TECHNICAL PAPERS

Amino Acid Impairment in Casein Heated With Glucose

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In all cases of "heat damage" to amino acids in protein foods or feeds which have come to our attention, reducing sugars have been present or potentially available. We have previously demonstrated the partial inactivation of free amino acids and amino vitamins resulting from glucose-heat treatment (*1, 3*). This results, apparently, from combination of the reactive groups with aldoses, their aldehyde degradation products, or (most probably) polymers of the latter. The validity of our conclusions has been corroborated in at least two other laboratories (*2, 6*).

With increasing use of protein hydrolysates and crystalline amino acids for food and feed fortification, such glucose-heat damage to free amino acids may become important. At present, however, amino acids are consumed chiefly in combined form as proteins, and the evidence cited above does not prove that amino acids are similarly attacked in intact proteins. This phase of the problem has been investigated in the following manner:

Vitamin Test Casein (G.B.I.), free from dialyzable nitrogen, was refluxed 24 hrs in a 5% N-free glucose solution at 96.5° C. Aliquots of this glucose-heat treated sample, freed from glucose, were used for comparison with the untreated casein on an equivalent weight basis. Both untreated and treated casein were hydrolyzed by refluxing 30 hrs in 6 N HCl. For tryptophan assay, the samples were digested with pepsin, papain and Taka-diastase, and trypsin. Nine essential amino acids were determined microbiologically by the method of Stokes, *et al.* (*7*), using *Str. faecalis R*, modified by substituting sucrose for glucose in the media to prevent further inactivation (*1*).

The results, shown in Table 1, indicate that, of the 9 essential amino acids assayed, only lysine, arginine, and tryptophan were inactivated to an appreciable extent by the glucose-heat treatment. Analyzed by Student's pairing method, the data show statistically significant decreases for certain of the other amino acids. However, if the actual percentage decrease is examined, it will be noted that the decreases are of questionable significance nutritionally. The heat treatment used in these tests was purposely mild; it is quite possible that more drastic heat in the presence of an aldose would cause greater inactivation. Theoretically, lysine, arginine, tryptophan, and possibly histidine would be expected to be most susceptible to reaction with aldehydes, since these amino acids contain functional nitrogen groups unattached in polypeptide linkages.

TABLE I

AMINO ACID CONTENT OF UNTREATED AND GLUCOSE-HEAT TREATED CASEIN

Amino acid	Sampling distribution			Significance	
	Untreated $\overline{A} \pm S_A$	Treated $\overline{B} \pm S_B$	Decrease $\overline{A-B} \pm S_{\overline{A-B}}$	t	Probability
	(%)	(%)			
Lysine	5.07 ± 0.50	3.75 ± 0.55	1.322 ± 0.353	10.575	999 : 1,000
Arginine	3.30 ± 0.30	2.29 ± 0.22	1.008 ± 0.164	17.351	999 : 1,000
Tryptophan	0.95 ± 0.07	0.58 ± 0.08	0.370 ± 0.133	7.857	999 : 1,000
Histidine	3.05 ± 0.15	2.84 ± 0.03	0.210 ± 0.130	3.950	99 : 100
Methionine	2.29 ± 0.20	2.32 ± 0.10	− 0.030 ± 0.167	3.999	99 : 100
Valine	6.09 ± 0.22	6.24 ± 0.28	− 0.146 ± 0.298	1.388	77 : 100
Leucine	8.43 ± 0.10	8.20 ± 0.09	0.226 ± 0.106	6.072	999 : 1,000
Isoleucine	5.36 ± 0.31	5.05 ± 0.24	0.314 ± 0.258	3.434	99 : 100
Threonine	2.94 ± 0.08	3.12 ± 0.05	− 0.183 ± 0.108	4.149	99 : 100

We feel it is no coincidence that Riesen, *et al.* (*4*) found that the amount of each of the essential amino acids liberated by acid hydrolysis from soybean oil meal, *except lysine, arginine, and tryptophan,* was unaffected by heat treatment. Soybeans contain ample aldose to explain these results on the basis of the glucose-heat reaction we have observed. In part, at least, the inactivation of lysine in baked products, "puffed" cereals, and casein (*8*), as well as other foods, may be due to this reaction. Even pure casein contains a reducing sugar (galactose) as an integral part of the molecule (*5*).

It thus appears that heat processing of foods or feeds in the presence of reducing sugar results in partial inactivation of free amino acids, amino vitamins, and at least three essential amino acids (lysine, arginine, and tryptophan) in protein. In light of these findings, one may question the effect on nutritive value of adding commercial products containing reducing sugar (glucose, maltose, etc.) to foods which are to be heat processed. Likewise, one may question the advisability of adding glyceraldehyde to ordinary sugar for the purpose of preventing dental caries, as has been proposed (*9*).

