and may not exist as such "in vivo".

The purothionin homologue from barley was named "hordothionin" by Redman and Fisher (1969). Accordingly, the rye homologue described in this paper could be designated "secalethionin". However, this class of proteins seems to be more widely distributed in the plant kingdom than previously suspected, since they have been already found in species of the genera *Aegilops*, *Triticum*, *Hordeum*, and *Secale*, as well as in a species as phylogenetically distant from the Gramineae as the European mistletoe (*Viscum album*, L.). Moreover, several species might have the same genetic variant. For these reasons, the present nomenclature does not seem to be adequate. Therefore, we propose the general designation of *thionins* for this type of proteins, followed by the name of the species from which it has been isolated, and by a greek letter, if more than one variant is present in a given species.

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Digestibility and Lysine Values of Proteins Heated with Formaldehyde or Glucose

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Experiments were done with a blend of lactalbumin and ovalbumin with ca. 13% moisture content. Heating the protein for 2 h at 80 °C with either 1% formaldehyde or 6% glucose (to supply the equivalent quantity of carbonyl groups) resulted in approximately a 15% loss of total lysine and 25–35% decrease in reactive lysine in either case. Neither product appeared toxic, but the protein heated with HCHO showed a 25% decline in digestibility and a 50–60% decrease in "available lysine" value for both rats and chicks. The protein heated with glucose showed unchanged digestibility and only a 30% decrease in available lysine value. The more general damage with HCHO is consistent with it reacting first with lysine and then forming cross-linkages by the Mannich reaction. It confirms the need for caution in using HCHO to preserve or harden animal protein materials prior to drying.

In an earlier study comparing fish meals prepared in different ways (Carpenter and Opstvedt, 1976), the application of formalin as a firming agent immediately prior to processing, to ease the process of pressing liquor out of the fish after cooking, gave the poorest product as judged by growth assays with rats or chicks with lysine as the limiting amino acid. This inferiority, however, was not predicted by chemical tests. When glucose had been added to proteins before processing the same chemical tests had proved sensitive detectors of damage to lysine (Hurrell and Carpenter, 1974). Since both chemicals, i.e., formaldehyde and glucose, are thought to react with proteins by virtue of their carbonyl group, it might be thought that they would have similar effects. The present study was designed to investigate this by direct comparison.

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MATERIALS AND METHODS

Test Materials. Two-hundred-gram portions of a mixture of ovalbumin and lactalbumin (3:2 by weight) (cf. Hurrell and Carpenter, 1974) were added to 200 mL of formaldehyde solution containing either 0.75, 1.0, or 1.5% formaldehyde or to 200 mL of a 6% glucose solution. The pH of all mixes was approximately 5.2. Each slurry was mixed for 1 h in a food mixer, freeze-dried, and then ground in a hammer mill with a 1-mm screen. The freeze-dried materials were adjusted to 15% moisture before sealing into McCartney bottles which were stored immediately at −20 °C (control), or either heated in an oven for 2 h at 80 °C or held for 5 days at 37 °C before being stored at −20 °C. The intermediate concentration

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of formaldehyde (i.e., 1%) and the glucose level (6%) were chosen each to provide 0.33 g mol of reactant/kg of albumin mix. This amount would be theoretically sufficient to react with approximately 75% of the lysine units in the protein on a mole per mole basis.

Chemical Analyses. Nitrogen, fluorodinitrobenzene (FDNB)-reactive lysine by the "direct" and by the "difference" procedures, total lysine after borohydride treatment, furosine, dye-binding capacity (DBC) for Acid Orange 12, and Cr_2O_3 were determined by procedures described previously (Hurrell and Carpenter, 1974, 1975; Hurrell et al, 1976). In the "direct" FDNB-reactive lysine procedure, a factor of 1.09 was used to correct for loss of DNP lysine during acid hydrolysis. Basic amino acids were determined by the procedure of Spackman et al. (1958) using a 15-cm ion-exchange column. 6-Amino-*n*-hexanoic acid was used as an internal standard (Bates, 1971). Dye-binding lysine (DBL) was determined from the difference in dye-binding capacity of test materials with and without prior treatment with propionic anhydride to block lysine groups specifically (Hurrell and Carpenter, 1976). Moisture content was taken to be the weight loss incurred on heating test materials in a hot-air oven for 3 h at 105 °C.

