Effect of Lysine Provided by Different Routes on Cariogenicity of Lysine Deficient Diet. (23559)

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Evidence of a cariogenic role of heat-processed skim milk powder in diets of white rats has been presented(1-5). In lysine deficient diets a dietary supplement of L-lysine resulted in a reduction in caries(6,7). An unanswered question pertaining to the caries inhibitory effect of lysine under these conditions is its mode of action, *i.e.*, is lysine acting within the oral cavity or does it have a systemic effect which in turn influences susceptibility to dental caries. To obtain information relative to this question, the current experiments have compared the effect of lysine ingested (a) in food, (b) in drinking water, (c) by intubation, and (d) by intraperitoneal injection.

Methods. The general plan of these studies as well as the preparation of the lysine-deficient basal diet 636 are described elsewhere(1,2). Holtzman rats from the National Institutes of Health colony, at weanling age were distributed as litter mates between control and test groups, and continued on experiment sixty days. Teeth were examined under low power (10X magnification) as previously described(4) and only that caries scored, which was observed on buccal and lingual surfaces. The essential data and plan of the experiments appear in Table I. In Experiments 1-a, –b, –c, –d and –e, 5 littermates from 40 litters were housed 2/cage. A regimen of approximately equalized diet intake of paired control and test littermate rats was followed by giving equal quantities of diet to the 2 rats/per cage. Under this regimen, followed also in experiments 2–a and –b, 3–a and –b, rats which had no supplemental lysine consumed *ad libitum* a quantity of diet which established the level of food intake for their littermates receiving supplemental lysine. Rats in both 1–a and –b served as controls, the latter group receiving 1 ml distilled water by intubation 5 days per week. One ml of a solution containing 75 mg L-lysine* was intubated in Exp. 1–c, 5 days/week. The diet and drinking water of rats in group 1–d and –c contained 1.5% of L-lysine. In Exp. 2–b (2–a served as control) one ml of L-lysine solution (75 mg/cc) was intubated 6 days/week. Food was withheld from 5 P.M. to 9 A.M. Two hours after feeding at 9 A.M., the rats were intubated. With this regimen it seemed probable that the intubated L-lysine would be available when food was present in the digestive tract. In experiments 3–b and 4–b, the rats were injected intraperitoneally twice daily 5 days/week (once on sixth day) with one ml of a solution containing 100 mg L-lysine. Rats in 3–a and 4–a were injected with 1 ml of distilled water. In experiments 4–a and –b the rats were housed individually and daily food intakes approximately equalized, for each pair of control and test rats. Food consumption was *ad libitum* in Exp. 5-a, -b, -c, and –d. One ml of a solution containing 100 mg of L-lysine was intubated or injected twice daily 5 days/week (once on sixth day) in Exp. 5–c and –d respectively. The diet in 5–b contained 1.5% of L-lysine. A littermate group of control rats was compared with groups of test rats, each group containing 40 rats and housed 2/cage. All the rats in all experiments were weighed weekly. The growth data for this last experiment are shown in Fig. 1. The approximate daily intake of supplemental L-lysine was as follows: 1–c (intubation) 55 mg, 1–d (diet) 75-80 mg, 1–e (drinking water) 150-180 mg, 2–b (intubation) 65 mg, 3–b, 4–b, 5–c and 5–d (injection or intubation) 160 mg, 5–b (diet) 150-180 mg. It may be noted that in one previous study on the effect of L-lysine added to the diet, 0.25%, 0.50% and 2.50% L-lysine were about equally effective

* As the monohydrochloride.

TABLE I. Effect of Lysine on Cariogenic Property of Diet 636 Containing Autoclaved Skim Milk Powder.

Exp.	Diet regimen and supplement	No. of rats	Avg daily gain* (g)	Caries experience: Rats with caries (%)	Caries experience: Carious lower teeth/rat	Caries experience: Carious areas/rat	Caries experience: Caries score/rat
1 a	Control	38	.55	94.7	4.6 ± .4†	9.1 ±1.0	10.6 ±1.5
b	Water by intubation	37	.57	100.0	5.3 ± .6†	10.7 ±1.4	13.7 ±1.9
c	L-lysine by intubation	29	.88	72.4	2.0 ± .4†	3.2 ± .7	3.4 ± .8
d	" : 1.5% in diet	34	1.00	32.4	.9 ± .3†	1.4 ± .5	1.5 ± .5
e	" : 1.5% in drinking water	33	1.00	45.5	1.1 ± .3†	1.8 ± .5	1.9 ± .5
2 a	Control	30	.75	84.6	2.9 ± .3	5.5 ± .7	6.3 ± .9
b	L-lysine by intubation	39	1.12	38.5	.5 ± .2	1.1 ± .3	1.2 ± .3
3 a	Control	39	.52	87.2	2.4 ± .4	3.6 ± .7	4.0 ± .8
b	L-lysine by intraper. inj.	36	.79	66.7	1.9 ± .3	2.9 ± .6	3.4 ± .7
4 a	Control	40	1.15	47.5	1.0 ± .2	1.3 ± .3	1.4 ± .3
b	L-lysine by intraper. inj.	39	1.35	48.7	1.4 ± .3	2.2 ± .2	2.4 ± .7
5 a	Control	38	.72	100.0	4.9 ± .3	11.0 ± .9	18.7 ±2.0
b	L-lysine: 1.5% in diet	37	2.12	81.1	2.6 ± .3	4.8 ± .6	5.4 ± .8
c	" by intubation	39	1.70	94.9	3.7 ± .3	7.1 ± .7	10.8 ±1.4
d	" by intraper. inj.	38	1.69	100.0	4.7 ± .2	9.4 ± .7	13.0 ±1.3

* Food intake was approximately equalized in all experiments except 5 a, 5 b, 5 c and 5 d.
† Both upper and lower teeth were diagnosed.

in reducing the caries which develops on diet 636(6). The estimated lysine intake in these later studies(6) was 40-200 mg daily.