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Newly Discovered Outcrops of the Cannonball Formation in North Dakota[1]

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About 60,000,000 years ago, near the beginning of the Tertiary period, western North Dakota was inundated by the readvance of a sea or an arm of the sea that had been in existence in the same general region and to the eastward since late Cretaceous time. The 300′ of brown, sandy, fossiliferous sediments that were deposited in this sea in the vicinity of Bismarck and southwestward were first recognized as a marine unit by E. Russell Lloyd in 1912 and named the Cannonball marine member of the Lance formation in 1914 from typical exposures along the Cannonball River, southwest of Mandan. However, the discovery in 1907 by A. G. Leonard, former state geologist of North Dakota, of an oyster bed, interbedded with the lignitic strata of the Fort Union formation exposed in the bluffs of the Little Missouri River just south of Yule, North Dakota, had indicated the existence of an early Tertiary sea toward the east. This oyster bed is now regarded as evidence of a brackish-water estuary leading into the Cannonball sea.

A map showing the distribution of the Cannonball deposits and a description of the fauna contained therein were published in 1921 by T. W. Stanton (*2*), who, although recognizing a few species with Tertiary aspect, assigned the fauna to the late Cretaceous because of the large percentage of forms theretofore identified as Cretaceous. This age assignment served to continue the already warm debate concerning the Cretaceous-Tertiary boundary in the Rocky Mountains and Plains region. In 1940, S. K. Fox, Jr., and R. J. Ross, Jr. (*1*), reported that an analysis of 64 species of foraminifers from the Cannonball showed clear relationships to those of the Midway strata of the Gulf Coast and indicated Paleocene age. On this evidence the U. S. Geological Survey in 1944 formally adopted the name Cannonball formation, with assignment to the Paleocene series.

Interest in the Cannonball formation continues because its outcrops provide readily identifiable stratigraphic markers and because clues are sought to determine whether the Cannonball sea had Arctic or Gulf of Mexico connections. As no marine fauna of Cannonball time has ever been found in the Canadian region toward the Arctic Ocean, the Midway aspect of the Cannonball fauna, for lack of competitive comparison, must be regarded as one-sided evidence that the connections of the Cannonball sea were with the Gulf of Mexico.

Eastward and northeastward from the Missouri River at Bismarck the bedrock strata of the Plains are for the most part concealed beneath a mantle of glacial drift, so that only in few places can satisfactory outcrops be seen, but not many miles east of Bismarck late Cretaceous strata appear at the surface, showing that the Cannonball deposits, if they were ever present there, were eroded before Wisconsin glaciation. The probability, however, that some of the Cannonball deposits are preserved beneath the glacial cover is confirmed by the fact that in July 1947 the writers came upon such outcrops on the south side of the Souris River, in road cuts on U. S. Highway 52 about 1½ miles east of Sawyer (SW¼ sec. 12, T. 153 N., R. 81 W.) and about 2½ miles east of Velva (SW¼ sec. 18, T. 153 N., R. 79 W.), North Dakota, respectively. These localities are approximately 55 miles north of the nearest hitherto reported outcrops of the Cannonball on the Missouri River, near Washburn. The exposures, at altitudes of 1,540′ and 1,520′, respectively, may be near the top of the Cannonball, because they are overlaid to the northwest at a slightly higher level by lignitic strata whose stratigraphic position and fossil content suggest equivalence to the Tongue River member of the Fort Union formation of regions to the westward.

The thinly bedded brown sands and sandy shales of the outcrops yielded the foraminifers (identified by J. A. Cushman) *Dentalina gardnerae* (Plummer), *Nodosaria affinis* (Reuss), *Robulus wilcoxensis* Cushman and Ponton var. *dissentia* Cushman and Todd, *Robulus* cf. *inornatus* (D'Orbigny); the mollusks (identified by J. B. Reeside, Jr.) *Drepanochilus americanus* (Evans and Shumard) var. *pusillus* Stanton, *Polynices* sp., *Dentalium* sp., *Nucula* sp., *Nuculana* sp., *Trigonarca?* sp., *"Corbula" mactriformis* Meek and Hayden, *Neptunella gracilis* (Stanton), *Neptunella newberryi* (Meek and Hayden), *Fasciolaria* (*Mesorhytis*) *dakotensis* Stanton; the worm *Serpula* sp.; the ostracodes (identified by F. M. Swain) *Cytheridea* cf. *fornicata* Alexander, *C.* cf. *ruginosa* Alexander, *C.* cf. *multipunctata* Alexander, *Cythereis* cf. *prestwichiana* Jones, *Brachythere* cf. *interrasilis* Alexander; and shark teeth (identified by D. H. Dunkle) *Odontaspis* sp.

From these outposts of the Cannonball formation one may perhaps look hopefully toward the glaciated country north and northeastward with the expectation that still other outcrops or subsurface evidence may be found that will provide further information about Cannonball paleogeography and the line of retreat of the Cannonball sea in the latter half of the Paleocene. Since that date

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