Original

THE MODE OF INHIBITING ACTION OF Ca/P RATIO IN A CARIES-INDUCING DIET, 6PMV, IN RATS***

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Abstract: Of three caries inhibiting factors, Ca, P, and Ca/P ratio, in caries inducing diets recommended by different authors, Ca/P ratio functioned as the primary determinant. It is considered that Ca-and P-components could form a cubic concave surface mode of action against caries incidence, and the proposed model reconciled the previous arguments on this subject.

Key words: Ca/P ratio, Rat, Experimental caries, Diet

Introduction

The caries preventing effect of Ca and P, which almost all diets contain in various ratios, has been so conflictingly reported by different authors that one group ascribes the caries reducing activity to Ca^{1,2)}, a second to phosphates^{3,4)}, and a third to the Ca : P ratio⁵⁾.

McClure⁶⁾ and Dalderup⁷⁾ claimed that no caries reduction was caused by increasing Ca in the diet which they used. Nizel and Harris⁴⁾ reviewed papers published until 1962, emphasizing the P effect, even when it was influenced by the kind of phosphate and the way of administration, and they stressed the importance of an optimum dosage of phosphorus in the diet. Stanton⁵⁾ demonstrated the most effective Ca and P ratio to be 0.55 to 0.65 for average American adults by analysis of the foods which they ate in one week. McClure and McCamm⁸⁾, however, found that changing the Ca/P ratio of the diet not influence caries susceptibility in rats when they were placed on a caries-inducing diet after weaning. Sobel et al⁹⁾ stressed the good effect of P in the diets designed with several Ca/P ratios. Wynn et al¹⁰⁻¹¹⁾ concluded from serial studies that a larger amount of Ca and P in diets with a constant Ca/P ratio of 1.0 resulted in a larger reduction of caries incidence.

However, we¹²⁾ obtained results similar to those of Stanton in a diet containing a modified McCollum salt mixture by which we were able to obtain various Ca/P ratios by replacing CaCO₃ with NaHCO₃.

The objective of the present studies, therefore, was to reconcile the incompatible claims presented by the different authors working with the Ca and P problem in caries-inducing diets and to represent the mode of action of these diets.

Material and Methods

From the pure strain colony of Wistar rats kept at the Department of Preventive Dentistry, Tokyo Medical and Dental University, Faculty of Dentistry, 180 rats 21 days old were divided into 9 groups containing 20 rats each. 4 groups were used for the 1st experiment testing the effect of a Ca-constant-but-P-variable

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Table 1 Components of 6PMV.

Sugar	600 g
α -Starch	210 g
Casein	100 g
Salt mixture*	50 g
Panvitan	10 g
Olive oil	30 ml
* Modified McCollum's salt mixture	
CaCO_3	544.08 g
KH_2PO_4	212.00 g
KCl	112.00 g
NaCl	69.00 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	32.80 g
MgCO_3	25.00 g
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	51.00 g
$\text{FePO}_4 \cdot 4\text{H}_2\text{O}$	20.50 g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1.46 g
$\text{Al}(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$	0.31 g

salt mixture in the caries inducing diet, 6PMV¹³⁾, and 5 groups were used for the 2nd experiment to test the effect of Ca and P in the same diet system. Modification of Ca and P content was done by changing the amount of CaCO_3 and/or KH_2PO_4 in the McCollum salt mixture in the 6PMV. The actual amounts of Ca and P are found in Table 2 for the 1st experiment and in Table 3 for the 2nd experiment. All rats freely drunk the deionized water provided.

At the end of 30 days caries time, they were decapitated under chloroform anesthesia, and the jaws were placed in 10% neutral formalin solution for the fixation. Later, three molars from one side, usually the right side, were sawed into three serial sections about 100 μm thick with a Bronwill's apparatus.

Table 2 Caries production on a Ca-constant-but-P-variable diet during the 30 days caries time.

Rats groups	Diet system		Ca/P	No. of rats surviving	Body weight		Caries production	
	Ca(g/K)	P(g/K)			Wo(g)	ΔW (g)	Prevalence (Frequency)	Extent
1	12.01	10.20	1.2	18	36.2	56.9	0.50	1.22
2	12.01	8.70	1.4	19	37.4	49.8	0.44	1.11
3	12.01	6.45	1.9	18	37.3	49.0	0.42	1.17
4	12.01	4.25	2.8	18	35.9	44.2	0.61	1.56

Wo: Body weight at the beginning of the experiment.

ΔW : Body weight gain during the caries time.

Table 3 Caries production on a Ca- and P-variable diet during the 30 days caries time.

Rats groups	Diet system		Ca/P	No of rats surviving	Body weight		Caries production	
	Ca(g/K)	P(g/K)			Wo(g)	ΔW (g)	Prevalence (Frequency)	Extent
5	29.58	19.40	1.5	18	34.1	26.4	0.22	0.44
6	16.38	8.14	2.0	20	34.3	45.9	0.30	0.45
7	13.08	5.29	2.4	18	33.7	41.5	0.50	1.00
8	11.88	4.33	2.7	19	34.4	35.3	0.74	2.34
9	10.80	3.40	3.2	19	34.2	32.4	0.88	4.53

Induced lesions were detected on a film shadowed by soft X-Ray under a low power microscope. The lesions, which were usually found at the dento-enamel junctions under the bottom of the fissures and on the contact areas were scored and averaged, giving the caries extent of the group. The carious rate of the surviving rats of the group was also counted as the caries inducing capacity of the diet, giving the caries prevalence of the group. The prevalence of the group is convertible to the logarithm of the extent

based on the fact : $P=0.625 \log E+0.512^{14)}$, where P represents the prevalence and E represents the extent.

