The effect of Maillard reaction products on zinc metabolism in the rat

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The effect of giving Maillard reaction products (MRP) on zinc metabolism was investigated in the rat. In Expt 1, MRP were prepared by incubating casein with either glucose or lactose under controlled reaction conditions, and were quantified as either 'early' or 'advanced' after estimation of lysine loss and lysine destruction respectively. In Expt 2, the effect of the purified early MRP fructose–lysine (FL) on Zn metabolism was studied. The experimental diets containing 20 mg Zn/kg were given to weanling rats for 21 d. Zn balance was assessed over 9–14 d (Expt 1), or 1–14 d (Expt 2). Femur, liver, kidney and serum Zn concentrations were determined at 21 d. The major effect of the MRP in the casein–sugar mixtures was on urinary Zn excretion. The casein–glucose MRP induced up to a 6-fold increase in the quantity of Zn excreted in the urine. The magnitude of the hyperzincuria increased with the extent of the Maillard reaction. Similar dietary levels of casein–lactose MRP increased urinary Zn loss 2-fold. Free FL had no effect on urinary Zn. Faecal Zn, Zn retention, liver, femur and serum Zn were generally not influenced by giving MRP from casein–sugar mixtures or by giving free FL, although kidney Zn was decreased in rats fed on FL. It was concluded that although urinary Zn excretion can be increased by the presence of MRP in the diet, this is only a minor excretory pathway and would have little influence on overall Zn nutrition in individuals fed on a diet adequate in Zn.

Maillard reaction products: Zinc metabolism: Rat

The Maillard reaction between proteins and reducing sugars is primarily responsible for the loss in nutritional value of food proteins during processing and storage (Mauron, 1981; Hurrell, 1984). The reaction may be divided into two stages. In the early Maillard reactions, the amino acid lysine reacts with reducing sugar to form the colourless 'Amadori' (ϵ-deoxyketose) derivative. On further heating, in the advanced phase of the reaction, this compound breaks down to form a variety of secondary low molecular weight derivatives which are colourless or pale yellow. These secondary derivatives react with other amino acid side chains (Hurrell *et al.* 1983) and condense to form the coloured, higher molecular weight melanoidins.

Many anti-nutritional and anti-physiological effects of the Maillard reaction in foods have been reported following animal studies (Adrian, 1974). Most of these are not linked to the presence of the Maillard reaction products (MRP) *per se*, but result from a protein inadequacy due to decreased lysine bioavailability and reduced protein digestibility (Pintauro *et al.* 1983). Other studies have indicated that there may be an effect of orally or parenterally administered MRP on mineral metabolism (Stegink *et al.* 1975; Adrian & Boisselot-Lefebvres, 1977; Johnson *et al.* 1983; Andrieux & Sacquet, 1984) and particularly on zinc (Stegink *et al.* 1975, 1981; Johnson *et al.* 1983; Furniss *et al.* 1986; Lykken *et al.* 1986). In these studies it was suggested that MRP chelated Zn and increased its excretion via the urinary or faecal pathway, or both. In adult humans fed on MRP, Zn retention was

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reduced but there was no effect on urinary Zn loss (Johnson *et al.* 1983; Lykken *et al.* 1986), except when administered by the intravenous route (Stegink *et al.* 1975, 1981). In contrast, feeding rats with MRP led to significant hyperzincuria (Furniss *et al.* 1986), although tissue concentrations of Zn were not altered after 2 months.

In the previously mentioned studies, the chemical nature of the MRP with Zn-chelating potential was not established, although the urinary excretion of Zn was positively correlated with the dietary level of fructose–lysine (FL), the early Maillard derivative of lysine and glucose (Furniss *et al.* 1986). The diversity of the MRP in terms of chemical and physical properties, the difficulty in their quantification, together with the lack of information on the metabolic fate of MRP in humans have complicated this issue. The interpretation of the experimental results may, however, be facilitated by the use of well-defined models of the Maillard reaction. In a previous study (Furniss *et al.* 1986), we reported the hyperzincuric effect of MRP from casein–glucose mixtures prepared under controlled reaction conditions to contain almost exclusively MRP of the early type. The aims of the present study were to investigate further the nature of the Maillard compounds that induce hyperzincuria, and to assess the nutritional significance of the effect by measurement of Zn balance and status. Two experiments were performed. In Expt 1, casein was heated together with glucose or with lactose to form MRP of the early, and of the advanced type. The reaction conditions were carefully controlled so that the predominant MRP formed in the casein–glucose or casein–lactose mixtures were the deoxy-ketose derivatives of lysine, and lactose–lysine (LL). These early MRP, and their advanced Maillard degradation products, were quantified using the furosine technique (Finot *et al.* 1981), Nitrogen balance was also measured in order to determine the N digestibility and the N retention coefficients of the Maillard diets. In Expt 2, the effect of giving the purified MRP, FL was investigated.

