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The participation of methionine and cysteine in the formation of bonds resistant to the action of proteolytic enzymes in heated casein

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1. The influence of temperature, moisture content and the presence of glucose on the level of available methionine and cysteine in casein was studied.

2. Differences between total and available methionine and cysteine contents of heated casein (90° for 24 h) were determined by an in vitro method. The maximum losses in total and available methionine content were 22 and 51% respectively. The losses in total and available cysteine content were 24 and 100% respectively.

3. The results indicated that for heated casein the release of amino acids by proteolytic enzymes was less complete than for native casein.

4. The results of rat growth assays suggested that diets containing oxidized casein are less well utilized by rats than those containing native casein. The decrease in body-weight of rats receiving the diets containing oxidized casein could be counteracted by the addition of methionine and 20 g unoxidized casein/kg diet.

5. There was a lower level of some available amino acids (determined after enzymic hydrolysis using pancreatopeptidase E (*EC* 3.4.4.7), leucine aminopeptidase (*EC* 3.4.1.1) and prolidase (*EC* 3.4.3.7)), including those essential for the rat, in oxidized casein as compared with native casein.

6. Cysteic acid, in oxidized casein, probably makes impossible the utilization of the amino acids in its neighbourhood.

7. From the differences in the available amino acid contents of the native, oxidized and heated casein it was concluded that the oxidation of casein causes the formation of complexes in the polypeptide chain, resistant to enzymic hydrolysis, but to a much lesser extent than does heating.

The effects of high temperatures and the presence of reducing sugars during processing are the most common reasons for the reduction in the biological value of proteins of food products. This reduction is only to a small extent correlated with destruction of amino acids, and is mainly the result of a decrease in the available amino acid content associated with the formation of polypeptide fragments resistant to the action of proteolytic enzymes (Miller, Hartley & Thomas, 1965).

The changes which occur in proteins as a result of technological processing are generally measured by methods based on changes in available lysine content, as this amino acid is very easy to determine both chemically and biologically.

It is known that the decrease in available lysine in proteins subjected to thermal processing is due to the blocking of ϵ-amino groups, which occurs both in the presence and absence of reducing sugars, although the mechanism in the latter instance is not known.

Some workers have suggested that in the absence of carbonyl components character-

istic of the Maillard reaction, the formation of glutamyl-lysine or aspartyl-lysine in drastically heated proteins could explain the decrease in biological value (Bjarnason & Carpenter, 1969, 1970).

However, Waibel & Carpenter (1972) have reported that these types of bonds are hydrolysed by proteolytic enzymes and the liberated lysine can be utilized by the organism. Thus reactions of this type cannot be responsible for the decrease in the available lysine content and, as a consequence, the biological value of the protein. Although the available amino acid content can be determined microbiologically, this method cannot be used to study the mechanism of changes occurring in the other amino acids in proteins, which lead to a decrease in biological value.

Therefore because of procedural difficulties little is known about the effects of processing on the formation, in proteins, of forms of amino acids which are not available to the organism, e.g. methionine sulphone or cysteic acid (Njaa, 1962; Carpenter & Bjarnason, 1968) and the participation of amino acids other than lysine in the formation of polypeptide fragments resistant to the action of proteolytic enzymes.

In the present work both in vivo and in vitro experiments were done to study the effects of the presence of oxidized forms of sulphur amino acids (methionine sulphone and cysteic acid) in casein on the liberation of amino acids, and how methionine and cysteine in casein subjected to high temperatures participate in the formation of bonds resistant to hydrolysis by proteolytic enzymes.

EXPERIMENTAL

Materials and methods

Casein (BDH Ltd, Poole, Dorset) was oxidized by the performic acid method of Toennies (1942). Air-dried casein and casein containing 800 mg moisture/g were heated in an oven at 90° for 24 h in the presence or absence of D-glucose (50 mg/g casein).

Enzymic hydrolysis of casein using pancreatopeptidase E (*EC* 3.4.4.7) (K & K Laboratories Inc., California, USA) was done by the method of Pieniążek, Rakowska, Szkiłądziowa & Grabarek (1975). After 24 h hydrolysis the pH of the hydrolysate was adjusted to 2·0 using 1 M-HCl and the sample was evaporated to dryness; 5 ml 0·05 M-Tris–HCl (pH 8·5), 0·1 ml toluene, 0·1 ml 0·04 M-$MnSO_4$ and 0·2 ml leucine aminopeptidase (*EC* 3.4.1.1) (Sigma Chemical Corp., St Louis, Missouri, USA) and 0·1 ml of a solution containing 10 mg prolidase (*EC* 3.4.3.7) (isolated from pig kidney by the method of Smith (1955))/ml, were added and the mixture was incubated at 40° for 24 h with continuous shaking. After hydrolysis, the samples were desalted using Dowex 50W × 8 (Serva Feinbiochemica, Heidelberg, Germany).