Animal Assays. *Chick Lysine Assay.* The preexperimental and basal diets used in the assay were those described by Hurrell and Carpenter (1974). One hundred and eighty male "Ross White" chicks (Ross Sterling Poultry, Woodhall Spa, Lincoln, England), one-day old on arrival were kept for 3 days on a commercial diet and then transferred to the preexperimental diet for a further 12 days. The chicks were weighed and, after rejecting those at either extreme, 168 were randomized into 56 cages with three chicks in each cage. Four cages were allotted to each of 14 dietary treatments. The test materials were each fed at two levels designed to contribute 1.75 and 3.50% crude protein, respectively, and were added to the basal diet at the expense of zein and starch so as to keep the diets isonitrogenous. The response of the chicks over 9 days was measured both as "g of weight gain" and as "g of weight gain/g of food eaten" relative to the level of supplementary lysine in the diet. The potency of the test materials was compared to that of lysine using the slope-ratio method of Finney (1964).

Toxicity Test with Chicks. The basal diet and preexperimental diets were the same as used in the chick lysine assay described above and 60 "Ross White" chicks were similarly reared on a commerical ration for 4 days, followed by a preexperimental ration for a further 7 days. At 12 days the chicks were weighed and 48 of average weight were randomized into 16 cages with three chicks/cage. Four cages were allotted to each of four dietary treatments (Table IV) in which the test materials were added at levels contributing 3.5% crude protein (at the expense of zein) to the basal diet supplemented with 1.25% lysine HCl. The lysine supplement was calculated to satisfy the chicks requirement for growth, and the basal diet had already been designed to meet the requirements for all other nutrients. The response of the chicks over 10 days was measured as "g of weight gain" and as "g of weight gain/g of food eaten".

Ileal Digestibility Studies with Chicks. The method used was similar to that described by Varnish and Carpenter (1975). The N-free basal diet contained (g/kg): arachis oil, 50; vitamin mix, 31.3; choline chloride, 1.5; inositol, 1; $CaCO_3$, 20; $CaHPO_4$, 25; chromium bread, 10 (300 g Cr_2O_3/kg; Kane et al., 1950); and maize starch to 1000. The vitamin and mineral mixes were as used in the chick lysine assay. The test materials were added at levels to supply 200 g of crude protein (N × 6.25)/kg. The 48 chicks used in this experiment had previously been used in the toxicity test described above and then fed a commerical ration for 4 days before being rehoused individually in 48 cages. The commercial ration was fed for a further 2 days, but only for two periods in each day (9.00 to 11.00 h and 15.00 to 17.00 h) so that the birds became accustomed to eating their food rapidly. After an overnight fast, 12 birds were allocated at random to each of three test diets and to the N-free diet. They were allowed to eat for 2 h and after a further 2.75 h they were killed by direct intracardial injection of Nembutal (Abbot Laboratories Ltd., Queenborough, Kent, England). Each bird was dissected immediately and the undigested material removed from the ileum (Varnish and Carpenter, 1975). The ileal contents from six chicks on each dietary treatment were pooled so that there were two independent mixed samples per treatment; these were immediately frozen and freeze-dried for analysis. N and Cr_2O_3 determinations were made in duplicate, but total lysine estimations were not replicated. Using the nutrient:Cr_2O_3 concentration ratios for food and for digesta, true digestibility coefficients for N and for lysine were calculated (cf. Varnish and Carpenter, 1975).

