Amino-acid content of raw and heat-sterilized cow's milk

By SHEILA PAYNE-BOTHA* and E. J. BIGWOOD

Department of Biochemistry and Nutrition, Faculty of Medicine, Brussels University

(*Received* 14 *November* 1958—*Revised* 10 *July* 1959)

It is well known that the nutritive value of milk proteins is reduced by heating, for example when milk is brought to, and kept for some time at, its boiling point. The reduction may be due either to the breakdown of certain amino acids under the influence of heat, or to combination of amino acids with other chemical constituents of milk, as in the formation of the so-called Schiff bases due to the condensation of free amino groups with the aldehyde group of sugars. This condensation, in turn, leads to the Maillard reaction, often referred to as the 'browning reaction'. It takes place in heat-sterilized milk when it is exposed for a sufficiently long time to a high temperature. Amino acids may eventually be released from certain of these condensation products by acid hydrolysis, which does not necessarily mean that the nutritive value of the heated product has not been impaired since digestive enzymes may fail to hydrolyse these protein derivatives and digestibility may thereby be reduced (Lea & Hannan, 1950). Our purpose has been to determine the effect of sterilization by heat on the amino-acid content of partly skimmed milk.

EXPERIMENTAL

A sample of partly skimmed raw milk was divided into two parts: one was hydrolysed immediately, the other was sterilized in an autoclave at 122–124° for 20 min. This process produced no visible browning. Two samples of the unsterilized and two of the sterilized milk, each containing 40–50 mg protein, were hydrolysed by refluxing with 200 ml of 6N-HCl (ammonia-free) for 22 h. The amino-acid content of the hydrolysates was determined by Moore & Stein's (1951) original chromatographic method on ion-exchange columns (Dowex 50 × 8), with the modified procedure of Schram, Dustin, Moore & Bigwood (1953). Cystine was determined separately (Schram, Moore & Bigwood, 1954). In the course of acid hydrolysis under our conditions, methionine is partly converted into its sulphoxide, which is allowed for in the calculation (Schram *et al.* 1953). Tryptophan was estimated separately in the hydrolysate by the microbiological method of Henderson & Snell (1948). Total nitrogen was estimated in duplicate in each sample by Kjeldahl's method.

* On leave from Professor Platt's Human Nutrition Research Unit, Medical Research Council (London), on a grant from the Anglo-Belgian Cultural Agreement for Exchange of Scientific Workers.

Table 1. *Amino acid content (mg/100 ml) of skim cow's milk before and after heat sterilization*

Amino acid	Raw: Mean of two determinations (*a*)	Raw: Deviation (±) of each value from mean (%)	Sterilized: Mean of two determinations (*b*)	Sterilized: Deviation (±) of each value from mean (%)	*b* as a percentage of *a*
Aspartic acid	261	3·5	253	2	97·1
Threonine	146	3	144	2	98·5
Serine	197	3·3	187	3·4	95·0
Glutamic acid	731	6·5	734	2	100·1
Proline	330	8·4	318	0·5	96·5
Glycine	66	5·1	66	2	100·0
Alanine	110	5	116	7	105·5
Cystine	21	8	18	9	85·7
Valine	202	6	204	2	101·0
Methionine	86	9	81	2	94·2
Isoleucine	165	11	162	3	98·2
Leucine	326	6	312	2	95·7
Tryptophan	23	—	26	—	—
Tyrosine	172	9	182	0·5	106·0
Phenylalanine	171	1	162	0·5	94·5
Histidine	84	2	87	12·0	103·6
Lysine	268	1	242	0·2	90·3
Arginine	107	4	105	5·5	98·0
Ammonia	65	5	63	3·0	97·0
Total nitrogen (mg/100 ml)					
In the amino acids and ammonia	490·7	—	479·0	—	—
In milk (by Kjeldahl)	495·2	—	490·0	—	—
Recovery in the amino acids and ammonia (%)	99·1	—	97·7	—	—
Protein (g/100 ml):					
Total amino-acid residues	3·00	—	2·94	—	—
N × 6·25	3·09	—	3·06	—	—

RESULTS

The total nitrogen content (Kjeldahl) amounted to 495·2 mg/100 ml for the sample of raw skim milk and to 490·0 mg for the autoclaved one. Each of these values is the mean of duplicate determinations which in both instances deviated from the mean by ±0·5%. Autoclaving had therefore not altered significantly the volume of the milk. The mean amino-acid composition of the raw and sterilized milks is given in Table 1. The values obtained fall within the range of individual variation observed by us in some twenty samples of bulked cow's milk (unpublished findings).

