4.8	2.7	
Leucine (19)	14.4	10.1	14.8 (8), 12.1 (19)	12.2	8.7		17.4	12.4	17.2 (8), 14.3 (11)	16.7	11.9	
Isoleucine (19)	5.2	3.6	5.1 (8)	6.3	4.6		4.2	3.0	4.2 (8)	4.3	3.1	
Histidine (20)	2.0	3.6	1.9 (8), 1.7 (1), 1.8 (3)	2.0	3.6	1.5 (1), 1.8 (3)	1.4	2.6	1.8 (1), 1.4 (3, 8)	1.5	2.8	1.7 (1), 1.2 (3)
Lysine (21)	6.0	7.5	5.9 (8), 6.1 (1), 5.8 (26)	5.6	7.2	5.5 (1), 5.2 (3)	6.2	8.0	6.3 (1), 5.2 (3), 5.9 (8)	6.6	8.5	6.6 (1), 5.8 (3)
Threonine (22)	4.6	3.5	4.6 (8), 4.4 (22)	4.5	3.5		4.3	3.4	4.5 (8)	4.0	3.1	
Methionine (23)	3.1	1.9	2.9 (1), 3.0 (8, 23)	2.3	1.5	2.7 (1), 2.2 (3)	2.4	1.5	2.3 (1), 2.2 (3)	1.7	1.1	1.4 (1), 1.9 (3)
Tryptophane (7)	1.3	1.2	1.4 (2), 1.1 (3), 1.2 (8)	1.5	1.4	1.1 (1), 1.7 (3)	2.1	1.9	2.0 (8), 1.9 (2), 1.8 (3)	2.3	2.1	2.5 (2), 2.3 (3)
Valine (19)	5.3	4.2	5.2 (8), 7.0 (19)	5.0	4.0		4.0	3.2	4.0 (8)	4.1	3.3	
Total	99.1	80.4		96.5	79.5		90.6	75.7		91.3	78.4	

DISCUSSION

The data obtained in this investigation contribute to a better understanding of infant nutrition. It has been the practice to use cow's milk diluted to about 50 per cent of its original protein content when human milk is not available for young infants. As shown in Table II, there are differences between the total amino acids in human milk and those in diluted cow's milk. This table shows the amino acid content, in mg. per 100 cc., of cow's milk, human milk, and cow's milk diluted to 50 per cent of its protein content. Although the total protein content as well as the proportion of

TABLE II

Comparison of Amino Acid Content of Cow's Milk and Human Milk from Their Protein Analyses

Figures are mg. per 100 cc.

Amino acid	Cow's milk, 2.8 per cent casein, 0.5 per cent lactalbumin	Human milk, 0.5 per cent casein, 1.0 per cent lactalbumin	Diluted cow's milk, 1.4 per cent casein, 0.25 per cent lactalbumin
Tyrosine	172	73	86
Alanine	75	35	37
Glycine	11	0	6
Proline	250	80	125
Glutamic acid	680	230	340
Aspartic "	166	116	83
Serine	160	69	80
Cystine	27	41	14
Arginine	127	67	64
Phenylalanine	177	77	88
Leucine	490	228	245
Isoleucine	167	75	83
Histidine	63	25	32
Lysine	200	94	100
Threonine	151	63	76
Methionine	99	29	50
Tryptophane	47	31	24
Valine	171	66	86

casein to lactalbumin varies in milk, the average composition is reported to be approximately 2.8 per cent casein, 0.5 per cent lactalbumin in cow's milk, and 0.5 per cent casein, 1.0 per cent lactalbumin in human milk (5, 6, 27, 28).

It is expected that cow's milk should contain larger total quantities of all of the amino acids than human milk since the protein content is twice as great. This holds true with the exception of cystine. However, in the group of essential amino acids there are noteworthy differences between

human milk and the diluted cow's milk. Here, the human milk contains 3 times as much cystine as does the diluted cow's milk. On the other hand, the diluted cow's milk proteins contribute over $1\frac{1}{2}$ times as much methionine to the milk as do the human milk proteins. In the case of the human milk, the lower methionine content probably does not present a nutritionally critical condition since the larger amount of cystine may spare the requirement of methionine (29). To put the comparison on a quantitative basis, the proteins in 100 cc. of human milk contain 0.36 mM of sulfur amino acids (cystine = 0.17, methionine = 0.19), while the diluted cow's milk proteins contain 0.38 mM (cystine = 0.05, methionine = 0.33).

Another important difference is the higher content of tryptophane in human milk as compared to the diluted cow's milk. Of the other essential amino acids, there are to be noted differences of about 20 per cent in the case of histidine, threonine, and valine, the human milk being lower. However, it has been shown that histidine is not required by adults to maintain nitrogen equilibrium over an experimental period of about a week (30).

SUMMARY

1. The casein and lactalbumin from human and cow's milk were isolated and the amino acid content for eighteen amino acids determined.

2. In a comparison of human milk and cow's milk diluted to 2 volumes, the analyses indicate that there are over 3 times as much cystine in the human milk. However, there was less methionine found in the human milk. On the basis of millimoles of total sulfur amino acids, there is no significant difference between the two.

3. The diluted cow's milk was shown to contain higher percentages of valine, threonine, and histidine while human milk was found richer in tryptophane.

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