THE CARIES REDUCING EFFECT OF NARINGENIN AND OF PROTAMINE IN HAMSTERS

by

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With the aim of finding agents effective in the prevention of dental caries in man a series of compounds have been tested *in vitro* for their inhibition of acid production by oral microorganisms. A simple screening technique is to measure the reduction of the amount of acid produced from glucose in saliva from caries susceptible individuals or by lactobacilli or streptococci in a synthetic medium.

In connection with the Vipeholm Dental Caries Study (1) and using such techniques *Gustafsson* (2, 3) investigated flavonoles and their glucosides and demonstrated that at equimolar concentrations naringenin, naringenin-chalcone, quercetin, hesperitin and floretin had a stronger inhibitory effect than sodium azide and sodium fluoride. The corresponding glucosides had no effect. Working in the same laboratory *Krasse* (4) found a strong effect of 5-fluornicotinamide, protamine and guanazolo on the acid production of oral microorganisms *in vitro*.

As the flavonoids and protamines are normally present in some widely consumed food-stuffs and therefore presumably non-toxic, the scope of the present investigation was limited to assessment of the effect of a flavonoid (naringenin) and of protamine on experimental caries in hamsters.

MATERIAL AND METHODS

The material consisted of male Syrian hamsters from a stock known from other studies (*Strålfors* 1956) to be caries susceptible (6).

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The animals were kept in large galvanized steel cages with mesh wire bottoms. Eight to nine animals were kept in each cage.

In the experimental groups the diet was supplemented by 0.2 % naringenin and 0.1 % protamine-sulphate*) respectively.

The naringenin was prepared by hydrolysis of naringin (Eastman Kodak) by boiling for 3 hours 100 g of this compound and 40 ml concentrated sulphuric acid in 1000 ml of distilled water. After cooling, the precipitated naringenin was filtered, repeatedly washed with water, and dried. The diet, which was similar to that used by *Keyes* (5) and by *Strålfors* (6), was given in a dry state and had the following composition: Skim milk powder 30, confectioner's sugar 20, whole wheat flour 20, alfa-alfa meal 5, potato flour 25. Distilled water was provided from bottles with glass spouts, and, like the food, was given *ad libitum*.

The animals were placed on the abovementioned diet at an age of 20—25 days and the length of the experimental period was 125 days. The animals were sacrificed by bleeding from the heart in ether anaesthesia. Autopsy was performed with registration of any gross pathological changes. The heads were autoclaved, the soft tissues removed, and the teeth were examined under a dissecting microscope ($\times$ 16).

The dental examinations were made independently by both investigators. The recordings were afterwards compared and discrepant findings were reexamined and corrected. The caries incidence was expressed as the mean number of carious teeth, carious surfaces and cavities per animal.

RESULTS

Throughout the experimental period the general health of the animals was good and no animal died. After a slight difference of about 20 % in body-weight (Fig. 1) between the control and the experimental groups at the start and during the first weeks of the experimental period, the naringenin and protamine groups gained more weight than the control group. At the end of the experiment the weights were practically identical. No gross pathological changes were observed at the autopsies.

*) The protamine-sulphate was kindly supplied by AB Vitrum, Stockholm.