The available methionine and cysteine contents of casein were determined by the in vitro method of Pieniążek *et al.* (1975).

The total amino acid content of casein was determined by the acid-hydrolysis method of Moore, Spackman & Stein (1958). The amino acid contents of enzymic hydrolysates and acid-hydrolysates were estimated using an amino acid analyser (TSM; Technicon Instruments Corporation, Tarry Town, New York, USA).

Table 1. *Composition (g/kg) of diets containing 110 or 180 g protein/kg, with casein or oxidized casein as the sole source of protein, for rats*

Ingredients	Diet§							
	D1	D'1	D2	D'2	D3	D'3	D4	D5
Casein	140	230	—	—	—	—	20	20
Oxidized casein	—	—	140	230	140	230	120	120
Wheat starch	416·5	327	406	316	402	310	406	403
Potato starch	50	50	50	50	50	50	50	50
Sucrose	200	200	200	200	200	200	200	200
Soya-bean oil	120	120	120	120	120	120	120	120
Cod-liver oil	20	20	20	20	20	20	20	20
Mineral salts*	40	40	40	40	40	40	40	40
Vitamin B supplement†	10	10	10	10	10	10	10	10
Amino acid supplements‡:								
L-methionine	—	—	—	—	3·5	5·7	—	3·0
L-cysteine	3·5	3·2	4·0	4·0	4·0	4·0	4·0	4·0
L-tyrosine	—	—	8·0	8·0	8·0	8·0	8·0	8·0
L-tryptophan	—	—	2·0	2·0	2·0	2·0	2·0	2·0
Energy (MJ (Mcal)/kg diet)	16·7 (4)	16·7 (4)	16·7 (4)	16·7 (4)	16·7 (4)	16·7 (4)	16·7 (4)	16·7 (4)

* Hawk, Oser & Summerson (1947), modified as described by Kunachowicz (1970).
† Prepared as described by El-Maraghi, Platt & Stewart (1965).
‡ Cysteine, tyrosine and tryptophan were added in amounts recommended by Schweigert & Guthneck (1954).
§ D1–D5, 110 g protein/kg; D'1–D'3, 180 g protein/kg.

Rat assays

Wistar rats, 25 d old, (from the Institute colony) were divided into groups of five and given diets containing 110 or 180 g protein/kg, with casein or oxidized casein as the sole protein source, for 11 or 14 d. The compositions of the diets are shown in Table 1.

Diets D2–D5 and D'2–D'3 were supplemented with tryptophan, tyrosine and cysteine (Schweigert & Guthneck, 1954) and diets D3-D5 and D'3 were supplemented with methionine, because oxidation of casein is associated with the complete destruction of tryptophan, the partial destruction of tyrosine, and the conversion of cysteine to cysteic acid and methionine to methionine sulphone, neither of which is utilized by the organism (Miller & Samuel, 1968; Ellinger & Palmer, 1969).

The methionine supplement was equivalent to the methionine content of diets D1 and D'1 respectively. Food intake and growth rate were recorded after 11 or 14 d.

RESULTS

Effects of temperature, moisture content and the presence of glucose on the available and total methionine and cysteine contents of casein

The available methionine and cysteine contents of casein with various moisture contents heated (90° for 24 h) in the presence or absence of D-glucose were determined after enzymic hydrolysis of the casein using pancreatopeptidase E (Pieniążek

