Nutritional and Gastrointestinal Response to Heated Nonfat Dry Milk

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Nonfat dry milk (NFDM) that had been autoclaved for 30 or 45 min to produce Maillard products was incorporated into experimental rat diets; unheated NFDM served as the control. Food intake and body weight gain were reduced in the groups fed heated NFDM. Relative to body weight, enlargement of the kidney, stomach, small intestine, and large intestine but not the pancreas or liver was observed in the heated NFDM groups. Trypsin activity was significantly elevated in the intestine and pancreas whereas amylase was significantly lower. The results indicate that the poor nutritional quality of proteins containing Maillard products is not due simply to the loss of indispensable amino acids but may also reflect the need to adapt and metabolize the nondigestible material in the context of inadequate dietary protein availability.

Nonenzymatic browning or the Maillard reaction is known to occur in foods containing protein and reducing sugar when exposed to elevated temperatures and humidity. In nonfat dry milk the primary reaction appears to be between lactose and the ϵ-amino group of lysine, resulting in deterioration of the protein quality (Block et al., 1946; Ford, 1975; Mauron et al., 1955). Several factors appear to contribute to the poor nutritional quality of proteins containing Maillard products. One obvious factor is the unavailability of indispensable amino acids that have been complexed with the carbohydrate. In addition, the heated protein accumulates in the small intestine, suggesting that the presence of these products can interfere with the overall digestibility of the peptide chain and that the Maillard products are poorly absorbed and compete for amino acid absorption sites in the small intestine (Erbersdobler, 1976; Nesheim and Carpenter, 1967; Mori and Nakatsuji, 1977; Mori, 1978; Valle-Riestra and Barnes, 1970; Lee et al., 1977a,b). The poor quality of heated proteins involves more than just the reduction of amino acid content because supplementation with the unavailable amino acids does not necessarily completely restore the nutritional quality of the protein (Sgarbieri et al., 1973). In a previous paper we demonstrated that rats fed severely damaged casein, heated without carbohydrate, accumulated secreted pancreatic enzymes in the small intestine after a meal, yet heat-treated casein is not a more potent stimulant of pancreatic secretion than unheated casein (Percival and Schneeman, 1979a,b). The results of that study suggested that part of the poor nutritional quality of a severely damaged protein may be due to enhanced loss of endogenously secreted protein.

The objective of the present study was to determine if consumption of heat-treated milk by experimental animals under conditions less severe than the previous work would lead to similar enzyme accumulation and adaptation of the gastrointestinal tract.

EXPERIMENTAL SECTION

The composition of the experimental diet is shown in Table I. Nonfat dry milk (NFDM) was added to provide 15% protein in the diet based on the composition of NFDM published in "Agricultural Handbook 8-1" (1976). The NFDM was unheated or had been autoclaved (121 °C, 2 psi) for 30 min (mild) or 45 min (severe). The 30-min treatment resulted in a very lightly browned product whereas the 45-min treatment resulted in a dark brown product. Male Wistar rats (Simonsen Animals, Gilroy, CA) with an average initial body weight of 91 g were randomly assigned to each dietary treatment for a 4-week period. Food intake was measured during the first week of the study and during week 4. Rats were housed in stainless steel wire mesh cages with a 12-h light/dark cycle. During the last 10 days of the study, food was provided at 1000 h and removed at 1700 h to synchronize diurnal variations during which period the dark cycle was between 1000 and 2200 h. Half of each group was killed 12 h after their last meal and are designated the unfed group. The other half, designated the fed group, were killed 3 h after consuming a meal of their respective diets.

Table I. Diet Composition

	%
nonfat dry milk[a]	42.0
corn starch	37.0
cellulose	5.0
corn oil	8.0
salt mix[b]	6.0
vitamin mix[b]	2.0
BHT	0.02

[a] Provides 15 g of protein and 21 g of carbohydrate. [b] Refer to Schneeman and Gallaher (1980) for composition.