Net Protein Ratio (NPR) and Digestibility for Rats. Three test materials were assayed for their value as sole protein sources for young rats using the NPR test (Bender and Doell, 1957) and for the fecal digestibilities of N and lysine. The experimental procedure was similar to that described by Hurrell et al. (1976). Thirty-two 21-day-old female rats of CFY strain (Anglia Laboratory Animals, Huntingdon, England) were placed into 16 cages so that each contained two rats. Four cages of rats were randomly allocated to each of the three experimental diets and to the N-free diet. The N-free diet contained (g/kg): sucrose, 840; cellulose powder, 40; maize oil, 50; mineral mix, 52; trace element mix, 3; vitamin mix, 5; chromium bread, 10. The test materials were added at a level of 100 g of crude protein (N × 6.25)/kg at the expense of sucrose. The mineral mix contained (g/kg): KH_2PO_4, 334.8; $CaHPO_4$, 92.9; $CaCO_3$, 222.5; NaCl, 244.4; $MgSO_4 \cdot 7H_2O$, 91.5; and maize starch to 1000. The trace element mix contained (g/kg): $CuSO_4 \cdot 5H_2O$, 8.00; NaF, 3.33; KIO_3, 0.13; ferric citrate pentahydrate, 100; $MnSO_4 \cdot 4H_2O$, 81.33; $ZnCO_3 \cdot 2ZnO \cdot 3H_2O$, 10.27; Na_2SeO_3, 0.03; and maize starch to 1000. The vitamin mix contained (g/kg): "Rovimix A" (Roche Products Ltd., Welwyn, Herts; containing 150 mg of retinol/g), 3; "Rovimix D_3" (10 mg of cholicalciferol/g), 0.56; "Rovimix E" (250 mg of DL-α-tocopheryl acetate/g), 48; menapthone, 0.4; nicotinic acid, 3.34; calcium D-pantothenate, 2.4; riboflavin, 1; thiamin hydrochloride, 0.8; pyridoxine, 1.56; choline chloride, 200; cyanocobalamin, 0.004; and maize starch to 1000. Each diet was fed ad libitum for 10 days and NPR was calculated by adding the weight loss of the N-free group to the weight gain of the test group and then dividing by the weight of crude protein (N × 6.25) consumed by the latter. Feces were collected daily over the last 3 days of the trial and immediately frozen. Duplicate samples were obtained by pooling the feces of two groups of four rats within each dietary treatment. All analyses were made in duplicate except for the total lysine estimations, where the duplicate collections were analyzed only once. True digestibility coefficients of N and of total lysine were calculated.

RESULTS AND DISCUSSION

Analytical Results. Furosine was found only in acid hydrolysates of material to which glucose had been added;

0.68 g/16 g of N in the sample kept for 2 h at 80 °C and 0.45 g/16 g of N in that kept 5 days at 37 °C. All the other analytical values on the test materials are set out in Tables I and II. It is seen from Table I that the effect of formaldehyde in reducing lysine values is progressively greater with increasing concentration. With glucose the effect on lysine is considerably greater after 2 h at 80 °C than after 5 days at 37 °C, but with formaldehyde the difference is less (though still in the same direction) and at the highest level of formaldehyde more than half of the effect has been obtained even when the material was freeze-dried immediately after mixing. For the six treatments using formaldehyde followed by storage at 37 °C or heating at 80 °C, the overall loss of total lysine was 17%. The corresponding loss of reactive lysine was 22% as measured by the direct procedure and 30% as measured by the "difference" procedure.

The greater sensitivity of the "difference procedure" in the protein–formaldehyde series is in contrast to results with protein–glucose samples both here and in a previous study (Hurrell and Carpenter, 1974). Finot and Mauron (1972) have shown that the reaction of FDNB with modified proteins is more complex than was assumed to be the case when the "difference" procedure was first proposed (cf. Carpenter, 1973).

We had originally used the borohydride procedure as an analytical tool in studying protein damage after Means and Feeney (1968) had shown its usefulness in measuring the attachment of formaldehyde to the lysine groups in protein by converting the compounds to acid-stable methyl–lysine units. In the present instance the "borohydride" values for the protein–formaldehyde samples have decreased essentially to the same extent as the values determined by the direct FDNB procedure. With the protein–glucose samples the borohydride values have been slightly lower than those obtained with FDNB; this agreed with earlier results (Hurrell and Carpenter, 1974). The lower values have been explained by Finot et al. (1977) as coming from the Schiff's bases present when protein and glucose are heated or stored under mild conditions; the lysine units in these are still nutritionally available and still react with FDNB, but after reduction with borohydride they do not contribute to "total lysine".

From Table II it is seen that total histidine and arginine levels were virtually unaffected by the treatments. The dye-binding capacity has been reduced for the samples heated with 1% formaldehyde but not for the samples similarly heated with glucose. The reduction with formaldehyde corresponds roughly to the fall in reactive lysine in these samples as measured in other ways. This could be taken as evidence that the lysine units binding to formaldehyde (in contrast to the early Maillard products with glucose) have lost their basicity, but it could also merely reflect a reduced ability of the relatively large dye molecule to attach itself to all the basic groups in the proteins "hardened" with formaldehyde.

The specific DBL procedure has also given lower values for material heated with glucose despite the DBC values being unchanged. This was because the dye-binding capacity after treatment with propionic anhydride was higher for the "glucose" samples than for "controls", presumably because the lysine–glucose reactions prevented further reaction of lysine units with propionic anhydride even though the same units still had the capacity to bind the dye.