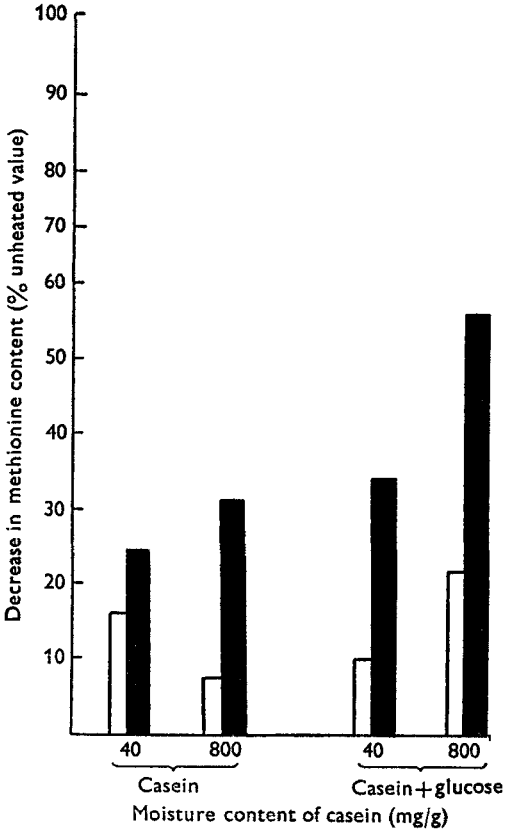

Fig. 1

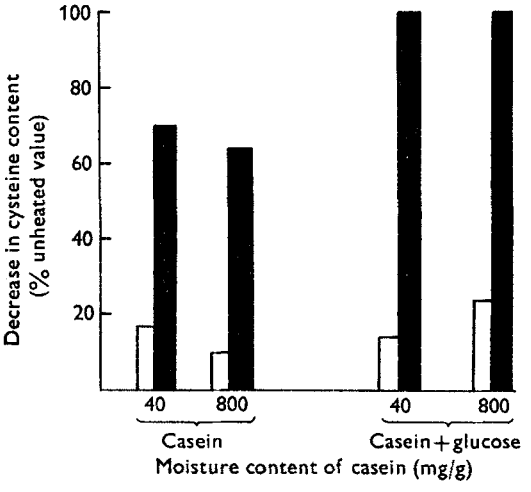

Fig. 2

Fig. 1. The effect of D-glucose and moisture content on the total (□) and available (■) methionine contents of casein heated at 90° for 24 h. Casein containing 40 or 800 mg moisture/g was heated in the presence or absence of 50 mg D-glucose/g casein. Total and available methionine contents were determined after acid-hydrolysis (Moore, Spackman & Stein, 1958) and by the in vitro method of Pieniążek, Rakowska, Szkiłądziowa & Grabarek (1975) respectively.

Fig. 2. The effect of D-glucose and moisture content on the total (□) and available (■) cysteine contents of casein heated at 90° for 24 h. Casein containing 40 or 800 mg moisture/g was heated in the presence or absence of 50 mg D-glucose/g casein. Total and available cysteine contents were determined after acid-hydrolysis (Moore, Spackman & Stein, 1958) and by the in vitro method of Pieniążek, Rakowska, Szkiłądziowa & Grabarek (1975) respectively.

et al. 1975), and the total methionine and cysteine contents were estimated by the method of Moore *et al.* (1958).

There was a reduction in the amounts of available and total methionine and cysteine (Figs. 1 and 2).

The total methionine content of heated casein ranged from 8 to 22% of the value for unheated casein, with the greatest reduction occurring when casein containing 800 mg moisture/g was heated in the presence of glucose. Values for available methionine content ranged from 24 to 51% of the value for unheated casein, and both moisture and glucose caused a reduction in the available methionine content.

The total cysteine content decreased by 10–24% when casein was heated and, as

Table 2. *The total amino acid content of unheated casein, and of casein with different moisture contents, heated at 90° for 24 h in the presence or absence of* D-*glucose (50 mg/g casein), determined after acid-hydrolysis**

(Mean values for two determinations)

	Casein								
	Unheated	Heat-treated				Heat-treated + glucose			
Moisture content (mg/g) ...		40		800		40		800	
	Content (g/kg CP)	Content (g/kg CP)	Loss† (%)	Content (g/kg CP)	Loss† (%)	Content (g/kg CP)	Loss† (%)	Content (g/kg CP)	Loss† (%)
Amino acid									
Cysteic acid	4·6	3·8	17	4·2	10	4·0	14	3·5	24
Methionine sulphone	31·5	26·2	16	29·0	8	28·5	10	24·5	22
Aspartic acid	82·1	83·2	0	82·4	0	82·9	0	81·6	1
Threonine	39·0	40·0	0	38·4	4	39·7	0	38·7	0
Serine	61·3	55·8	9	55·5	10	59·7	3	59·0	4
Glutamic acid	176·0	176·5	0	176·8	0	177·3	0	176·9	0
Proline	107·0	103·4	3	98·9	8	100·8	6	107·2	0
Glycine	20·2	22·4	0	22·9	0	22·4	0	24·2	0
Alanine	31·8	34·6	0	35·4	0	32·0	0	34·1	0
Valine	59·8	59·2	1	60·6	0	58·9	2	55·4	7
Isoleucine	48·5	49·1	0	48·8	0	48·3	0	45·0	7
Leucine	88·5	90·0	0	88·8	0	86·4	2	85·8	3
Tyrosine	59·8	53·8	10	55·1	8	52·8	12	54·9	8
Phenylalanine	53·8	51·3	5	48·6	10	50·0	7	52·6	2
Lysine	80·0	69·4	13	66·4	17	62·5	22	66·1	18
Histidine	29·3	30·6	0	29·8	0	31·2	0	32·1	0
Arginine	39·4	35·3	10	35·5	10	34·5	12	36·1	8