Animals were anesthetized with ether, and the blood was removed by heart puncture. The stomach, stomach contents, small intestine, small intestinal contents, large intestine, kidney, pancreas, and liver were removed as described previously (Schneeman and Gallaher, 1980). Serum was obtained from the blood and analyzed by a clinical laboratory (CBL Laboratories, Sacramento, CA) for glucose, total protein, albumin, and urea nitrogen with a Technicon SMAC analyzer. The wet weights of the kidney and liver were obtained. The dry weights of the gastrointestinal organs and contents were obtained after lyophilizing the samples. The activity of trypsin, chymotrypsin, amylase, lipase, and total protein in the small intestine and pancreas were estimated as previously described (Schneeman and Gallaher, 1980).

Statistical analysis of the data was by one-way analysis of variance with orthogonal contrast to compare each of the heat-treated NFDM groups to the unheated NFDM group and with LSD to compare each group with the others (Nie et al., 1975).

RESULTS

Food intake, final body weight, and plasma glucose, protein, albumin, and urea nitrogen are shown in Table II. The rats consuming unheated NFDM increased their

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Table II. Food Intake and Final Body Weight and Plasma Parameters[a]

	0-NFDM	30-NFDM	45-NFDM
body weight, g	189 ± 8[a] (14)	92 + 3[b] (14)	75 ± 2[c] (14)
food intake, g			
week 1	55 ± 2[a] (14)	39 ± 2[b] (14)	38 ± 2[b] (14)
week 4	79 ± 3[a] (14)	33 ± 1[b] (14)	23 ± 1[c] (14)
plasma			
glucose, mg/dL	158 ± 12[a] (6)	80 ± 5[b] (7)	111 ± 9[c] (6)
urea nitrogen, mg/dL	11 ± 1[a] (6)	13 ± 1[a] (7)	23 ± 2[b] (6)
total protein, g/dL	6.6 ± 0.1[a] (6)	5.9 ± 0.1[b] (7)	5.2 ± 0.2[c] (6)
albumin, g/dL	3.5 ± 0.1[a] (6)	3.1 ± 0.1[b] (7)	2.9 ± 0.1[c] (6)

[a] Values with different superscripts are significantly different. NFDM = nonfat dry milk; 0, 30, or 45 indicates the minutes of autoclaving. The N per group is in parentheses.

Table III. Organ Weight per 100 g of Body Weight from Unfed Rats[a]

	0-NFDM (6)	30-NFDM (7)	45-NFDM (7)
	Wet Weight, Grams		
kidney	0.98 ± 0.02[a]	1.26 ± 0.08[b]	1.37 ± 0.03[b]
liver	3.36 ± 0.20	3.23 ± 0.08	3.52 ± 0.08
	Dry Weight, Milligrams		
stomach	147 ± 2[a]	184 ± 12[b]	192 ± 13[b]
pancreas	107 ± 8	119 ± 5	135 ± 14
small intestine	260 ± 10[a]	275 ± 20[a]	426 ± 61[b]
large intestine	438 ± 38[a]	587 ± 23[b]	557 ± 35[b]

[a] Values without common superscripts are significantly different. NFDM = nonfat dry milk; the 0, 30, or 45 indicates the minutes of autoclaving. The N per group is in parentheses.

food intake and gained weight during the 4-week feeding period, those fed the mildly heated NFDM did not increase food intake and were just able to maintain their initial body weight, and those fed the severely heated NFDM reduced their food intake and lost weight during the experimental period. Fasting glucose, total protein, and albumin were all reduced in the rats consuming heat-treated NFDM, and urea nitrogen was twice as high in the severely heated NFDM group.

Organ weight was measured in the unfed groups and is expressed per 100 g of body weight (table III). Kidney but not liver weight was increased in the rats consuming the heated NFDM. The dry weight of the stomach and large intestine was greater in the heated NFDM groups and the small intestinal dry weight was greater in the severely heated NFDM group relative to the unheated control.

Pancreatic enzyme activity and protein was measured in the intestinal tissue and contents of animals that had been fed a meal prior to being killed. The activities are expressed per gram of intestine plus contents (Table IV). Trypsin and chymotrypsin activities were both about 1.5 times greater in the intestine of rats consuming the heated proteins, amylase was about one-third lower, and lipase was low in the 30-NFDM relative to the 45-NFDM. The intestinal contents of rats fed severly heated NFDM contained more protein but the intestinal tissue protein content was similar. The weight of intestinal contents was slightly elevated in the mildly heated group relative to the unheated control ($p < 0.10$).