Results of Animal Studies. The chick lysine assay results are shown in Table III. The potencies of the test materials as sources of lysine were calculated by the

Table I. Total and Reactive Lysine Content (g/16 g of N) of the Albumin Mix Processed under Different Conditions Prior to Freeze-Drying (Values in Parentheses Are the Proportions of the Unheated Control Value Remaining after Heat Treatment)

Additive	Process	Sample no.	% moisture	% crude protein (N × 6.25)	Total lysine	Total lysine after borohydride	Bound lysine	FDNB-reactive lysine[a] "By difference"	FDNB-reactive lysine[a] "Direct"
None	None	Z225	13.7	71.4	8.44	8.12	0.28	8.16	8.39
	heated 2 h at 80 °C	Z242	14.0	70.2	8.15 (0.97)	8.11 (1.00)	0.44	7.71 (0.94)	8.45 (1.01)
	stored 5 days at 37 °C	Z229	13.6	69.0	8.62 (1.02)	8.36 (1.03)	0.39	8.29 (1.02)	8.58 (1.02)
0.75% formaldehyde	none	Z235	12.4	70.8	8.02 (0.95)	nd[b]	0.80	7.22 (0.88)	8.14 (0.97)
	heated 2 h at 80 °C	Z236	12.2	71.2	7.31 (0.85)	nd	1.06	6.25 (0.77)	7.28 (0.87)
	stored 5 days at 37 °C	Z237	12.1	71.0	7.53 (0.89)	nd	0.96	6.57 (0.81)	7.51 (0.90)
1% formaldehyde	heated 2 h at 80 °C	Z238	14.4	70.8	6.98 (0.83)	6.20 (0.76)	1.29	5.69 (0.70)	6.47 (0.77)
	stored 5 days at 37 °C	Z239	14.5	70.0	7.41 (0.88)	6.89 (0.85)	1.24	6.17 (0.76)	6.93 (0.83)
1.5% formaldehyde	none	Z226	13.6	71.1	7.41 (0.88)	nd	1.28	6.13 (0.75)	6.58 (0.78)
	heated 2 h at 80 °C	Z228	13.1	71.0	6.30 (0.75)	nd	1.68	4.62 (0.57)	5.38 (0.64)
	stored 5 days at 37 °C	Z230	13.6	68.6	6.56 (0.78)	nd	1.67	4.89 (0.60)	5.87 (0.70)
6% glucose	heated 2 h at 80 °C	Z245	14.8	66.3	6.88 (0.82)	5.10 (0.63)	0.54	6.34 (0.78)	5.81 (0.69)
	stored 5 days at 37 °C	Z246	14.2	67.2	7.54 (0.89)	6.26 (0.77)	0.51	7.03 (0.86)	6.91 (0.82)

[a] FDNB, fluorodinitrobenzene. [b] nd, not determined.

Table II. Dye-Binding Values of the Test Materials in Relation to Their Contents of Basic Amino Acids (All Values in mmol/16 g of N)

Additive	Heat treatment	Sample no.	"Histidine + arginine" (H + A)[a]	Dye-binding capacity (DBC)	DBC minus "H + A"[b]	FDNB-reactive lysine (direct)	Dye-binding lysine (DBL)
None	None	Z225	44.7	109	64.3	57.5	53.8
	2 h @ 80 °C	Z242	43.9	106	62.1	57.9	55.5
	5 days @ 37 °C	Z229	45.3	112	66.7	58.8	57.6
1% formaldehyde	2 h @ 80 °C	Z238	43.0	86	43.0	44.3	40.9
	5 days @ 37 °C	Z239	47.2	87	39.8	47.5	38.1
6% glucose	2 h @ 80 °C	Z245	44.2	108	63.8	39.8	40.3
	5 days @ 37 °C	Z246	44.7	109	64.3	47.3	46.5

[a] The mean overall value for histidine was 2.38 g/16 g of N and for arginine 5.07 g/16 g of N. [b] These values are calculated because earlier work showed that in many materials the DBC value approximates to the sum of "H + A" and "reactive lysine".

Table III. Response of Chicks Receiving a Lysine-Deficient Diet to Either Lysine or to the Test Materials; and the Estimated Lysine Potency (g/16 g of N) of Those Test Materials (Values in Parenthesis Represent the Potency of the Test Materials as a Proportion of the Potency of the Heated Control Z242)