The deviation of each duplicate determination from the mean value, expressed as a percentage of the mean, is given in Table 1. The concentrations of amino acids in the sterilized sample, expressed as percentages of the corresponding values in the raw sample, are also shown in the table.

DISCUSSION

Duplicate determinations of an amino acid in a given sample may easily differ by ± 5 or 6 % of their mean value; they are considered to check satisfactorily when the difference does not exceed that amount. We consider therefore that differences between raw and sterilized samples are insignificant and negligible when they do not exceed 5 or 6 %. On this basis of interpretation, the only amino acid for which there was a definitely significant drop in content due to sterilization was lysine. With the amino acids listed below duplicate determinations checked less satisfactorily.

Proline. In the sample of raw milk the repeated estimations gave poor agreement with the original one. This finding is not surprising since this amino acid gives an unusual colour reaction with ninhydrin (yellow instead of purple).

Alanine. In the sterilized sample duplicate determinations showed rather poor agreement. The apparent difference before and after sterilization was insignificant.

Cystine. Duplicate determinations made on both samples gave poor agreement, though the method used usually yields more consistent results than it did here. The apparent drop of 14·3 % in cystine content on sterilization cannot, therefore, be taken to be definitely significant. We believe that there probably was some destruction of cystine due to the sterilization process, but this observation needs to be confirmed.

Methionine. Duplicate estimations on the raw sample gave poor agreement. Technical difficulties are known to exist with this method for methionine because of its partial oxidation to its sulphoxide (Schram *et al.* 1953). Although the amount of sulphoxide formed was allowed for in the calculation, the apparent drop on sterilization of 5·8 % in methionine content cannot be considered as significant.

Isoleucine and tyrosine. The duplicate determinations on the raw samples gave poor agreement.

Histidine. The duplicate determinations on the sterilized samples gave poor agreement.

Phenylalanine. The duplicate determinations on both raw and sterilized samples showed satisfactory agreement (± 1 % or less). The drop of 5·5 % after sterilization may therefore be significant, but the result is marginal since the drop does not exceed 5 or 6 %.

Lysine. It was the only amino acid that showed a definitely significant drop of the order of 10 %; the duplicate determinations on each of the two samples of both raw and sterilized milk agreed to within ± 1 % or less.

Pasteurization is known not to affect the nutritive value of milk proteins (Milk Nutrition Committee, 1937; Kay, 1939–40). Our experiments show that in heat sterilization at 122–124° for a relatively short time (15–20 min), that is under conditions still mild enough not to result in visible browning, the concentration of one amino acid, lysine, fell. There was possibly a similar fall in concentration of cystine, although it cannot be stated firmly. Mauron, Mottu, Bujard & Egli (1955) have studied carefully the Maillard reaction, particularly in its relation to loss of lysine under the effect of heat in the manufacture of spray-dried and roller-dried milk and of evaporated and sweetened condensed milk. The reaction takes place at the ϵ-NH_2 end-groups of

lysine in protein peptide chains and the first condensation product (aldosylamine) obtained is reversibly hydrolysable with HCl, whereas it is resistant to pepsin and pancreatic proteolysis, hence the decrease in digestibility and nutritive value for growing rats. The further steps of the reaction leading to the production of Schiff bases and to the browning reaction (production of melanoidines) are irreversible by acid. Mauron *et al.* (1955) have studied in vitro the enzymic digestibility of the products, omitting, however, to check whether the aldosylamine is also resistant to erepsin in the gut; they studied the action of pepsin followed by that of pancreatin. The action of erepsin should be studied also in such in vitro tests to ensure that they simulate completely conditions in the digestive tract. It appears, however, that in the experiments with rats of Mauron *et al.* (1955) lysine was irreversibly combined by heat treatment in some of the milk products analysed. They detected no differences in lysine content between samples of fresh and boiled milk, whereas we found a slight but definite drop of the order of 10%.

Keeney & Bassette (1957) have also studied the browning reaction in samples of dried milk under the influence of heat or of water after reconstitution. The distinction between available and unavailable lysine has also been extensively studied by various other workers, e.g. Eldred & Rodney (1946), Carpenter & Ellinger (1955), Carpenter, Ellinger, Munro & Rolfe (1957); Carpenter (1958*a*, *b*).