Results

The Findings are shown in Tables 2 and 3 and plotted in Figure 1, where the Ca/P is plotted on the abscissa and caries prevalence and extent are plotted on the ordinate.

In the 1st P-variable-but-Ca-constant diet experiment, the caries incidence curve reached the lowest point, showing the most effective dose of phosphorus against the constant Ca amount in the diet. In the 2nd Ca-and-P-variable diet experiment, caries incidence was decreased by increasing both Ca and P in the diet, suggesting that the minimum point was approached.

Disucssion

To reach an undisputed conclusion from the data obtained, it is necessary to consider the quantitative relations of Ca, P, and Ca/P ratio at the minimum point of caries incidence. It would be reasonable to combine the data obtained in our previous work on Ca-variable-but-P-constant conversion of the 6PMV, even though the caries lesions developed in this case were evaluated by counting open cavities on each fissures after 90 days of caries time, as shown in Table 4 and Figure 1.

Table 4 Cavity production on a Ca-variable-but-P-constant diet during 90 days caries time.*

Rats group	Diet system		Ca/P	No. of rats surviving	Body weight		Cavity production	
	Ca(g/K)	P(g/K)			Wo(g)	ΔW(g)	Prevalence (Frequency)	Extent
1	1.67	3.45	0.48	30	34.5	182	0.30	0.7
2	3.34	3.45	0.96	24	35.7	190	0.08	0.3
3	6.70	3.45	1.94	30	35.0	169	0.00	0.0
4	10.88	3.45	3.15	28	35.8	148	0.36	1.4

* All data are reproduced from results obtained in a previous experiemient.¹²⁾

As Tables 2, 3, and 4 show, the actual concentrations of Ca and P at the least caries incidences are scattered widely the most effective Ca concentration was 29.6g/k diet in the 2nd experiment and 6.7g/k diet in the previous experiment, the most effective concentration of P was 6.45g/k diet in the 1st experiment and 19.4g/k diet in the 2nd experiment. Considering the counter minerals contained in the same diets, however, the wide variability of the Ca and P content in the diet meets in the narrow range of Ca/P ratios 1.4-1.9. These results, therefore, indicate that the Ca/P ratio is the primary determinant in all diets.

The above discussion can be expressed by a cubic concave image, in other words, a valley-shaped surface constructed from three axis of the caries incidence, Ca-content, and P-content of a diet (Fig. 2).

The caries incidences obtained from previous data on a Ca-variable diet, however, have to be enlarged to compare them to the caries incidences obtained in the present experiments, because open cavities are much less estimable than the closed early lesions found in the dentinoenamel junction of the molars detectable on microradiograms¹⁵⁾. If the above presumptions are accepted, the cubic concave mode of action expresses the findings of all the various authors cited in the introductory paragraph of this paper.

However, further discussion is needed on the discrepancy found between our most effective Ca/P ratio (1.4-1.9) and Stanton's (0.55 to 0.65). This could be explained by the differences in test objects (rats vs. human adults) and different of approaches (synthetic diet vs. reports on food consumed for one week). Based on the proposed model, speaking in general, in order to decrease caries incidence in both countries, it is

Figure 1 Interrelationships between produced caries and Ca/P ratios in three experiments.

● and ○ denote data from Ca-constant-but-P-variable diet, ● and ○ data from Ca-and P-variable diet, and ● and ○ data from Ca-variable-but-P-constant diet

P: Prevalence of caries

E: Extent of caries

Figure 2 The proposed model of caries inhibiting mode of action between Ca/P ratio, Ca and P amount, and caries incidence.

CI axis: Caries incidence represented by caries prevalence.

Ca axis: Ca content in g/kilogram diet.

P axis: Phosphorous content in g/kilogram diet.

▲: Ca-constant, but-P-variable diet.

○: Ca-and P-variable diet.

●: Ca-variable-but-P-constant diet.

necessary eat more Ca-containing foods in Japan, where the diet is low in Ca (Ca/P 0.31 in 1963¹⁶⁾), and Stanton suggested need of additional phosphorus in U. S. foods.

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概要: 鼠のう蝕誘発食餌, 6PMV 中の Ca/P 比率のう蝕阻止作用様式, 大西正男*, 尾崎文子**

食餌に含まれている Ca, P とその Ca/P 比率のいずれが, 鼠う蝕誘発能力を抑制するかを知るために, 合成う蝕誘発食餌 6PMV の Ca と P 成分を変えることによって, Wistar 系鼠に発生するう蝕数の変化を観察した。その結果う蝕抑制の第一次決定因子は, Ca/P 比率であることがわかった。食餌中の Ca, P 量はう蝕発生量に対して立体的な凹面模型で示されることが考察され, このモデルは従来のこの問題に対する 3 つの学説を統一した。

索引用語: Ca/P 比率, 鼠, 実験う蝕, 食餌