Supplement	Level of inclusion (g/100 g diet)	Av wt gain, g[a]	Av wt gain (g)/g of food eaten	Lysine potency	
				by weight gain	by gain/food
None		24.1	0.077		
L-Lysine hydrochloride[b]	0.175	78.8	0.218		
	0.35	165.1	0.327		
	0.525	257.7	0.436		
Albumin mix, heated 2 h at 80 °C (Z242)	2.49	101.4	0.241	9.99	9.91
	4.99	212.5	0.377		
Albumin mix + 1% formaldehyde, heated 2 h at 80 °C (Z238)	2.47	40.7	0.123	4.02 (0.40)	3.81 (0.38)
	4.94	87.9	0.211		
Albumin mix + 1% formaldehyde, stored 5 days at 37 °C (Z239)	2.50	55.5	0.151	3.64 (0.36)	3.39 (0.34)
	5.00	70.8	0.181		
Albumin mix + 6% glucose, heated 2 h at 80 °C (Z245)	2.64	77.9	0.199	6.94 (0.69)	7.26 (0.73)
	5.28	142.5	0.301		
Albumin mix + 6% glucose, stored 5 days at 37 °C (Z246)	2.60	83.1	0.206	7.75 (0.78)	7.98 (0.81)
	5.61	161.6	0.324		
Pooled estimate of SE of treatment means		±7.2	±0.010	±0.36	±0.38

[a] Per cage containing three chicks. [b] L-Lysine hydrochloride containing 80% lysine.

Table IV. Toxicity Test: Response of Chicks to a Nutritionally Adequate Diet Supplemented with the Heated Test Materials at a Level of 3.5% Crude Protein (N × 6.25)

Test supplement	Av wt gain,[a] g	Av wt gain (g)/g of food eaten
None	372.1	0.517
Albumin mix, heated 2 h at 80 °C (Z242)	358.0	0.505
Albumin mix plus 1% formaldehyde heated 2 h at 80 °C (Z239)	356.4	0.489
Albumin mix plus 6% glucose, heated 2 h at 80 °C (Z245)	377.1	0.519
Pooled SE of treatment means	±12.0	±0.011

[a] Per cage containing three chicks.

slope-ratio technique (Finney, 1964) which requires that the response to each supplement in relation to the levels of supplementation should not differ significantly from linearity and that when the response lines are extrapolated down to zero level of supplementation, they all intersect at a common point. With our "gain/food" data both these conditions were satisfied. With the "weight gain" data the point of intersection was found to differ significantly ($p < 0.05$) from the measured response at zero supplementation and the results at zero supplementation were therefore excluded from the analysis (cf. Carpenter et al., 1972). Similar absolute estimates of lysine potency were obtained using either series of data. For the control material heated alone at 80 °C (Z242), the values were about 20% higher than the chemical assay values (Table I).

This phenomenon, i.e., of peptide-linked lysine in a good quality protein being apparently more effective than the equivalent quantity of free lysine, has been observed in several earlier studies with no satisfactory explanation (e.g., Miller et al., 1965; Hurrell and Carpenter, 1974).

Table V. Net Protein Ratio (NPR) Values and Digestibility Coefficients of N and of Lysine for Test Materials Heated for 2 h at 80 °C

Test material (addition to albumin)		Net protein ratio (rats)	Digestibility coefficients			
			Rats (based on fecal analysis)		Chicks (based on ileal analysis)	
			N	Lys	N	Lys
Z242	(no addition)	5.0	0.96	0.97	0.96	0.97
Z238	(1% formaldehyde)	2.6	0.70	0.71	0.73	0.70
Z245	(6% glucose)	4.7	0.95	0.96	0.92	0.97
	Pooled SE of treatment means	±0.21	±0.016	±0.007	±0.016	±0.011

With both sets of data, the responses of the chicks to the two formaldehyde-treated materials were as low as or lower than their response to the lowest level of synthetic lysine. This is unsatisfactory, particularly since the weight gain response had not intersected at the zero supplementation point. However, even if there is some doubt about these potency values for the formaldehyde-treated materials, it is clear that they are very much lower than for the corresponding samples treated with glucose. The loss of biologically available lysine in the heated albumin–glucose mixes was in close agreement with that predicted by the chemical tests (Table I), especially the direct FDNB-reactive lysine test; however, the loss of available lysine in the albumin–formaldehyde mixes was far greater than that predicted by chemical tests. The low potency of the "formaldehyde" samples does not appear to have been due to any growth-depressant effect. As is seen in Table IV, comparable chicks receiving the same diets fortified with additional lysine showed only a small and nonsignificant difference between diets containing material heated alone or with either formaldehyde or glucose.

The chick ileal digestibility coefficients for the three test materials heated for 2 h at 80 °C are shown in Table V. The digestibility of lysine and of N in the glucose–albumin mix was similar to the albumin mix heated alone and almost complete. However, in the heated albumin–formaldehyde mix the digestibility of both components had been reduced to about 70%. These results are in almost exact agreement with those for the rat digestibility test based on fecal analyses as set out in Table V.

