Lysine and Cariogenicity of Two Experimental Rat Diets

Smooth surface dental caries in white rats resulted from a diet containing heat-processed cereal foods (*1*) and also from diets containing commercial roller- and spray-process skim-milk powders (*2*). An additional "dry autoclaving" of both roller-dry and spray-dry skim-milk powders augmented their cariogenic property (*2, 3*). The more severe the heat treatment during preparation of skim-milk powders, the greater the apparent cariogenic effect in rats' diets (*4*).

In these previous experiments, growth failure resulted from feeding both diet 586 (*1*) and diet 636 (*2*), but growth was greatly improved by a lysine supplement. A deficiency of lysine in diet 586 was due to its cereal content as well as to an additional heat treatment of the cereal foods (*5*). Lysine was inadequate in diet 636, because of the dry autoclaving of the milk powder (*6*). The effect of lysine on the cariogenic potential of these diets thus became a matter of great interest, and this report presents current results from this continuing experimentation (*7*).

The general plan of this kind of caries study, as well as the preparation of the diets, is described elsewhere (*1–3*). The rats were of Holtzman and Sprague-Dawley strains from the National Institutes of Health. The dental caries was essentially all the smooth surface type, although considerable occlusal fissure caries, along with smooth surface caries, has been observed by other investigators who have fed diet 636 (*8*). Mixed thoroughly into the dry ration, lysine was the only variable in the test versus control diets. Weanling-age litter-mate rats distributed equally according to sex were compared on control and test diets for either 60 or 90 days. In most of these studies, food allotments of pairs (in one instance tetrads) of control and test rats were equalized. The pertinent data are presented in Table 1.

In the first of these studies (diet 586, Table 1) a supplement of 2.5-percent DL-lysine accounted for a significant reduction in both incidence and severity of caries. Following these initial studies major interest centered on diets containing skim-milk powders (*3, 4*) including diet 636, which was lysine deficient. These results have proved especially interesting because a notable reduction in caries occurred on the addition of L-lysine to diet 636. This reduction was consistent in 10 comparisons of diet 636 fed with and without L-lysine supplement (Table 1). On the average, L-lysine reduced caries approximately 59 percent in incidence, 78 percent in number of carious teeth, and 83 percent in severity score.

To throw further light on the possible specificity of L-lysine as an anticaries agent in diet 636, L-arginine monohydrochloride (0.5 percent) and L-histidine monohydrochloride (1.0 percent) were added independently to diet 636. Some loss of these two basic amino acids occurred when skim-milk powder had been dry autoclaved (*9*). However, no inhibition of caries resulted from these two supplements. Additional experimentation also compared L-lysine monohydrochloride with D-lysine monohydrochloride, L-ornithine monohydrochloride, and cadaverine dihydrochloride, which were added independently at a level of 0.25 percent to diet 636. This study evaluated compounds similar to L-lysine in composition and structure, for speculation on this entire problem had considered the possibility that free L-lysine, per se, might have an anticaries effect independent of its nutritional requirement. Related compounds, therefore, might have a similar anticaries property. Whereas L-lysine was highly inhibitory of caries, D-lysine had no effect on caries and, as was expected, did not promote growth. Neither did ornithine nor cadaverine inhibit the caries produced by diet 636.

In view of the striking reduction in the cariogenicity of diet 636 by L-lysine and of diet 586 by DL-lysine, similar studies with diet 635 were undertaken. Although this latter diet contains unautoclaved milk powder and is not deficient in lysine, insofar as may be indicated by its ability to support growth, it nevertheless has proved to be distinctly cariogenic (*2, 3*). Thus far the addition of L-lysine to diet 635 at levels of 2.00 and 2.50 percent has given somewhat variable results. The data indicate strongly that L-lysine does not significantly inhibit the smooth surface caries produced by this diet, which may possibly be reconciled by the fact that it is adequate in lysine. This result does not support the idea that free L-lysine, per se, has anticaries properties, but it does suggest that the cariostatic effect of L-lysine is somehow dependent on its dietary deficiency, at least as produced during heat processing of a skim-milk powder.