Protein and enzyme activity in the pancreas were measured in unfed animals to determine if any changes in the composition of the stored enzymes had occurred (Table V). Protein and chymotrypsin activity were unaffected. Trypsin activity was elevated in the groups fed heated NFDM; this difference was statistically significant ($p < 0.05$) in the 30-NFDM group and was close to significant in the 45-NFDM group ($p < 0.10$). Amylase was reduced; lipase was elevated only in the mildly heated group.

Table IV. Protein and Enzyme Activity in the Intestine of Fed Rats[b]

	0-NFDM (8)	30-NFDM (7)	45-NFDM (7)
	Enzyme Units[a] per Gram of Intestine plus Contents		
trypsin	499 ± 44[a]	772 ± 43[b]	751 ± 42[b]
chymotrypsin	527 ± 37[a]	763 ± 63[b]	753 ± 50[b]
amylase	3330 ± 98[a]	2770 ± 110[b]	2582 ± 89[b]
lipase	1420 ± 130[ab]	1060 ± 84[a]	1860 ± 310[b]
	Milligrams of Protein		
per g of contents	247 ± 10[a]	288 ± 5[ab]	348 ± 22[b]
per g of intestine	781 ± 58	760 ± 49	744 ± 52
	Milligrams of Intestinal Contents		
weight	263 ± 25	328 ± 28	300 ± 9

[a] Enzyme units: trypsin = μmol of TAME hydrolyzed/min; chymotrypsin = μmol of BTEE hydrolyzed/min; amylase = μmol of maltose released/min; lipase = μequiv of OH^- titrated/min. [b] Values without common superscripts are significantly different. NFDM = nonfat dry milk; 0, 30, or 45 indicates the minutes of autoclaving. The N per group is in parentheses.

Table V. Protein and Enzyme Activity in Pancreatic Tissue of Fasted Rats[b]

	0-NFDM (6)	30-NFDM (7)	45-NFDM (7)
protein, mg	0.86 ± 0.06	0.95 ± 0.04	0.90 ± 0.06
trypsin, units[a]/mg of tissue	1.57 ± 0.26[a]	2.50 ± 0.35[b]	2.44 ± 0.25[ab]
chymotrypsin, units[a]/mg of tissue	3.41 ± 0.27	4.27 ± 0.54	4.35 ± 0.46
amylase, units[a]/mg of tissue	22.7 ± 0.90[a]	14.5 ± 1.5[b]	11.7 ± 1.4[b]
lipase, units[a]/mg of tissue	16.9 ± 1.1[a]	20.0 ± 0.8[b]	18.1 ± 1.0[ab]

[a] For enzyme units, see Table IV. [b] Values without common superscripts are significantly different. NFDM = nonfat dry milk; 0, 30, or 45 indicates the minutes of autoclaving. The N per group is in parentheses.

DISCUSSION

The poor nutritional quality of protein that has undergone the Maillard reaction by heat treatment was confirmed in the present study. Two heat treatments to produce mildly and severly heated NFDM were used; however, both were inferior to the unheated milk diet. The effect on weight gain and food intake was proportional to the severity of the heat treatment in that the animals consuming the mildly treated NFDM were able to maintain their initial body weight whereas the group consuming the severely treated NFDM lost weight during the 4-week period. The poor weight gain in these two groups was associated with a reduction in food intake. Although palatability may be in part responsible for the reduction

in food intake, a primary factor appears to be the reduction in amino acid availability. Amino acid imbalances and poor protein quality are known to reduce food intake (Harper, 1976). Supplementation with lysine can result in increased food intake with improved weight gain (Block et al., 1946).