Also summarized in Table V are the results of the evaluation of the three heated test materials for supporting the growth of young rats as their sole source of protein. Again the NPR value of the glucose–albumin mix was little or no different from that of the albumin mix heated alone. This would be expected where glucose has had no effect on overall digestibility of the proteins, if its only effect was on the lysine and the level of this amino acid remaining available were still sufficient for it not to have become the first limiting amino acid. In the present instance the level of lysine in the initial albumin mix was in excess of that required by rats and, even if it had effectively fallen by some 30% as indicated by the various measures of reactive lysine given in Table I and by the chick lysine assay (Table III), this should still be sufficient to meet the rats requirement (cf. Bender, 1965).

In contrast, the NPR value of the albumin–formaldehyde mix was 52% of that for albumin alone. We would, of course, have expected a change proportional to the change in overall digestibility of the protein in this material, but it would appear that there has also been a fall in the quality of the portion of the protein that had been digested.

Surprisingly, we have found only one other published experiment on the protein quality for rats of protein treated with formaldehyde (Hove and Lohrey, 1976). In this study casein was stirred with aqueous formalin in excess, then washed and dried at 60 °C. The dry product was estimated to contain 3% formaldehyde. The apparent digestibility of the protein had been reduced from 93 to 66% by the treatment, i.e., the value for the treated material was approximately 70% that of the untreated. From the weight gain data given it is also possible to estimate that the NPR values were approximately 1.4 and 3.5, respectively, i.e., the treated sample had 40% of the value of the control. Further, feeding the formaldehyde material at a very high level overcame the poor growth, so that it was clearly not growth depressant. There is, therefore, a close agreement between these results and our own.

Table VI. Relative Effects of Equimolar Additions of Formaldehyde and Glucose to Protein Subsequently Heated for 2 h at 80 °C (All Values for the Supplemented Proteins Are Expressed as Proportions of the Corresponding Results for Proteins Heated Alone)

	1% formaldehyde	6% glucose
Growth assays		
Lysine (chicks)	0.40	0.69
Overall protein quality (NPR; rats)	0.52	0.94
Digestibility of nitrogen[a]		
Ileal level (chicks)	0.76	0.96
Faecal level (rats)	0.73	0.99
Chemical tests		
Total lysine	0.86	0.84
FDNB-lysine (by difference)	0.74	0.82
FDNB-lysine (direct)	0.77	0.69
Lysine after borohydride	0.76	0.63
Dye-binding lysine (DBL)	0.74	0.73
Dye-binding capacity (DBC)	0.81	1.02
DBC minus HA[b]	0.69	1.03

[a] As can be seen in Table V, the corresponding values for lysine were in no case significantly different from those for N. [b] Calculated as explained in Table II by subtracting histidine and arginine values from the DBC value.

Hove and Lohrey (1976) also report the results of a trial with lactalbumin treated with more dilute formalin solutions and then washed and freeze-dried. Even at the highest level of formaldehyde used (corresponding to 2.7% of the crude protein in the mix) there was only a 13% decrease in protein efficiency ratio (equivalent to approximately an 8% fall in NPR) and a 3.5% decrease in digestibility of N. It is tempting to conclude that the small degree of damage in these experiments is to be explained by no heat treatment having been applied.

Possible Mechanisms. The relative effects of 1% formaldehyde and its equivalent of glucose (6%) in changing the sensitivity of the protein to heat damage under our conditions are summarized in Table VI. This brings out again the point that the formaldehyde has had a much more deleterious effect in all the biological tests. On the other hand, the various chemical tests for reactive lysine would seem to indicate similar damage from the two additives, with the difference going in one direction or the other according to which test is used.