EXPERIMENTAL

Preparation of MRP

Expt 1. Casein–glucose and casein–lactose samples were prepared by mixing casein, 4 kg (E. Merck, Type Hammersten, Darmstadt, W. Germany) with either glucose monohydrate, 5·6 kg (Merck), or lactose monohydrate, 5·8 kg (Merck), and 20 litres distilled water. The suspensions were homogenized, spray-dried (Niro Atomizer, Copenhagen, Denmark), canned and stored at 4 °C. One batch of spray-dried casein–glucose samples received no further treatment. The second batch was heated for 3 d at 60°. Casein–lactose samples were further heated for 1 d at 50° or for 3 d at 60°. The final water content of the samples was 6·5 g/kg.

Expt 2. Free FL was synthesized by the procedure of Finot & Mauron (1969), but the conditions of purification were simplified in order to prepare larger quantities for the animal test. After the reaction and the evaporation of methanol, α-formyl-fructosyl-lysine dissolved in water was passed through a column of Dowex-50 × 4 (form H^+), washed with water to remove glucose and eluted with 0.5 M-ammonium hydroxide. The fractions containing α-formyl-lysine and lysine were discarded. The deformylation was performed in 2 M-hydrochloric acid at 80° for 2 h. The excess of HCl was removed on a column of Dowex 1 × 4 (form CH_3COO^-) and the eluting solution was treated with active charcoal for discoloration and ε-fructosyl-lysine acetate precipitated. It was slightly brown and contained trace quantities of ε-difructosyl-lysine.

Diet composition

Expt 1. The experimental diets consisted of two control diets made with either glucose or lactose, and four test diets made from the spray-dried casein–glucose mixture, the same

mixture heated 3 d at 60°, the spray-dried casein–lactose mixture heated for 1 d at 50° and for 3 d at 60°. The control diet contained (g/kg): casein 226, glucose or lactose 162, sucrose 250, arachis oil 100, minerals 53 and vitamins 12·5 (as described by Temler *et al.* (1984)), cellulose 20, and maize starch to 1000. The diets containing the heated casein–sugar mixtures had the same composition as the control diet except that half the casein component and half the respective sugar component were replaced by the heated casein–glucose or casein–lactose mixtures.

Each test diet contained 100 g crude protein (N × 6·25)/kg from unreacted casein and 100 g/kg from the heated casein–sugar mixtures and had a lysine level adequate for optimum growth (Warner & Brewer, 1972). The final Zn level in the diets was adjusted to 20 mg/kg with $ZnSO_4.7H_2O$.

Expt 2. The control diet was identical to that of Expt 1. The test diet contained a supplement of 5 g FL (in the acetate form, molecular weight 368)/kg added at the expense of the starch component.

Experimental design

Male weanling rats of the Sprague-Dawley strain (Voss, Tuttlingen, W. Germany), weighing approximately 45 g, were housed individually in plastic metabolism cages. Animals were maintained in a room with a 12 h light–dark cycle, at a temperature of 23–24° and relative humidity of 55%. Animals were fed *ad lib.* on the control or test diets at all times, and received distilled, deionized water. Before the experiment animals underwent a 2 d adaptation period and were fed on the control diet. At the end of this period animals were weighed and assigned to the experimental diets to form groups of six rats of equal average weights.