Although the cariogenic potential of a diet containing dry autoclaved skim-milk powder was greatly inhibited by as little as 0.25-percent L-lysine, this does not justify the conclusion that a lysine deficiency alone causes the cariogenicity of this diet. A combination of the complex changes identified with heat processing of milk powders may be involved. On the other hand, it is of interest to note a recent report in which degenerative changes in incisor dentine, alveolar bone, and the mandibular condyle of rats are attributed to a dietary deficiency of lysine (*10*). Additional studies are required to determine whether the lysine deficiency of diet 636 has a similar adverse effect on rats' dental structures and whether the defects are related to the caries. In control and L-lysine-supplemented pairs of rats, in which food allotments were equalized, caries reduction was not related to the growth-promoting effect of L-lysine.

Table 1. Effect of a lysine supplement on the smooth surface rat caries produced by diets 586 and 636

L-Lysine added* (%)	Rats (No.)	Daily gain (g)	Carious rats (%)	Carious teeth per rat	Severity score
		Diet 586			
0.00	45	0.3‡	62.2	1.7	5.0
2.50†	59	0.5‡	18.6	0.5	0.8
		Diet 636			
0.00	37	1.0‡	89.2	3.5	8.4
2.50	37	1.2‡	51.4	1.3	2.0
0.00	40	0.7‡	60.0	1.3	2.1
2.00	38	1.0‡	7.9	0.1	0.2
2.00	39	1.8	23.1	0.4	0.6
0.00	38	0.6	92.1	6.4	21.3
2.00	37	1.5	78.4	1.5	4.1
0.00	16	0.5‡	87.5	4.4	11.6
2.00	19	0.7‡	42.1	0.6	0.8
0.00	38	0.6‡	94.7	4.6	10.5
1.50	34	1.0‡	32.4	0.9	1.5
0.00	34	0.9‡	82.4	3.3	6.9
0.25	36	1.1‡	61.1	1.6	2.7
0.00	43	0.3‡	83.7	2.6	5.4
0.25	43	0.4‡	20.9	0.5	0.8
0.50	44	0.5‡	20.5	0.4	0.6
2.50	44	0.5‡	9.1	0.2	0.3

* Fed as the monohydrochloride. † DL-lysine monohydrochloride. ‡ Food allotment of paired control and test rats was equalized.

The inactivity of arginine and histidine strongly suggests that a deficiency in lysine is at least one basic cause of the caries potentiality of diet 636. The specificity of L-lysine as a compound having caries-inhibitory effects in diet 636 is also supported by the evidence that D-lysine in particular, as well as ornithine and cadaverine, failed to reduce the caries produced by this diet.

Continuing studies will attempt to resolve some of the questions raised by these latest results and particularly to clarify the possible role played by L-lysine. The properties of these cariogenic experimental diets permit somewhat new approaches to the resolution of the possible relationship of dietary factors to the etiology of dental caries.

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Interaction between Oxygen and Oxygen-Carryng Proteins

In a recent article I. M. and T. A. Klotz (*1*) state that they have elucidated the nature of the electronic changes responsible for color and the oxygen-carrying ability of certain metal-proteins. Inspection of the observations and deductions of these authors leads to a somewhat different impression.

The article (*1*) starts with a summary of earlier studies of hemocyanin, a copper-containing protein that carries oxygen. Although the general inference is drawn that the protein contains cuprous copper, it is correctly stated that the evidence from magnetic and spectrophotometric data, as well as from the experiments of the authors on the oxidation-reduction potentials of the protein, is inconclusive. Some chemical evidence is then discussed. The experiments were carried out by the authors in the following fashion. Hemocyanin and oxyhemocyanin were separately prepared, and the copper content of the proteins was estimated by means of diquinolyl, a reagent for cuprous copper. A separate experiment was made so that the total copper content of the protein was known. Then, the authors continue, the difference between total copper content and copper content determined by diquinolyl gives the content of cupric copper.

