AMINO ACID COMPOSITION OF HUMAN MILK*

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The fundamental importance of human milk in infant nutrition makes it desirable to have available as complete information as possible on the amounts of amino acids contributed to the diet from this source (Williamson (1), Block and Bolling (2, 3), and Macy (4)). In the present study, hydrolysates of human milk have been analyzed by ion exchange chromatography (Moore and Stein (5)) as applied in this laboratory to the analysis of foods (Schram et al. (6)). The applicability of the method to materials of high carbohydrate content has been demonstrated by Dustin et al. (7).

EXPERIMENTAL

Samples for Analysis—The chromatographic analyses were performed on two mixed samples of human milk, Samples A and B. Sample A represented pooled milk from twenty donors from the urban area of Brussels, obtained in the middle of the winter. Sample B was from forty donors from the same area and was collected at the end of the winter season (1950-51). In both instances the raw milk was centrifuged on the day of collection. The skimmed, relatively fat-free milk was pasteurized by heating it to 70° for 35 minutes. After storage of the samples in a refrigerator at about 0° for 48 hours, aliquots of the milk were taken for hydrolysis and analysis. The protein level, calculated as 6.25 X total N, was 1.1 per cent, a figure which is the same as the average value reported by Macy (4, 8) for samples of human milk collected over the period from 15 days to 15 months post partum. The hydrolysis was carried out by the prescribed procedure (6) on 5 ml. of milk (about 50 mg. of protein) in 200 ml. of 6 N HCl. One-tenth of the quantity hydrolyzed was used for each Dowex 50 chromatogram (5, 6). Cystine is unstable when hydrolysis is performed

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1 The samples were obtained through the generous cooperation of the Lactarium de l'Œuvre Nationale de l'Enfance of Brussels. All of the donors were carefully selected normal persons under medical supervision.
in the presence of carbohydrate and hence was determined as cysteic acid by the method of Schram et al. (9) on a separate 5 ml. sample oxidized by performic acid and hydrolyzed for chromatography on Dowex 2. Tryptophan was determined microbiologically\(^2\) after alkaline hydrolysis.

**Analytical Results**—Effluent curves obtained with Sample B on 100 cm. and 15 cm. Dowex 50 columns are shown in Fig. 1. During acid hydrolysis carbohydrates give rise to products which yield a red color in the ninhydrin reaction (6, 7). The peak given by the decomposition products of lactose is obtained at about 55 ml. of effluent. There are two ninhydrin-positive peaks, Peaks A and B in Fig. 1, which appear in addition to those expected at the positions of emergence of the common amino acids. The material responsible for the color in the range of Peak A is eluted at the point of emergence of the buffer of pH 4.25. By advancing the point of change of the eluent, almost all of the material could be shown to move fairly well ahead of the methionine position, which is immediately in front of isoleucine. The value for methionine has been based upon the methionine sulfoxide peak, assuming almost complete oxidation during hydrolysis (6), and is a minimal value subject to revision when a method becomes available which is free from some of the uncertainties that have been shown to be present in the determination of the methionine content of foods (6). Peak B in Fig. 1, occurring at the position known to be assumed by amino sugars (5), is receiving further study.

**Conclusions**

The amino acid composition of human milk determined chromatographically (Table I) shows glycine, alanine, proline, and glutamic and aspartic acids to be considerably higher than they were previously thought to be. Earlier tabulations (4, 8) have routinely listed the glycine content as zero (1). For most of the amino acids, the values obtained by the different methods are in fair agreement. As is true of most foods, the four nutritionally important amino acids present in the smallest amounts are cystine, methionine, tryptophan, and histidine, but all of these are present in human milk at about the 2 per cent level. (For purposes of comparison with other proteins, the percentage composition figures for the amino acids of human milk can be obtained from Table I by dividing the mg. per 100 ml. values by 10, since the total quantity of amino acids present is 1 gm.)

The analyses account for 88 per cent of the total N in terms of amino acids and ammonia. A considerable proportion of the ammonia in the hydrolysate arises from the urea of human milk (4). The level of urea N

\(^2\) We are greatly indebted to Professor J. H. Bouckaert and his associates at the University of Ghent for the microbiological determination of tryptophan in alkaline hydrolysates by the method of Henderson and Snell (10).
Fig. 1. Chromatographic analysis of an acid hydrolysate of human milk (Sample B, Table I). The acidic and neutral amino acids were determined on a 100 cm. Dowex 50 column (Curve 1), and the basic amino acids were determined on a 15 cm. column (Curve 2). The sample for analysis corresponded to 0.5 ml. of milk. The proline concentrations have been corrected for the low color yield to bring the peak into scale on a molar basis with those of the other amino acids.
### Table I