In Expt 1 Zn balance and N digestibility were measured over days 9–14. In Expt 2 Zn balance was measured over days 1–14, but N digestibility was not determined. Food intake and weight gain were recorded every 3 d, and over the balance period each 2 d. Urine and faecal collections were made over 2 d periods. Before each collection period, cages were thoroughly washed, soaked in EDTA solution (10 g/l) and rinsed in distilled, deionized water. Urine was collected into acid-washed polypropylene containers and then stored at −20°. Faeces were collected into cleaned plastic vessels and transferred to polyethylene bags for storage. At the end of the Zn balance, animals were transferred into individual stainless-steel mesh cages until death and removal of tissues for the assessment of Zn status at 21 d. Animals were killed after drawing blood from the aorta under diethyl ether anaesthesia. The liver and kidneys were removed, thoroughly rinsed in deionized, distilled water and blotted dry. Organs were stored at −20° in individual plastic bags with air-tight seals. Femurs were stored in small polypropylene containers at −20°.

Analytical methods

MRP and amino acids. After acid hydrolysis, the untreated and heated casein–sugar mixtures were analysed for total lysine, reactive lysine, FL and LL by ion-exchange chromatography (Biotronik LC 7000, Munich, W. Germany) using the furosine technique of Finot *et al.* (1981). Reactive lysine refers to those lysine units that have not combined with other food components during processing and are therefore still potentially chemically reactive. The term 'reactive' lysine is synonymous with the term available lysine but is preferred when referring to chemical estimates of lysine (Hurrell & Carpenter, 1981). The same materials were analysed for total amino acids by ion-exchange chromatography (Biotronik LC 7000) and for methionine and cystine after performic acid oxidation (Moore, 1963). Tryptophan was not determined. Free FL was measured in urine by ion-exchange chromatography. The undiluted urine was centrifuged at 2000 rev./min for 15 min and the

pellet was discarded. The clear supernatant fraction was then filtered (0·45 μm Millipore) and diluted in the sample loading buffer. The analysis of free FL was carried out using the physiological fluids standard programme recommended by the manufacturer. The standard FL was synthesized according to the method of Finot & Mauron (1969). Free LL was not measured as the standard material was not available.

Zn analyses. The kidney and liver were lyophilized, and the faecal samples were oven-dried before being ground to a fine powder within polyethylene bags. The homogenized tissue and faeces were then wet-digested using a programmable Tecator digestion system (model 1016, Högenäs, Sweden) in 8 ml nitric acid ('instra-analysed'; Baker Deventer, Holland)/500 mg dry weight tissue. The temperature was gradually increased from 30° to 120° over a period of 8 h, before heating finally to 150° for 15 min, or until the samples were pale-yellow and non-viscous. Samples of bovine liver reference standard (National Bureau of Standards, Washington) were simultaneously digested to check the recovery of Zn. Diets and cleaned femurs were processed by dry-ashing in quartz crucibles in a muffle furnace (Solo, Biel) at 450° for 24 h. Diet samples were treated with a mixture (1:1, v/v) of nitric and hydrochloric acids; samples were evaporated to dryness and heated at 450° for a further 24 h and finally dissolved in nitric acid. Femur samples were solubilized in 1 ml concentrated HCl. Zn was determined in the diluted samples by flame atomic absorption spectrophotometry (model AA-975; Varian International AG, Zug).

N. N was determined in the diets, the urine and the faeces by the method of Kjeldahl.

Calculations. Apparent Zn retention over the metabolic balance period was calculated in mg and as a percentage of the dietary intake.

$$\text{Zn retention (mg)} = \text{Zn ingested (mg)} - (\text{urinary} + \text{faecal Zn})\ \text{(mg)},$$

$$\text{Zn retention (\%)} = \frac{\text{Zn intake} - (\text{urinary Zn} + \text{faecal Zn})}{\text{Zn intake}} \times 100.$$

Apparent N digestibility (AD) and retention coefficient (RC) were calculated as described by Eggum (1973):

$$\text{AD} = \frac{(\text{N intake} - \text{faecal N})}{\text{N intake}},$$

$$\text{RC} = \frac{(\text{N intake} - (\text{faecal N} + \text{urine N})}{\text{N intake}}.$$

Statistical analysis. The results were analysed by Student's *t* test. The levels of significance were 5, 1 or 0·1 %. The values for FL excretion were analysed by analysis of variance followed by the multiple-range test of Duncan (1955).