How can this contrast be explained? Reviewers of the reactions between formaldehyde and proteins emphasize (a) that there are many reactions and their relative importance varies with the conditions used and (b) that formaldehyde is capable of cross-linking reactions, with the formation of methylene bridges between amino acid units, a property not shared with other monoaldehydes (French and Edsall, 1945; Walker, 1964; Feeney et al., 1975; Cheftel, 1977, Galembeck et al., 1977). It would appear that, although it can also react with sulfhydryl and other groups, the first major reaction of formaldehyde with proteins under cold, aqueous conditions close to a neutral pH is with the ϵ-NH_2 group of lysine units to form a methylol compound and that this can then react further and more slowly by the Mannich reaction to form bridges with carbon in the ring structures of tyrosine and histidine units, or with other unreacted lysine units (Fraenkel-Conrat and Olcott, 1948; Bizzini and Raynaud, 1974). Cross-linking between lysine residues has been reported

to occur more readily at neutral pH, whereas cross-linking between lysine and tyrosine residues occurred more readily at pH 5.0 (Warren et al., 1974); our mixture had a pH of 5.2. This is not the only form of reaction, however, since methylene bridges between serine and either glutamine or asparagine have also been introduced into proteins by formaldehyde treatment (Myers and Hardman, 1971). It is these later reactions that are thought to produce the hardening and reduced solubility of proteins made use of in tanning (Walker, 1964) and in the modification of feeds for ruminants so as to reduce their susceptibility to microbial degradation (cf. review by Broderick, 1975).

Acid hydrolysis releases lysine from the methylol compounds but not from the cross-linked compounds with other amino acids (Bizzini and Raynaud, 1974). The methylol compounds are reduced to the acid-stable methyl-lysine with borohydride (Means and Feeney, 1968), but the relatively small further decrease in lysine recovered from our samples after this treatment suggests that most of the compounds formed with the lysine units had gone on to the cross-linkage stage. This would fit with the observation of severely reduced digestibility of the protein–formaldehyde samples. Similarly, low digestibility was found with severely heated pure proteins in which cross-linkages are thought to have been produced between lysine units and the amide groups of asparagine and glutamine units (Bjarnason and Carpenter, 1970), whereas the protein–glucose samples, where cross-linkages were not expected to be present, did not show a significant fall in digestibility in the present study, nor did protein whose lysine units had been made largely unavailable by propionylation (Varnish and Carpenter, 1975).

Galembeck et al. (1977) have made the interesting observation that the slowed in vitro digestibility with α-chymotrypsin of bovine serum albumin given a mild treatment involving formaldehyde or acetaldehyde could be partly explained by the release of peptides that had an inhibitory effect on the enzyme.

Glutamyl-lysine isopeptides have been shown to be completely available as a source of lysine for rats (Mauron, 1970; Waibel and Carpenter, 1972). It seems possible, however, that the cross-linked compounds formed as a result of the formaldehyde–protein reactions (e.g., lysine–CH_2–tyrosine, lysine–CH_2–histidine, and lysine–CH_2–lysine), which are acid resistant, will also be biologically unavailable as sources of lysine. Their fate in the rat could then be similar to that of lysine–glucose compounds (Finot et al., 1977) which, although absorbed from the gut, are not utilized but excreted in the urine. Such a mechanism would account for the digestibility of the lysine in our albumin–formaldehyde mix being greater than its availability as judged by growth assays.

Lastly, under our conditions, there has been no indication that anything approaching 75% of the lysine units had reacted with formaldehyde; this was the theoretical maximum if each formaldehyde molecule had reacted with the free amino group of a lysine unit. Nor were there indications of reaction with arginine or histidine. It remains possible therefore that compounds had been formed with other groups in the protein to a greater extent than had been expected and that these contributed significantly to the changes in nutritional value of the proteins. Certainly the low NPR value of the protein–formaldehyde sample as the sole source of protein for the rat suggests that the availability of some amino acid(s) other than lysine is reduced to a greater extent than can be explained by the decrease in overall digestibility of the protein.

Practical Implications. This small study is a quite inadequate basis for drawing broad generalizations about the effect of formaldehyde on the nutritional value of proteins. But it does serve to confirm the suspicions aroused from earlier work (Wiechers and Laubscher, 1962; Carpenter and Opstvedt, 1976) that the nutritional damage resulting from its use may be considerably greater than can be predicted from analyses for total or reactive lysine, even though it is thought that this is the amino acid with which it reacts to the greatest extent.

Formalin has been used both as a preservative and as a firming agent for wet proteinaceous material that is to be processed into protein concentrates. But it seems, for example, that even a quantity corresponding to 0.5% (w/w) of the fish protein can be significantly damaging, i.e., reducing protein quality by more than 10%, when used as a firming agent in fish meal manufacture (Carpenter and Opstvedt, 1976) and that manufacturers are right to avoid such levels. Although we have no direct evidence, it also seems a possibility that some commercial samples of blood meal which have shown lower protein quality than could be explained by either their reactive lysine values or the results of model experiments in heating and drying blood (Waibel et al., 1977) could have had formalin added as a preservative while they were awaiting processing.