CP, crude protein (nitrogen × 6·25).

* Method of Moore, Spackman & Stein (1958). † Relative to unheated value.

for methionine, the total cysteine content was affected by moisture and glucose and the effect was greatest when casein containing 800 mg moisture/g was heated in the presence of glucose (24% reduction). The reduction in the available cysteine content was very high (64–100%) and although moisture content did not affect the cysteine content, glucose caused a marked decrease.

Differences between the total and available methionine and cysteine contents of casein were not caused by the lowering of the total values in the sample during enzymic hydrolysis, since the recovery of nitrogen was 96–98%.

To study changes which could occur in the amino acid composition of casein as a result of heating, and which could cause some differences between the total and available S amino acid contents, the amino acid compositions of the test samples were determined after acid-hydrolysis, and after enzymic hydrolysis procedures using pancreatopeptidase E, leucine aminopeptidase and prolidase. The results are shown in Tables 2 and 3. There was a slight decrease in the total amount of some amino acids when casein was heated, and moisture content and glucose affected only the S amino acid contents, which were lowest in casein containing 800 mg moisture/g heated at

Table 3. *The available amino acid content of unheated casein, and of casein with different moisture contents heated at 90° for 24 h in the presence or absence of* D-*glucose (50 mg/g casein), determined after enzymic hydrolysis with pancreatopeptidase E* (EC 3.4.4.7), *leucine aminopeptidase* (EC 3.4.4.1) *and prolidase* (EC 3.4.3.7)

(Mean values for four determinations for unheated casein and for two determinations for heated casein)

	Casein								
	Unheated	Heat-treated				Heat-treated + glucose			
Moisture content (mg/g) ...		40		800		40		800	
		Content		Content		Content		Content	
	(g/kg CP)	(g/kg CP)	Loss† (%)	(g/kg CP)	Loss† (%)	(g/kg CP)	Loss† (%)	(g/kg CP)	Loss† (%)
Amino acid									
Methionine	30·1	7·5	75	9·3	70	3·6	88	2·6	91
Aspartic acid	17·8	11·4	36	4·8	73	10·1	43	3·4	81
Threonine	42·9	26·6	38	18·7	56	23·2	46	17·6	60
Serine	32·3	18·2	44	11·9	63	12·5	61	10·6	67
Glutamic acid	73·3	55·7	24	18·9	74	39·8	46	19·5	73
Proline	96·3	82·6	14	18·4	81	64·6	23	17·0	82
Glycine	21·0	15·2	28	7·4	65	10·1	52	5·8	72
Alanine	25·9	22·2	14	14·9	42	15·5	40	13·1	49
Valine + cysteine*	59·5	57·4	4	32·9	45	27·5	54	28·6	62
Isoleucine	44·8	46·1	0	21·3	52	18·6	58	17·1	62
Leucine	77·0	74·6	3	58·3	24	39·8	48	52·3	32
Tyrosine	54·6	55·4	0	53·6	2	37·1	32	41·6	24
Phenylalanine	43·7	46·6	0	42·2	3	37·1	15	33·4	24
Lysine	66·4	55·5	15	46·1	31	45·1	32	36·5	45
Histidine	25·9	19·5	25	17·9	31	14·0	46	4·6	82
Arginine	40·6	31·5	22	34·7	15	25·0	38	25·8	36

CP, crude protein (nitrogen × 6·25).
* Cysteine was not determined as it co-chromatographed with valine.
† Relative to unheated value.

90° in the presence of D-glucose. Higher levels of some amino acids (glycine, alanine and histidine) were found for heated casein.