RESULTS

MRP

Table 1 shows the levels of MRP formed in the heated casein–sugar model systems. The values for lysine as FL or LL give the level of MRP of the early type, whereas the values for lysine destruction reflect the formation of advanced MRP. The spray-dried casein–glucose mixture contained about 40 % of the original lysine units (92·0 mg/g crude protein) as FL, but less than 10 % of the original lysine units involved as advanced MRP. This sample was white in colour. Heating for 3 d at 60° increased the level of FL slightly to 51 % of the original lysine, but increased 3-fold the level of lysine combined as advanced MRP, to 26 % of the original lysine. The product was a pale brown colour. Reactive lysine

Table 1. *Reactive lysine, lysine as fructose–lysine or lactose–lysine*, and lysine destroyed†* (*mg/g crude protein* (*nitrogen* × 6·25)) *in unheated casein and in heated casein–sugar mixtures*

Samples	Reactive lysine	Lysine as fructose– or lactose–lysine		Lysine destroyed†	Final dietary level of lysine as Maillard products (g/kg)	
		Early		Advanced	Early	Advanced
Casein, unheated	92·0	—		—	—	—
Spray-dried casein–glucose mixtures						
Spray-dried	46·9	36·9		8·2	3·69	0·82
Heated for 3 d at 60°	20·6	47·3		24·1	4·73	2·41
Spray-dried casein–lactose mixtures						
Heated for 1 d at 50°	53·0	33·9		5·1	3·39	0·51
Heated for 3 d at 60°	21·9	38·9		31·2	3·89	3·12
SE of analytical values	1·3	0·9		2·0	—	—

* Measured by the furosine technique (Finot *et al.* 1981).

† Calculated as reactive lysine in untreated casein minus (reactive lysine + lysine as fructose–lysine or lactose–lysine) of heated caseins.

in this sample had fallen from 92·0 to 20·6 mg/g crude protein. The two heated casein–lactose mixtures contained similar levels of early and advanced MRP derived from lysine as the heated casein–glucose mixtures. The samples, heated for 1 d at 50° and for 3 d at 60°, were respectively cream and pale brown in colour.

Amino acids

No significant losses of amino acids other than lysine were found in the spray-dried casein–glucose or in the casein–lactose heated for 1 d at 50° (values not shown). However, the casein–glucose and casein–lactose mixtures heated for 3 d at 60° had respectively 45 and 30% losses of arginine and 18 and 21% losses of histidine.

Growth variables

In Expt 1 animals fed on the diet containing the casein–glucose sample heated for 3 d at 60° had diarrhoea over the initial 8 d of the experiment, although none of the other heated casein–sugar samples induced diarrhoea. No other adverse effects were noted.

Food intake, weight gain and food conversion efficiency (FCE) over 21 d are shown in Table 2. Food intake was reduced by giving the spray-dried casein–glucose ($P < 0·05$) or the casein–glucose heated for 3 d at 60° ($P < 0·01$). The heated casein–lactose diets did not significantly influence food intake. Weight gains at 21 d were reduced only in the groups fed on the 3 d heated casein–sugar samples. Values for FCE were not influenced by the heated casein–sugar diets, with the exception of the 3 d heated casein–lactose which caused a small but significant reduction in this variable ($P < 0·05$). In Expt 2, free FL had no effect on growth variables.

N balance (Expt 1)

AD. The increased faecal losses of N led to small reductions in N digestibility for all groups compared with the control (Table 2). The largest effects were noted in the groups fed on the most severely heated mixtures.

RC. This variable was not significantly reduced by giving the heated casein–sugar samples.

Table 2. *Expts 1 and 2. Average daily food intake (g/d), weight gain (g/d) and food conversion efficiency (FCE) (g/g) over 21 d of rats fed on Maillard reaction products in casein–sugar samples (Expt 1) or free fructose–lysine (Expt 2) and nitrogen balance values over 9–14 d (Expt 1)*

(Mean values with their standard errors)