The nutritional stress of the heated milk diets was reflected in the plasm measurements of total plasma protein and albumin, which were lower in these groups than the control (Table II). In an earlier study in which browned egg albumen was fed, a decrease in serum protein was seen after 12 months of the diet but not sooner (Kimiagar et al., 1980). However, in their study the control diet had a PER value similar to the heated diet; consequently, a difference from control may have taken longer to occur. The elevation in blood urea nitrogen that occurred in the severely heated NFDM group probably reflects the poor availability of the dietary protein. Since the animals lost weight during the study, tissue mass would have been catabolized. In addition, amino acids that cannot be used for protein synthesis due to the poor availability of indispensable amino acids would be excreted. The reduction in plasma glucose of the heat-treated groups may also be due to the nutritional stress of these diets. Since the rats reduce their food intake and, in the case of the severely treated NFDM group, are unable to maintain body weight, the lower levels observed in our study may reflect a greater period of starvation in these animals. Another possible factor leading to lower plasma glucose could be a reduction in carbohydrate digestibility as suggested by the lower amylase activity observed in the present study and the lower disaccharidase activities reported earlier (Lee et al., 1977a,b) in rats fed browned protein. The reason for the slight elevation in the blood glucose of the 45-NFDM group relative to that of the 30-NFDM is not understood at present.

The size of the kidney, stomach, small intestine, and large intestine but not the liver or pancreas relative to body weight increased in rats consuming the heated milk diets (Table III). The enchancement in kidney size may be due to the need to excrete nitrogenous compounds that are absorbed but not utilized (Nesheim and Carpenter, 1967; Lee et al., 1977a,b; Varnish and Carpenter, 1975). The enlargement in the gastrointestinal tract could be due to its handling a greater amount of nondigested material. The weight of the large intesting includes the tissue plus the cecal contents. The nondigested peptides can be fermented by the cecal bacteria (Varnish and Carpenter, 1975) and the increased weight probably reflects the increased proportion of undigested material passing into the cecum as well as increased bacterial mass. In addition, some enlargment of the tissue may have occurred (Kimiagar et al., 1980). Previous reports have suggested that browned proteins may delay gastric emptying (Kimiagar et al., 1980). Holding a large volume of food for long periods of time could lead to hypertrophy of the organ (Davenport, 1977). It is of interest to note that distension of the stomach is important in controlling food intake; hence, the filling and delayed emptying of the stomach may partially contribute to the reduction in food intake when heated protein is fed (Davenport, 1977). Enlargement of the small intestine relative to body weight was only seen in the group consuming the severely heated protein. This increase may represent a hypertrophy in the organ because it must handle a larger amount of undigestible material.

Intestinal contents were collected from animals fed a meal of their respective diet to determine the response to a meal (Table IV). The dry weight of contents was slightly greater in the mildly heated NFDM group but not in the severely heated NFDM. In both of the heat treated NFDM groups, the protein level of the intestinal contents was elevated; this difference was significant with the severe treatment and approached significance ($p < 0.10$) with the mild treatment. This increased protein reflects primarily the poor digestibility and absorption of the dietary protein. Several previous studies have indicated that the presence of Maillard reaction products interferes with hydrolysis of the peptide chain by digestive enzymes and with the absorption of digestion products. Neither the pancreatic (Table V) nor intestinal tissue had a significant reduction in protein per gram of tissue, suggesting that both of these tissues were able to maintain protein content. This finding agrees with our earlier report in which heat-damaged casein was fed to rats (Percival and Schneeman, 1979b, 1980). Lee et al. (1977a,b) reported that browned protein could lead to a reduction in disaccharidase activities in the intestinal tissue, but they did not report milligrams of protein per gram of tissue. The activity of the pancreatic digestive enzymes was, however, affected by the consumption of the heated NFDM.