Two points deserve consideration here. First, the reaction with diquinolyl is not instantaneous, on the authors' evidence, and no proof that the reaction goes to completion is given. It is less likely to go to completion with oxyhemocyanin than with hemocyanin, for the copper is bound with much greater affinity in the oxyhemocyanin. Diquinolyl can act only as a competing ligand for cuprous copper against the protein and oxygen, assuming, as the authors do, that there is a direct oxygen-copper bond, and it seems more reasonable to assume that the reagent fails to extract all the coppen than to assume that all it fails to extract is cupric ions. Klotz and Klotz do not test for cupric ions. If their assumptions are allowed, however, the conclusion is reached that half of the copper is as cupric and half as cuprous in the oxyhemocyanin. (In passing it should be observed that the figure of a half is derived from a comparison of the cuprous content of hemocyanin and of oxyhemocyanin, but the latter is only 39 percent of the *total* copper in the protein.) Surely, on removal of the cuprous copper from the oxygenated protein, the oxygen must be released, especially if the stability of the oxygen complex depends on the structures given. But the uptake of oxygen is reversible in the protein, and release of oxygen should lead to the "cupric" ions of oxyhemocyanin reverting to cuprous. Klotz and Klotz do not observe this. Does their procedure irreversibly oxidize the cuprous ion?

Turning next to hemerythrin, the authors present an identical argument. This protein is iron-containing, and the estimation of the ferrous and ferric content is made by use of phenanthroline, which gives a color with ferrous ions. No color developed in the reaction between the reagent and oxyhemerythrin; therefore, state Klotz and Klotz, all the iron is in the ferric state. The more likely conclusion is that the phenanthroline extracted no ferrous iron from the protein. The authors do not test for ferric ions. In hemerythrin itself about two-thirds of the iron was found to be ferrous by this phenanthroline "test."

If it is allowed that Klotz and Klotz have established a case for mixed valence states in the oxygen-containing proteins, then some of the structures proposed by them can be considered. At this point, however, the authors confuse two models. One is of the type discussed by McConnell and Davidson (*2*) in the mixed valency complexes, such as $Cu(II) \cdot Cl^- \cdot Cu(I)$ in solutions of which both cuprous and cupric ions could be detected, and the other is the no-bond complexes in which also charge-transfer forces play a part, for example, $Cu(I) \cdot O_2 \cdot Cu(I)$. Contribution to the stability of the latter type of complex is made by the structure $Cu(II) \cdot O_2^- \cdot Cu(I)$, but cupric ions are not detectable by chemical tests. These charge-transfer structures for oxygen-carrying proteins are contained in Pauling's and Mulliken's descriptions of double bonding in the transition metal complexes with unsaturated ligands and have been discussed with regard to hemocyanin (*3*). The evidence cited by Klotz and Klotz does not elucidate these matters.

Finally, turning to the absorption spectra of the oxygen-carrying proteins, the following observations are worth note. Ferrous and cuprous ions form complexes with aromatic diimines, which have very similar absorption spectra. The characteristics of these spectra have been explained on the assumption that the excitation involves the partial transfer of electrons from the cation to the ligand (*4*). Very similar absorption bands are found in certain cobaltous complexes. All three cations in other complexes appear to be able to carry oxygen, but the ferrous and cobaltous complexes do so only if, on the uptake of oxygen, there is a change in paramagnetic moment. Simultaneously with the change in paramagnetic moment, there is a large change in the absorption spectra of the complexes. This change in absorption is so like that described by Klotz and Klotz in hemerythrin on oxygen uptake that it is tempting to conclude that hemerythrin is a ferrous protein and paramagnetic but that it becomes diamagnetic on uptake of oxygen. It is also a mistake, on the evidence available, to assume that the absorption spectrum of oxyhemocyanin is not that of a cuprous protein (*1*) and (*3*). Many cuprous complexes do absorb strongly in the near ultraviolet, and many others have absorption bands in the visible. The final word on the electronic states of the oxygen-carrying proteins has not been said.

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