Dietary group	Food intake Mean	SE	Wt gain Mean	SE	FCE Mean	SE	Apparent N† digestibility Mean	SE	N retention† coefficient Mean	SE
Expt 1										
Casein–glucose										
Control, unheated	12·6	0·3	7·2	0·2	0·57	0·01	0·945	0·003	0·614	0·019
Spray-dried, unheated	11·6*	0·2	6·5	0·3	0·56	0·03	0·931**	0·003	0·607	0·024
Spray dried, heated for 3 d at 60°	10·2**	0·3	6·0**	0·2	0·59	0·01	0·909**	0·006	0·564	0·022
Casein–lactose										
Control, unheated	12·5	0·6	7·2	0·3	0·58	0·02	0·942	0·003	0·588	0·016
Spray-dried, heated for 1 d at 50°	12·4	0·3	7·1	0·3	0·57	0·01	0·928*	0·004	0·603	0·015
Spray-dried, heated for 3 d at 60°	12·1	0·3	6·4*	0·2	0·53*	0·01	0·893**	0·004	0·559	0·017
Expt 2										
Control	12·9	0·2	6·4	0·1	0·50	0·02	ND	ND	ND	ND
Fructose–lysine	12·7	0·3	6·5	0·2	0·51	0·03	ND	ND	ND	ND

ND, not determined.
Mean values were significantly different from the respective control group: * $P < 0·05$; ** $P < 0·01$.
† For details of calculations, see p. 742.

Table 3. *Expts 1 and 2. Total zinc excretion in the urine and faeces, Zn retention and weight gain during the balance period by rats fed on Maillard reaction products in casein–sugar samples (Expt 1) or free fructose–lysine (Expt 2)*

(Mean values with their standard errors)

Dietary group	Urinary Zn μg Mean	SE	Urinary Zn % intake Mean	SE	Faecal Zn mg Mean	SE	Faecal Zn % intake Mean	SE	Zn retained† mg Mean	SE	Zn retained† % intake Mean	SE	Wt gain (g) Mean	SE
Expt 1 (9–14 d)														
Casein–glucose														
Control, unheated	13·2	2·6	0·7	0·1	0·73	0·04	37·9	2·2	1·19	0·08	61·5	2·2	33·0	2·4
Spray-dried, unheated	63·7***	4·3	3·7***	0·3	0·70	0·03	40·7	2·6	0·97	0·07	55·6	2·6	36·6	2·4
Spray-dried, heated for 3 d at 60°	83·4***	7·9	4·8***	0·4	0·60*	0·03	34·8	1·3	1·03	0·04	60·4	1·2	30·0	1·8
Casein–lactose														
Control, unheated	25·6	5·2	1·2	0·2	0·70	0·10	34·6	4·3	1·28	0·10	64·2	4·4	33·6	1·8
Spray-dried, heated for 1 d at 50°	28·7	8·3	1·5	0·4	0·63	0·05	34·0	1·4	1·20	0·07	64·6	1·4	33·0	4·2
Spray-dried, heated for 3 d at 60°	42·3*	7·2	2·2*	0·3	0·82	0·06	44·7	4·3	0·99*	0·09	53·2	4·3	27·6*	1·8
Expt 2 (1–14 d)														
Control	32·9	5·3	1·01	0·2	1·40	0·07	43·9	2·4	3·89	0·07	55·1	2·5	80·6	2·0
Fructose–lysine	42·4	7·6	1·31	0·2	1·24	0·08	39·0	2·2	4·04	0·08	59·7	2·2	79·6	4·3

Mean values were significantly different from the respective control group: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
† Calculated as Zn intake − (faecal + urinary Zn).

Table 4. *Expts 1 and 2. Average daily fructose–lysine intake and daily urinary excretion during the balance period of rats fed on Maillard reaction products in casein–sugar samples (Expt 1) or free fructose–lysine (Expt 2)*

(Mean values with their standard errors)

Dietary group	Fructose–lysine intake (μmol/d)		Urinary fructose–lysine excretion			
			μmol/d		% intake	
	Mean	SE	Mean	SE	Mean	SE
Expt 1						
Casein–glucose						
Spray-dried, unheated	321	9	56	9	17·4	2·7
Spray-dried, heated for 3 d at 60°	349	32	33	3	9·8*	1·3
Expt 2						
Fructose–lysine	180	10	35	2	19·6	0·7

Mean value was significantly different from the spray-dried unheated value: * $P < 0.05$.