Results. As was to be expected(6), 1.5% L-lysine in the diet reduced caries significantly (experiments 1–d and 5–b). L-lysine also significantly reduced both incidence and severity of caries when given in the drinking water (experiment 1–e). In three trials, 1–c, 2–b and 5–c intubation of L-lysine also significantly reduced caries severity. The difference in the control score 18.7 (experiment 5–a) and a score of 10.8 (experiment 5–c) is statistically significant, $p < 0.01$. Severity scores were reduced by stomach-tubed L-lysine from 13.7 (1–b) to 3.4 (1–c) and from 6.3 (2–a) to 1.2 (2–b). Incidence of caries was also reduced by lysine intubation from 100.0% (1–b) to 72.4% (1–c) and from 84.6% (2–a) to 38.5% (2–b).

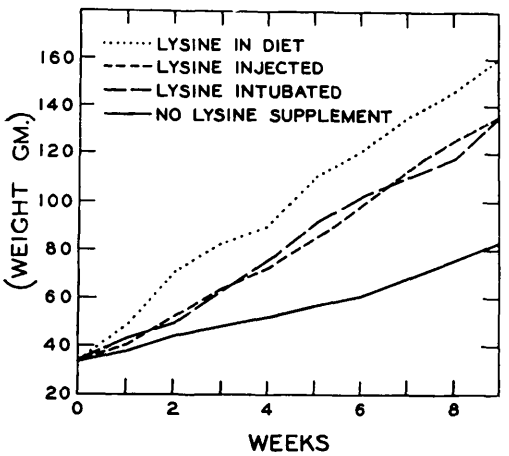

FIG. 1. Growth of rats receiving lysine by different regimens as a supplement to diet 636.

The results of administering L-lysine by intraperitoneal injection were variable, a caries reduction having occurred only in experiment 5–a *vs* 5–d and this was evidenced only in severity, $p < 0.02$. No change in caries was noted in experiments 4–a and –b. In connection with the low incidence of very mild caries observed in experiment 4–a, it may be noted that there is considerable variability in the caries in control groups of rats. This may be explained by differences in rats and by variations in the lysine content of the autoclaved skim milk powder. Different batches of skim milk powder were used and very slight changes in autoclaving conditions, moisture particularly, influence the lysine inactivation. It is generally true, also, that housing one rat/cage, and providing fresh diet daily (as required by control feeding) usually results in a low incidence of very mild caries. Food consumption which is increased by giving this diet fresh daily, as well as housing individually, would ap-

pear to account for the increased rate of growth in 4–a.

Discussion. Lysine in the diet and drinking water was about equally effective in inhibiting caries, followed by lysine administered by intubation. Under the conditions of these experiments, no consistent significant inhibition of caries resulted from injected lysine. Of special interest, however, is the finding of a significant caries-inhibitory effect of intubated lysine. It would seem that a systemic association could be the determining factor in this cariostatic action of lysine, in contrast to a direct effect of lysine localized within the oral cavity.

The possibility that an increase of lysine in rats' saliva could explain the effect of intubated lysine was considered and the following experiment was conducted to help resolve this question.† After injecting 1.0 ml of a solution containing 100 mg lysine intraperitoneally, saliva was collected according to the procedure of Benarde *et al.*(8) with slight modifications. The pooled salivas of 16 rats (about 1 cc per rat in 30 minutes) were analyzed for lysine using ion exchange chromatography on Dowex 50(9). The amount of lysine determined was less than 1.0 mg %, a quantity which seems insufficient to have brought about this significant effect of intubated lysine on the rats' caries experience.

The growth data in Fig. 1 show that under this regimen of *ad libitum* feeding dietary lysine gave the best growth improvement whereas intubated and injected lysine afforded about equal improvement in rate of growth. It is of interest that whereas injected lysine under the conditions of this experiment resulted in no consistent caries reduction, it was utilized for growth (Fig. 1). The result is in keeping with a previous suggestion(7) that the requirement of this amino acid for optimum growth may not coincide with other systemic requirements.

Finally in resolving the complex etiology of dental caries, it may be noted that there is need to extend our information regarding the role of the systemic metabolism beyond the immediate confines of the oral cavity. It is of particular interest, therefore, that a reduction in caries resulted from intubated L-lysine. In this connection it may be mentioned also that in preliminary studies from this laboratory, a caries potentiating effect of intubated EDTA (ethylene diamine tetra acetic acid) has been observed‡ and also according to other experiments,§ DHAS (sodium dehydroacetate) administered by stomach tube as well as by intraperitoneal injection, caused a significant increase in this particular experimental smooth surface rat caries.

Conclusions. (1) Administration of L-lysine in food, water and by intubation, to rats receiving a highly cariogenic lysine-deficient diet containing autoclaved skim milk powder, significantly inhibited smooth surface caries as diagnosed in these experiments. Although L-lysine as administered by injection was utilized for growth, it did not clearly inhibit caries. (2) When administered by intubation L-lysine was cariostatic. The result suggests a systemic involvement in this action of lysine on dental caries. This hypothesis gains support in the fact that injection of lysine resulted in less than 1 mg % lysine in rats' saliva.

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‡ Larson, R. H., Rubin, M., and Zipkin, I., unpublished data.

§ Zipkin, I. and McClure, F. J., unpublished data.

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