Further investigation of the availability of lysine is in progress in our laboratory, but it was essential first to determine accurately to what extent free lysine could be recovered by acid hydrolysis from sterilized liquid milk in the conditions described above. Our findings show a 90% recovery. It is difficult to decide whether the 10% loss represents a breakdown of the amino acid itself or whether its hypothetical derivative corresponding to unavailable lysine, presumably a derivative of the type R-NH.X on the ϵ-NH_2 end-group of the amino acid, is not completely hydrolysed by strong hydrochloric acid. This latter possibility is being further studied.

Table 1 shows that the nitrogen content calculated from the amino-acid analysis amounted to 99·1% of that obtained by direct Kjeldahl measurement with raw milk and to 97·7% with sterilized milk, a very satisfactory agreement. The table shows also the close similarity (within 3–4%) of the protein content obtained by addition of the amino-acid residues with that calculated by multiplying the value for the nitrogen content of the milk by the factor 6·25.

SUMMARY

1. After acid hydrolysis, the amino-acid content of fresh and of heat-sterilized cow's milk was estimated by the method of Moore & Stein (1951).

2. The milk was sterilized by autoclaving at 122–124° for 20 min.

3. Under these conditions the amino acids present in raw milk were all recovered quantitatively from sterilized milk except for lysine, which suffered a 10% loss; cystine was possibly also affected.

4. The significance of the loss in lysine is discussed.

The research was done with a grant from the Centre National de la Nutrition of the Belgian Institute of Food and Nutrition (I.B.A.N.).

REFERENCES

Carpenter, K. J. (1958*a*). *Proc. Nutr. Soc.* **17**, 91.
Carpenter, K. J. (1958*b*). *Voeding*, **19**, 152.
Carpenter, K. J. & Ellinger, G. M. (1955). *Biochem. J.* **61**, xi.
Carpenter, K. J., Ellinger, G. M., Munro, M. I. & Rolfe, E. J. (1957). *Brit. J. Nutr.* **11**, 162.
Eldred, N. R. & Rodney, G. (1946). *J. biol. Chem.* **162**, 261.
Henderson, L. M. & Snell, E. E. (1948). *J. biol. Chem.* **172**, 15.
Kay, H. D. (1939–40). *Nutr. Abstr. Rev.* **9**, 1.
Keeney, M. & Bassette, R. (1957). *Science*, **126**, 511.
Lea, C. H. & Hannan, R. S. (1950). *Biochem. biophys. Acta*, **5**, 433.
Milk Nutrition Committee (1937). *Milk and Nutrition.* Part 1. *The Effect of Commercial Pasteurisation on the Nutritive Value of Milk, as Determined by Laboratory Experiment*, p. 37. Reading: National Institute for Research in Dairying.
Mauron, J., Mottu, F., Bujard, E. & Egli, R. H. (1955). *Arch. Biochem. Biophys.* **59**, 433.
Moore, S. & Stein, W. (1951). *J. biol. Chem.* **192**, 663.
Schram, E., Dustin, J. P., Moore, S. & Bigwood, E. J. (1953). *Analyt. chim. acta*, **9**, 149.
Schram, E., Moore, S. & Bigwood, E. J. (1954). *Biochem. J.* **57**, 33.

Some blood coagulation studies in normal and scorbutic guinea-pigs

By P. BARKHAN

Department of Medicine, University of Cambridge

and A. N. HOWARD

Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council

(*Received* 3 *January* 1959—*Revised* 9 *May* 1959)

Haemorrhage is a feature of severe ascorbic-acid deficiency in both primates and guinea-pigs. The scorbutic picture in man is usually accompanied by a deficiency of other vitamins and nutrients, and the haemorrhagic state may reflect more than a lack of ascorbic acid. There is much evidence to indicate a capillary abnormality in scurvy (Findlay, 1921; Wolbach & Howe, 1926; Wolbach & Bessey, 1942; Lee & Lee, 1947), and the haemorrhage is generally believed to result from this vascular alteration. Other evidence indicates some change in the blood-clotting mechanism. Sullivan, Gangstad & Link (1943) observed a prolonged one-stage prothrombin time with scorbutic guinea-pig plasma. This finding was confirmed by Marx & Bayerle (1943), who in addition found an increase in the fibrinogen concentration. Both abnormalities disappeared after the administration of ascorbic acid.

To our knowledge there is no information of possible changes at the thromboplastic level of blood coagulation. In guinea-pigs, which we have used in these experiments, it is possible to produce a pure ascorbic-acid deficiency under controlled conditions. We have therefore studied some aspects of the coagulation mechanism in scorbutic and normal guinea-pigs with a view to extending the observations of these earlier workers.