Zn excretion and retention

Zn excretion and retention over the 6 d Zn balance period of Expt 1 (days 9–14) and over the 14 d balance period of Expt 2 (days 1–14), in absolute quantities and as a percentage of the dietary intake are shown in Table 3. Rats fed on the heated casein–glucose mixtures excreted significantly greater quantities of Zn in the urine than rats fed on the control diet. Urinary Zn excretion by the glucose-fed control rats represented 0·7% of the dietary Zn intake. Urinary Zn excretion by rats fed on the heated casein–glucose diets rose to 3·7 and 4·8% of the dietary intake for the groups fed on the spray-dried casein–glucose and the 3 d heated sample respectively. Urinary Zn excretion by lactose-fed control rats was 1·2% of the dietary Zn intake. Urinary Zn excretion was not significantly higher in the group fed on the casein–lactose sample heated for 1 d at 50°. However, the group fed on the more severely heated casein–lactose excreted almost twice the control level of urinary Zn.

Faecal Zn excretion ranged from 34% to 45% of the dietary intake. Zn retention (mg) over the 6 d balance period tended to be decreased in the groups fed on the heated casein–sugar mixtures. However, only the group fed on the casein–lactose heated for 3 d at 60° retained significantly less Zn ($P < 0.05$). When expressed as a percentage of the intake none of the groups fed on the diets containing the heated casein–sugar samples retained significantly more or less Zn than their respective control groups.

In Expt 2 giving FL had no significant effect on urinary and faecal Zn excretion or on Zn retention.

Urinary FL

Values for the average daily intake and urinary excretion of FL are shown in Table 4. The average daily intakes and excretion of FL by rats fed on the control diets were negligible (< 2 μmol/6 d). In Expt 1, the excretion of FL as a percentage of intake was significantly higher in the group fed on predominantly early MRP than in the group fed on more of the advanced type MRP ($P < 0.05$). The excretion of FL by rats fed on the free compound (Expt 2) corresponded to 19·6 (SE 0·7)% of the intake.

Table 5. *Expts 1 and 2. Zinc concentration of liver, kidney (μg/g dry wt), femur (total μg) and serum (μg/l), after 21 d of feeding Maillard reaction products in casein–sugar samples (Expt 1) or free fructose–lysine (Expt 2)*

(Mean values with their standard errors)

Dietary group	Liver		Kidney		Femur		Serum	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1								
Casein–glucose								
Control, unheated	90·9	3·4	85·7	4·5	43·4	1·0	1770	40
Spray-dried, unheated	98·4	8·3	109·0	17·8	41·9	1·0	1860	70
Spray-dried, heated for 3 d at 60°	84·2	5·3	87·3	5·6	40·7	1·4	1790	90
Casein–lactose								
Control, unheated	91·5	3·7	93·0	3·9	44·4	1·7	1930	50
Spray-dried, heated for 1 d at 50°	90·1	2·8	98·4	6·7	46·9	2·1	1940	70
Spray-dried, heated for 3 d at 60°	87·9	3·0	86·4	3·1	43·8	1·7	1710*	80
Expt 2								
Control	79·4	2·7	115·0	2·0	42·7	1·4	1560	100
Fructose–lysine	82·6	5·7	104·0**	2·0	41·4	1·3	1650	50

Mean values were significantly different from respective control group: * $P < 0·05$; ** $P < 0·01$.

Zn status

The variables measured are shown in Table 5. In Expt 1 no significant differences were found among groups for liver and kidney Zn concentrations (μg/g dry weight). One rat in the group fed on the spray-dried casein–glucose had abnormally high concentrations of Zn in the liver and kidney (2–3-fold the control level), which is the reason for higher standard errors of these variables for this group. In Expt 2 giving FL significantly depressed kidney Zn concentrations ($P < 0·01$). Total femur Zn was not significantly altered by giving the heated casein–sugar mixtures compared with their respective controls, nor by giving FL. However, in Expt 1 values were significantly higher for lactose-fed groups compared with glucose-fed groups ($P < 0·05$). Serum Zn levels were significantly lower in the group fed on 3 d heated casein–lactose (Expt 1), but no other significant differences were found.

DISCUSSION

The spray-drying of casein with glucose or lactose, followed by heating at 50° or 60°, induced Maillard reactions, which were characterized by a progressive browning, loss of reactive lysine (Table 1) and a fall in AD (Table 2). When these heated casein–sugar mixtures were added to rat diets to replace half the casein component, the rats ate slightly less food and grew slightly less well, although there was no decrease in apparent N retention and, except for rats fed on the most severely heated casein–lactose mixture, no reduction in FCE (Table 2). This means that for all three casein–glucose diets and two of the three casein–lactose diets, the same amount of food consumed resulted in similar weight gains and that no additional urinary loss of nutrients would be expected as a result of poor food utilization.