Amino Acid Composition of Human Milk

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Pooled Sample A</th>
<th>Pooled Sample B</th>
<th>Average values</th>
<th>Literature values, mg. per 100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg. amino acid per 100 ml.</td>
<td>N, per cent total N</td>
<td>Mg. amino acid per 100 ml.</td>
<td>Based on milk protein analyses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Whole milk</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>88.7</td>
<td>98.2</td>
<td>5.7</td>
<td>93</td>
</tr>
<tr>
<td>Glutamic</td>
<td>200.0</td>
<td>197.0</td>
<td>10.9</td>
<td>198</td>
</tr>
<tr>
<td>Glycine</td>
<td>22.9</td>
<td>24.0</td>
<td>2.5</td>
<td>23</td>
</tr>
<tr>
<td>Alanine</td>
<td>35.5</td>
<td>40.6</td>
<td>3.5</td>
<td>38</td>
</tr>
<tr>
<td>Valine</td>
<td>50.2</td>
<td>57.0</td>
<td>3.7</td>
<td>54</td>
</tr>
<tr>
<td>Leucine</td>
<td>92.9</td>
<td>107.0</td>
<td>6.2</td>
<td>100</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>48.8</td>
<td>58.4</td>
<td>3.3</td>
<td>54</td>
</tr>
<tr>
<td>Serine</td>
<td>50.8</td>
<td>46.6</td>
<td>3.8</td>
<td>49</td>
</tr>
<tr>
<td>Threonine</td>
<td>46.4</td>
<td>49.3</td>
<td>3.2</td>
<td>48</td>
</tr>
<tr>
<td>Cystine§</td>
<td>23.0</td>
<td>20.0</td>
<td>1.0</td>
<td>23</td>
</tr>
<tr>
<td>Methionine</td>
<td>87.6</td>
<td>83.8</td>
<td>1.1</td>
<td>20</td>
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<tr>
<td>Proline</td>
<td>38.9</td>
<td>42.2</td>
<td>2.0</td>
<td>41</td>
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<tr>
<td>Phenylalanine</td>
<td>46.5</td>
<td>46.3</td>
<td>2.1</td>
<td>46</td>
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<tr>
<td>Tyrosine</td>
<td>22.7</td>
<td>20.2</td>
<td>1.5</td>
<td>19</td>
</tr>
<tr>
<td>Histidine</td>
<td>65.2</td>
<td>63.1</td>
<td>7.1</td>
<td>64</td>
</tr>
<tr>
<td>Lysine</td>
<td>30.6</td>
<td>32.0</td>
<td>5.8</td>
<td>31</td>
</tr>
<tr>
<td>Arginine</td>
<td>28.1</td>
<td>33.6</td>
<td>14.6</td>
<td></td>
</tr>
</tbody>
</table>

* The samples represent pooled milk from twenty and forty donors, respectively. The nitrogen content of Sample A was 177.4 mg. per 100 ml. and that of Sample B was 168.3 mg. per 100 ml. The average N of 173 mg. per 100 ml. corresponds to a calculated protein content of 1.1 per cent (factor 6.25). The total N of the Brussels samples is the same as the mean value for the human milk reported by Macy (4, 8). For milk of higher or lower N content, approximate values for the amino acid levels can be calculated on the basis of the N content of the given sample.

† For comparison with the composition of other proteins, the values in mg. per 100 ml. can be divided by 10 to give percentage figures, since the total amino acids in the milk amount to 1.0 gm.

‡ For purposes of comparison, the values from the literature have been recalculated for milk of 173 mg. of N per 100 ml., assuming the precipitable proteins to represent 78 per cent of the total N (Beach et al. (12)).

§ Cystine was determined chromatographically as cysteic acid (9).

|| Tryptophan was determined by microbiological assay (see the text).
is known to be comparable to that of the blood plasma and may account for 7 to 10 per cent of the total N of the milk. Urea is about 80 per cent hydrolyzed to ammonia in boiling 6 N HCl after 20 hours. The small peak from residual urea (color yield only 0.03 (11)) may be masked by the carbohydrate decomposition products, which emerge close to the urea position (5). The inclusion of the residual urea N in the summation would account for about 90 per cent of the total N. The N of Peaks A and B would add about 5 per cent to the total, leaving 5 per cent for other unknown constituents and minor components such as creatine, creatinine, uric acid, and choline (4).

The total of 1.0 gm. of amino acids (Table I) corresponds to about 0.85 gm. of protein, when calculated in terms of the amino acid residues. Thus the conventional calculation (173 mg. of N × 6.25 = 1.1 gm.) overestimates the protein level in human milk by more than 20 per cent. The independently determined 20 per cent of non-protein N (4, 12) should be subtracted from the total N for the calculation of the protein content (138 mg. of N × 6.25 = 0.86 gm.).

Macy and associates (cf. (4)) have shown that the free amino acid nitrogen, at 5 mg. per cent, is also about the same as that of blood plasma and represents a contribution of less than 3 per cent of the total amino acids of the food. The chromatographic analyses include this fraction, together with the amino acids from any peptides that may be present. Separate chromatograms on hydrolysates of the dialyzable fraction of human milk have confirmed the fact that the amino acids of the non-protein N represent only a small percentage of the total composition in Table I.

SUMMARY

The amino acid composition of human milk represents a standard of reference in infant nutrition. Chromatographic analyses have been carried out to provide as complete a tabulation as possible of the amino acids which enter the diet from this source.

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