There was a significant decrease in the amino acid content (determined after enzymic hydrolysis) when casein containing 40 mg moisture/g was heated at 90° for 24 h. Valine, isoleucine, leucine, tyrosine and phenylalanine were exceptions and the levels of these amino acids were unchanged. The greatest decrease in amino acid content was found in samples containing 800 mg moisture/g, heated in the presence of D-glucose.

On heating there was a 75% decrease in the methionine content of casein containing 40 mg moisture/g, but no further reduction was found when the moisture content was increased, although heating in the presence of glucose produced an 88% reduction. The greatest decrease (91%) was found in the casein with 800 mg moisture/g, heated in the presence of D-glucose.

Table 4. *Body-wt gain for rats fed for* 11 *d on diets containing* 110 *g protein/kg, with casein* (D1), *oxidized casein* (D2) *or oxidized casein+methionine* (D3) *as the sole source of protein*

(Mean values for five rats/group)

	Body-wt gain	
Diet*	g/rat per 11 d	% D1 value
D1	+20	100
D2	−12	−60
D3	+6	30

* For details of diets, see Table 1.

Table 5. *Body-wt gain, food intake and food conversion efficiency for rats fed for* 11 *d on diets containing* 180 *g protein/kg, with casein* (D'1, *control*), *oxidized casein* (D'2) *or oxidized casein+methionine* (D'3) *as the sole source of protein*

(Mean values for five rats/group)

	Body-wt gain		Food intake	Food conversion efficiency	
Diet*	g/rat per 11 d	% D'1 value	(g DM/rat per 11 d)	g body-wt gain/ g food intake	% D'1 value
D'1	+43	100	84·6	0·508	100
D'2	−13	−30	20·6	−0·636	−125
D'3	+14	32	41·3	0·341	+67

DM, dry matter.
* For details of diets, see Table 1.

The effect of methionine sulphone and cysteic acid in oxidized casein on its utilization by rats

The values for body-weight gain for rats (in the 11 d experimental period) given diets D1–D3 (casein, oxidized casein and oxidized casein + methionine respectively) and weight gain as a percentage of that for rats given diet D1 are shown in Table 4. The weight gain for rats given diet D3 was only 30% of that for rats given diet D1.

To determine whether this low growth rate was associated with a toxic effect of the oxidized casein, the growth rate of rats fed on the same diets but with 180 g protein/kg (D'1–D'3) was studied; the results obtained are shown in Table 5.

The growth rate for rats given diet D'3 was 32% of that for rats given diet D'1 and the food conversion efficiency was 67% of that for rats given diet D'1. As growth rates obtained with diets containing 110 or 180 g protein/kg were similar, it was assumed that poorer growth of animals was not due to the toxic effect of oxidized casein, but to the amino acid deficiency in diets D3 and D'3 which had the same amino acid content (determined after acid-hydrolysis).

When 20 g oxidized casein in diets D2 and D3 were replaced by 20 g casein (diets D4 and D5 respectively), the weight gain and food conversion efficiency for rats given diet D5 were 76 and 93% respectively of that for rats given diet D1 (Table 6). These

Table 6. *Body-wt gain, food intake and food conversion efficiency for rats fed for* 14 *d on diets containing* 110 *g protein/kg, with casein* (*D*1, *control*), *oxidized casein+casein* (*D*4) *or oxidized casein+casein+methionine* (*D*5) *as the sole source of protein*

(Mean values for five rats/group)

	Body-wt gain		Food intake	Food conversion efficiency	
Diet*	g/rat per 14 d	% D1 value	(g DM/rat per 14 d)	g body-wt gain/ g food intake	% D1 value
D1	+55	100	102·8	0·534	100
D4	−11	−20	23·3	−0·475	−88
D5	+34	+76	69·6	0·489	+93

DM, dry matter.
* For details of diets, see Table 1.