Giving heated casein–glucose led to hyperzincuria, an excessive and constant loss of Zn in the urine, and confirmed our earlier findings (Furniss *et al.* 1986). Since FCE was not reduced, and there was thus no modification in the Zn requirements for growth, these results suggest either Zn chelation by the MRP or a more direct action of these compounds

on the kidney. Giving similar levels of MRP from casein–lactose had much less effect on urinary Zn and giving purified free FL had no effect, despite the urinary excretion of relatively large amounts of this compound (Table 4). Faecal Zn excretion and apparent Zn retention were generally little influenced by MRP from the casein–sugar samples or by purified FL, and Zn status at 21 d was not affected.

The hyperzincuria induced in the rat by the MRP was similar in magnitude to that reported by Freeman & Taylor (1977) and Hsu & Smith (1983) on giving histidine (500 mg/d) and cysteine (420 mg/d) respectively. As in the present study, the constant urinary Zn loss of about 6-fold the control level did not lead to changes in tissue Zn levels at the end of the study period. This is because, despite hyperzincuria, the urinary pathway still remains a minor route for Zn excretion in comparison with the faecal pathway. Zn homeostasis may be maintained by small adaptive changes in the quantity of Zn lost via the faecal route, either through increased Zn absorption or through decreased excretion of endogenous Zn, or both (Weigand & Kirchgessner, 1980; Wada *et al.* 1985). The nature of the MRP responsible and the mechanism of their effect on urinary Zn were not elucidated from the present experiments, although it may be assumed that the effect is due to the absorbed fraction of MRP. This includes the early MRP (Amadori derivatives) and the low-molecular-weight advanced MRP (Premelanoidin fraction), and excludes the high-molecular-weight advanced MRP that are not absorbed (Finot & Magnenat, 1981; Nair *et al.* 1981). In our study, 17·4% of the dietary protein-based FL and 19·6% of the free FL (Table 4) were excreted in the urine compared with 11 and 64% respectively reported by Finot & Magnenat (1981). From our results, the urinary loss of Zn cannot easily be attributed to the presence of either the early or the advanced type MRP. However, we should speculate that the premelanoidin fraction is responsible. This is based on the lack of effect of free FL, and on the observation that rats fed on the heated casein–glucose mixture, containing more advanced MRP and more FL than the same mixture spray-dried, excreted about 40% less FL in the urine (Table 4) but 30% more Zn (Table 3). The mechanism by which premelanoidins increase urinary Zn is unclear. They could chelate Zn, either in the intestine or after absorption, and be excreted still chelated in the urine, or they could have a direct effect on kidney Zn re-absorptive processes.

In spite of the virtual absence of effect on tissue Zn levels in these experiments, it cannot be assumed that the inclusion of MRP in diets low or marginal in Zn (< 10 mg/kg) would also be without effect on Zn nutrition. As with phytate and other inhibitors of Zn absorption, potential negative effects on Zn bioavailability may be masked by the presence of high levels of dietary Zn. In our studies rats were fed on diets containing 20 mg Zn/kg, which is somewhat higher than the rat requirement of 12 mg Zn/kg proposed by Warner & Brewer (1972). This level was chosen to be adequate for growth of the weanling rat, but low enough to permit changes in femur Zn concentrations which can increase with dietary levels up to 30 mg/kg (Weigand & Kirchgessner, 1980). It was not surprising, however, that the extra 4% of dietary Zn appearing in the urine of rats fed on Maillardized casein did not reduce their Zn status.

Maillard reactions are common in heat-processed foods. They occur especially in milk and milk-based food products, since milk contains a high level of lactose, but they also take place during such processes as bread baking, the roasting of coffee and meat and the production of breakfast cereals, and they are universally present in infant formulas as well as in some other infant foods and enteral feeds. Some infant formulas, for instance, may contain up to 10% of their lysine residues combined as lactose–lysine (Hurrell, 1984). While MRP (especially lactose-derived MRP) would be expected to have little influence on overall Zn nutrition of individuals consuming diets adequate in Zn, they do increase urinary Zn excretion and might be an aggravating factor in diets containing borderline

levels of Zn. The presence of MRP should, therefore, be taken into consideration when evaluating the nutritional value of Zn in processed foods.

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