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Utilization of Protein from Soy Flour and Soy Isolate by Adult Women

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Two nitrogen balance studies with adult women subjects were conducted to assess the effect of alkaline processing of soy products on protein nutritive value. Two soy flours (Soyafluff 200W and Soyafluff 200T) were compared with soy isolate (Promine D) alone, soy isolate supplemented with amino acids to match the flour, and in one study, soy isolate supplemented with sulfur amino acids. At nitrogen intakes of 5.2–5.3 g/day, nitrogen retentions were −0.06 ± 0.16, −0.30 ± 0.27, and −0.21 ± 0.71 g of nitrogen/day for Soyafluff 200W, Promine D, and Promine D plus amino acids, respectively, in study I, and −0.09 ± 0.65, 0.17 ± 0.38, 0.04 ± 0.98, and −0.32 ± 0.33 g of nitrogen/day for Soyafluff 200T, Promine D, Promine D plus matching amino acids, and Promine D plus sulfur amino acids, respectively, in study II. PER values determined for Soyafluff 200W, Promine D, and Promine D plus amino acids were 1.6 ± 0.2, 1.4 ± 0.2, and 1.5 ± 0.2, respectively (casein: 2.7 ± 0.3). Nitrogen retentions and PER values were not significantly different from each other.

The use of protein from soybean has increased dramatically in the last decade. This relatively abundant, inexpensive and good quality protein is being utilized in human foods in a variety of forms, including flour, soy protein concentrates, soy protein isolate, and spun soy fibers.

The preparation of soy protein isolates includes exposure to heat and mild alkali (Horan, 1974). Isolated soy protein may undergo more severe alkaline processing in the production of spun fibers used in the fabrication of meat analogues (Horan, 1974; Rosenfield and Hartman, 1974). In addition, during processing, certain protein fractions may be discarded. These treatments may influence the nutritive value of the final product. Several investigators have reported decreases in the nutritive value of soy protein isolated by an alkaline process (Bressani et al., 1967; Cogan et al., 1968; Badenhop and Hackler, 1970, 1973). The destruction of certain amino acids has been associated with alkaline processing (Cogan et al., 1968; DeGroot and Slump, 1969; Badenhop and Hackler, 1970; Robinson et al., 1971; and Woodard and Short, 1973). However, amino acid destruction alone does not sufficiently explain the decrease in protein quality in all cases. For example, DeGroot and Slump (1969) found that a decreased net protein utilization (NPU) resulting from alkaline treatment could not be completely alleviated by supplementation with either methionine or methionine plus lysine. Supplementation with threonine, which had not been destroyed on processing, plus methionine and lysine did improve NPU relative to supplementation with methionine and lysine. DeGroot and Slump (1969) suggested that a decreased utilization of threonine might be the result of isomerization from the L to D form. It seems clear that the availability of amino acids can be decreased under conditions of alkaline processing, and that the basic cause may be either racemization to less readily utilized forms (DeGroot and Slump, 1969) and/or destruction of amino acids and changes in the overall amino acid pattern (Harper, 1956; Kofranyi, 1972).

Data on the protein nutritive value of soy products, as measured in human subjects, are not as readily available as data from rat studies (Bressani, 1975). Furthermore, in studies with humans, the various forms of soy are usually tested in comparison to a non-soy protein source (Parthasarathy et al., 1964; Bressani et al., 1967; Kies and Fox, 1971, 1973; Korslund et al., 1973). These cited studies tested soy flour, textured vegetable protein, and a spun soy fiber product. Soy protein isolate has also been tested in human subjects, but again not in direct comparison to other soy protein sources (Zezulka and Calloway, 1976a,b).

The present studies were designed to compare, with human subjects, the relative protein quality of two soy flours which had not been processed with alkali and a soy protein isolate, which had received alkaline treatment. The subjects were young adult women, and nitrogen retention was used as the criterion for comparing the sources of protein. Study I compared Soyafluff 200W, which according to the manufacturer is a minimally moist-heat-treated flour, with a soy isolate (Promine D). Study II compared Soyafluff 200T, a toasted flour, to Promine D. These products were supplied by Central Soya Co., Chicago, Ill. Since changes in amino acid pattern could account for potential nutritive differences in flour and isolate, some experimental treatments were designed to obtain information about this possibility. In both studies, one group of subjects was fed soy isolate plus essential amino acids and a nonspecific nitrogen source. The combination of isolate and amino acids was designed to provide an essential amino acid pattern from isolate and supplemental amino acids identical with the soy flour pattern. In study II, one experimental group received

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