Table 7. *Amino acid content* (*g/kg crude protein* (*nitrogen* × 6·25)) *of casein and oxidized casein determined after acid-hydrolysis* and enzymic hydrolysis with pancreato-peptidase E* (EC 3.4.4.7), *leucine aminopeptidase* (EC 3.4.1.1) *and prolidase* (EC 3.4.3.7)

(Mean values for two determinations)

	After acid-hydrolysis			After enzymic hydrolysis		
Amino acid	Casein	Oxidized casein	Difference (%)	Casein	Oxidized casein	Difference (%)
Cysteic acid	4·5	4·4	0	—	0	—
Methionine sulphone	31·5	30·5	0	—	22·4	23
Methionine	—	—	—	30·1	—	—
Aspartic acid	82·1	80·0	3	17·6	16·6	7
Threonine	39·0	46·5	0	42·9	44·0	0
Serine	61·3	62·3	0	32·3	32·8	0
Glutamic acid	176·0	168·0	6	73·3	80·2	0
Proline	107·0	98·5	8	96·3	80·0	17
Glycine	20·2	20·4	0	21·0	17·3	18
Alanine	31·8	31·1	1	25·9	26·6	0
Valine	59·8	58·6	2	59·5	52·5	11
Isoleucine	48·5	45·5	4	44·8	41·9	6
Leucine	88·5	88·5	0	77·0	66·8	13
Phenylalanine	53·8	52·0	3	43·7	43·9	0
Histidine	29·3	27·4	6	25·0	23·1	11
Arginine	39·4	36·5	7	40·6	0	100
Tryptophan	22·4	—	100	†—	†—	—
Tyrosine	59·8	39·5	34	54·6	34·4	37
Lysine	80·0	79·4	1	66·0	66·0	0

* Method of Moore, Spackman & Stein (1958).
† Tryptophan was not determined after enzymic hydrolysis.

results suggested that the addition of 20 g casein/kg to a diet containing oxidized casein and methionine (D5) allowed growth at a level comparable to that of rats fed on the control casein diet (D1) and that the lower growth of rats given diet D3 (oxidized casein+methionine) compared with that for rats given diet D5 (oxidized casein+casein+methionine) was due to incomplete hydrolysis of oxidized casein by proteolytic

enzymes in vivo. Therefore in vitro studies of the action of proteolytic enzymes on casein and oxidized casein were done.

The amino acid contents of casein and oxidized casein were determined after enzymic hydrolysis with pancreatopeptidase E, leucine aminopeptidase and prolidase, and also after acid-hydrolysis as a control; the results are shown in Table 7.

Oxidation of casein was associated with the total destruction of tryptophan and 34% loss of tyrosine (determined after acid-hydrolysis) while values obtained after enzymic hydrolysis suggested that oxidized casein was less well hydrolysed than casein in respect of the release of amino acids such as proline, glycine, valine, leucine, histidine and arginine; the latter occurred only in trace amounts. Values for the release of cysteic acid and methionine sulphone were 0 and 77% respectively. Of the amino acids mentioned, valine, leucine, histidine and arginine are essential to the rat. It was assumed therefore that the deficiency of these amino acids in diet D3 (oxidized casein + methionine) was the cause of the poor growth rate of the animals.

DISCUSSION

Differences were found in the available and total contents of methionine and cysteine for casein heated at 90° for 24 h. In a further stage of the study an attempt was made to interpret these differences. The amino acid composition of casein containing different moisture contents, heated in the presence and absence of D-glucose, was estimated after acid-hydrolysis (to obtain the total amino acid contents) or after enzymic hydrolysis (to determine the amounts of released forms of the amino acids). It was found that the total amino acid content was not significantly affected by moisture content or by the presence of glucose. Similar results were reported by Bjarnason & Carpenter (1970) for heated bovine serum albumin, but they found no decrease in methionine content, although there was a 60% reduction in the cysteine content. Our results suggested that there was a decrease in both methionine and cysteine contents (Table 2), which in the latter instance was 17%. It should be stressed, however, that different workers have reported different values for losses of cysteine depending on the type of protein passing through the technological processes (Ellinger & Boyne, 1965; Miller *et al.* 1965). Increased levels of some amino acids have been reported by other workers (Dawson & Woodham, 1966; Bjarnason & Carpenter, 1970).

The use of pancreatopeptidase E, leucine aminopeptidase and prolidase for enzymic hydrolysis, as an optimal system for the liberation of amino acids in vitro, made possible a complete comparison of amino acid composition of heated and unheated casein.

A significant decrease was found in the amino acid content of casein on heating, and there was a further decrease in the presence of increased moisture and of glucose. The reduction in methionine content (Table 3) was 91% for casein containing 800 mg moisture/g, heated in the presence of glucose.

When amino acid contents were determined after acid-hydrolysis, heating of casein resulted in a slight destruction of amino acids but there was a significant lowering of the amino acid contents of heated casein analysed after enzymic hydrolysis, suggesting

that polypeptide fragments were formed which were resistant to the action of proteolytic enzymes.