In our previous study we had observed that both chymotrypsin and amylase activity were reduced in the pancreas of rats consuming heat-damaged casein because synthesis of these enzymes could not be maintained with the poor amino acid availability of the diet (Percival and Schneeman, 1979b). In contrast, total activity of all of the pancreatic enzymes measured was elevated in the intestinal contents of the group receiving heat-damaged casein, apparently due to a reduction in the ability to recycle endogenously secreted proteins. In the present study in which less severely heated protein was fed, the pancreatic response is similar but does not exactly duplicate the earlier observations. Within the pancreatic tissue trypsin activity was significantly elevated and amylase activity was significantly decreased in the heat-treated NFDM groups. Chymotrypsin activity tended to be elevated as well, but the probability values were 0.187 and 0.112 for the mild and severe groups relative to the controls, respectively. Amylase activity is known to adapt to the carbohydrate content of the diet with high carbohydrate or glucose infusion, leading to an elevation in activity (Snook, 1971; Corring, 1977). Insulin may be involved in the response by allowing entry of glucose and amino acids into the pancreatic tissue (Palla, 1968). It is unlikely that the small reduction in lactose content of the milk due to the Maillard reaction is a sufficient change in carbohydrate content to lead to adaptation; hence, either poor glucose absorption and uptake or inadequate amino acid availability to maintain enzyme synthesis must be responsible for the decreased activity.

Dietary factors that are potent stimulants of pancreatic enzyme secretion such as trypsin inhibitors or high protein can lead to adaptation of the proteolytic enzymes (Schneeman et al., 1977). This type of increased synthesis is probably mediated by the hormone cholecystokinin (Liener and Kakade, 1980). Hence, the significant increase in trypsin activity could reflect a greater stimulation of pancreatic secretion by the heated diets. In a previous study we demonstrated that heat treatment of casein with or without glucose does not change the acute pancreatic secretory response to the protein (Percival and Schneeman, 1979a). However, consumption of the heated NFDM diets could prolong the gastric and intestinal phases of digestion and the time that the gastrointestinal tract is responding to the presence of diet. The pattern of enzyme activity

in the intestine reflects to a certain extent that seen in the pancreas with significant elevation of trypsin and chymotrypsin and a reduction in amylase activities in the heat-treated groups (Table IV). The lower intestinal amylase activity is most likely due to the lower level in the pancreas available for secretion. The elevation in trypsin and chymotrypsin activity in the intestine could be due to increased secretin, decreased degradation as observed earlier (Percival and Schneeman, 1979b), or, most likely, a combination of these two factors.

Chichester and colleagues have argued that the poor nutritional quality of browned proteins is not simply due to the loss of amino acids but that other antinutritional effects appear to be involved in the physiological response to diets containing browned proteins (Kimiagar et al., 1980). The present study with heat-treated NFDM appears to support that argument. Enlargement of the kidney and of parts of the gastrointestinal tract represents not only a response to the presence of the poorly digested protein but also an increased demand for the protein needs of the animal. The changes in the pancreatic enzymes are similar to what one might expect if a high-protein diet were fed (Schneeman et al., 1977; Corring, 1977) yet in fact protein is less available in these groups and more of the dietary protein and perhaps the endogenously secreted protein is lost in the feces or by microbial degradation to unusable nitrogenous compounds (Pecival and Schneeman, 1979b; Varnish and Carpenter, 1975).

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Registry No. Trypsin, 9002-07-7; amylase, 9000-92-4; chymotrypsin, 9004-07-3; lipase, 9001-62-1.

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Acceleration of Nitrosamine Formation by Contaminant(s) in Sodium Chondroitin Sulfate Preparations

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Formation of nitrosamines from secondary amines and nitrite was markedly accelerated by sodium chondroitin sulfate (SCS) preparations. The nitrosation of dimethylamine in the presence of the SCS preparation was proportional neither to the nitrite concentration nor to the square of the nitrite concentration. Among dimethylamine, dibutylamine, pyrrolidine, piperidine, and morpholine, nitrosation of dimethylamine was most strongly accelerated. The nitrosation-accelerating activity was inhibited by cupric and silver ions. The nitrosation-accelerating substance in the SCS preparation was dialyzable and not chondroitin sulfate itself. The potency of the accelerating effect was about 18 times of that of a similar weight of sodium thiocyanate or potassium iodide. The importance of the substance in human exposure to carcinogens was suggested.

Secondary amines and other nitrosatable compounds easily react with nitrite in acidic medium and form nitroso compounds. The secondary amine that was most frequently found in foods was dimethylamine (DMA) (Neurath et al., 1977). Although the nitrosation rate of DMA by nitrite is rather slow (Mirvish, 1975), the rate is affected by many coexisting substances in the reaction mixture. Anions such as thiocyanate and iodide were reported to

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