It has been suggested that when high temperatures are applied to S amino acids they may be partly converted into their oxidized forms, which may result in the formation of peptide fragments resistant to enzymic digestion.

In the present work we found a lower growth rate for rats given a diet containing oxidized casein, compared to that for rats given the casein diet, although the total amino acid contents of the diets, as determined after acid-hydrolysis, were identical.

The use of enzymic hydrolysis (pancreatopeptidase E, leucine aminopeptidase and prolidase) to study the amino acid content of oxidized casein allowed this difference in the growth of rats to be interpreted. The concentrations of the available amino acids were found to be different for casein and oxidized casein; for oxidized casein the content of some essential amino acids, particularly arginine, was lower (Table 7). The reason for the lower growth rate of animals given the diet containing oxidized casein was presumably that the available amino acid contents were different. This was confirmed by the high food conversion efficiency obtained when 20 g oxidized casein/kg diet was replaced by 20 g casein (93 % of the value for the diet containing casein only).

The results of in vivo and in vitro studies have suggested that oxidation of casein not only causes the destruction of some amino acids (tryptophan, tyrosine) and the oxidation of S amino acids to forms (methionine sulphone and cysteic acid) not available to the organism, but also leads to the formation, in some fragments of polypeptide chain, of bonds resistant to the action of proteolytic enzymes, which in turn leads to a decrease in the concentration of available amino acids.

In the present work cysteic acid was not released from oxidized casein by proteases, and the available proline, glycine, valine, leucine, histidine and arginine contents of oxidized casein were lower than those for casein. These results are similar to those obtained by Wilkes, Bayliss & Prescott (1973), who found that no amino acids were released from peptides in the neighbourhood of cysteic acid.

Oxidation of casein was found to be associated with the formation of complexes resistant to proteolytic enzymes but to a considerably lesser extent than for heated casein (see Tables 3 and 7), suggesting that the decrease in available amino acid contents for heated casein was not correlated only with the presence of cysteic acid in the polypeptide chain.

REFERENCES

Bjarnason, J. & Carpenter, K. J. (1969). *Br. J. Nutr.* **23**, 859.
Bjarnason, J. & Carpenter, K. J. (1970). *Br. J. Nutr.* **24**, 313.
Carpenter, K. J. & Bjarnason, J. (1968). *Proc. I.B.P. Symp., Stockholm* p. 319.
Dawson, R. & Woodham, A. A. (1966). *Proc. Nutr. Soc.* **25**, ix.
Ellinger, G. M. & Boyne, E. B. (1965). *Br. J. Nutr.* **19**, 587.
Ellinger, G. M. & Palmer, R. (1969). *Proc. Nutr. Soc.* **28**, 42A.
El-Maraghi, N. R. H., Platt, B. S. & Stewart, R. J. C. (1965). *Br. J. Nutr.* **19**, 491.
Hawk, P. B., Oser, B. L. & Summerson, W. H. (1947). *Practical Physiological Chemistry*, p. 1163. Philadelphia: W. B. Saunders Co.
Kunachowicz, H. (1970). Investigation on net protein utilization coefficient (NPU) in relation to the growth rate of experimental animals. PhD Thesis, Institute of Food and Nutrition, Warsaw, Poland.
Miller, D. S. & Samuel, P. (1968). *Proc. Nutr. Soc.* **27**, 21A.
Miller, E. L., Hartley, A. W. & Thomas, D. C. (1965). *Br. J. Nutr.* **19**, 565.

Moore, S., Spackman, D. H. & Stein, W. H. (1958). *Analyt. Chem.* **30**, 1185.
Njaa, L. R. (1962). *Br. J. Nutr.* **16**, 571.
Pieniążek, D., Rakowska, M., Szkiłłądziowa, W. & Grabarek, Z. (1975). *Br. J. Nutr.* **34**, 175.
Schweigert, B. S. & Guthneck, B. T. (1954). *J. Nutr.* **54**, 333.
Smith, E. L. (1955). *Meth. Enzym.* **2**, 93.
Toennies, G. (1942). *J. biol. Chem.* **145**, 667.
Waibel, P. E. & Carpenter, K. J. (1972). *Br. J. Nutr.* **27**, 509.
Wilkes, S. H., Bayliss, M. E. & Prescott, J. M. (1973). *Eur. J. Biochem.* **34**, 459.

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