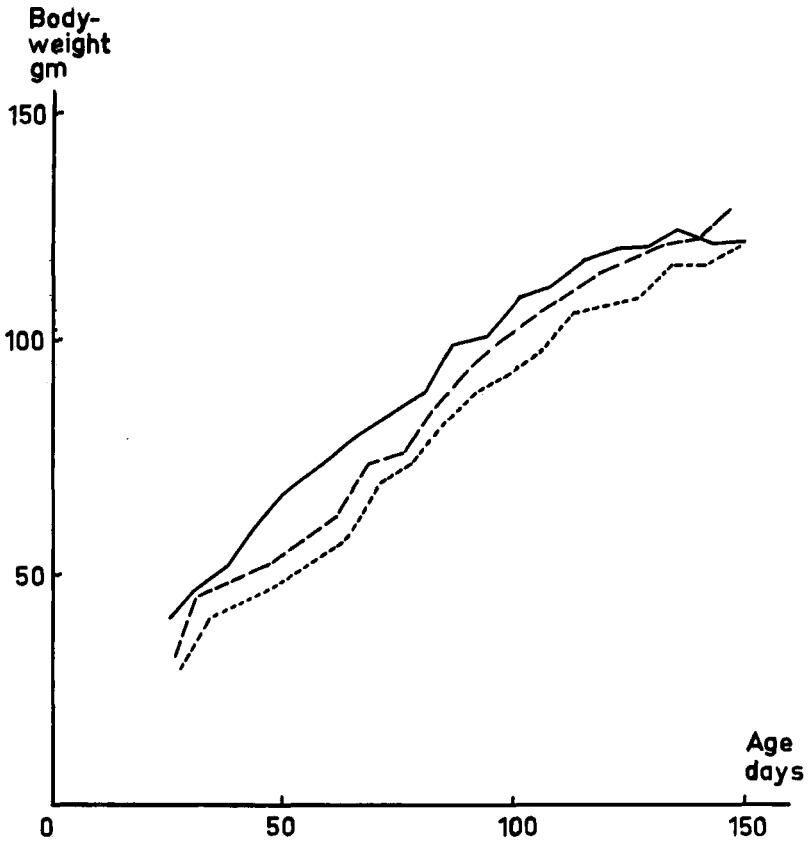

Fig. 1.
Growth curves of the animals in the control group ————, the naringenin group — — — —, and the protamine group .....

As is shown in Table 1, the caries incidence was higher in the control group than in the naringenin and the protamine groups. Further analysis (Table 2) shows that the differences between the control and the experimental groups are statistically significant with regard to the number of carious teeth and the number of cavities.

Table 3 shows the statistical analyses of the differences between the control and the experimental groups with regard to the development of caries on different tooth surfaces.

It is clear from Table 3 that, with regard to all carious surfaces, a statistically significant difference was found between

Table 1

The effect of dietary supplements of naringenin or protamine on dental caries incidence in hamsters.

	Control group	Naringenin group	Protamine group
Number of hamsters	17	23	24
Mean number of carious teeth	8.5 ± 0.5	5.3 ± 0.5	5.0 ± 0.4
Mean number of carious surfaces	16.1 ± 1.9	7.7 ± 0.7	9.6 ± 0.9
Mean number of cavities	9.8 ± 0.8	5.7 ± 0.5	5.8 ± 0.5
Mean number of carious occlusal surfaces	6.2 ± 0.6	2.6 ± 0.3	2.0 ± 0.3
Mean number of carious lingual, buccal and approximal surfaces	10.0 ± 1.2	5.1 ± 0.5	7.6 ± 0.7

Table 2

Statistical analysis of the differences between the control group and the experimental groups. Carious teeth and cavities.

Comparison	Carious teeth		Cavities	
	t-value	P	t-value	P
Control-naringenin	4.8	< 0.001	4.3	< 0.001
Control-protamine	5.5	< 0.001	4.2	< 0.001

the control group and the naringenin group. Between the control group and the protamine group the difference was probable. Table 3 also shows that the substances had a somewhat varying caries inhibiting effect on different surfaces: protamine gave a higher caries reduction on the occlusal surfaces and a lower caries reduction on the smooth surfaces than did naringenin.

Table 3
Statistical analysis of the differences between the control group and the experimental groups. Carious surfaces.

Comparison	All carious surfaces		Carious occlusal surfaces		Carious smooth surfaces	
	t-value	P	t-value	P	t-value	P
Control-naringenin	4.2	< 0.001	5.7	< 0.001	3.9	0.01-0.001
Control-protamine	3.2	0.01-0.001	6.4	< 0.001	1.8	0.1-0.05

DISCUSSION

The addition of two substances, naringenin or protamine, which have been found to inhibit acid production *in vitro*, produced a reduction of caries incidence in hamster experiments. As naringenin has a very low solubility at neutral or acid pH, its effect is somewhat surprising. *Fosdick et al.* (7) have pointed out that one of the most important requirements that has to be fulfilled by an anticariogenic compound is that it can be attached to the dental plaque for relatively long periods of time. Naringenin was not tested for this property, but in a pilot study in hamsters, in which naringenin was added in a soluble form to a cariogenic diet, no difference in caries incidence was found between the control and the experimental groups (2).

A tentative explanation of an attachment between protamine and the plaque is the possibility of the fixation of the basic compound protamine to acid groups of mucopolysaccharides of the plaque. The two compounds seem, however, to have different modes of action since protamine produces a higher reduction in caries on the occlusal surfaces than does naringenin. Naringenin on the other hand has a greater inhibitory effect than protamine on the approximal, lingual and buccal surfaces of the teeth.

In view of the good general health, the unimpaired growth of the animals and the absence of pathological changes at the autopsies extra-oral effects of naringenin and protamine seem unlikely. This is further stressed by the fact that flavonoids and protamines are widely distributed in plants and in animal tissues and thus are present in human diets. This fact may en-

courage further investigations into the possibilities of using these compounds as anti-caries agents in human beings.

SUMMARY

The addition of 0.2 % naringenin or 0.1 % protamine sulphate to a cariogenic diet in hamsters gave a significant reduction in the total caries experience. Naringenin had a greater reducing effect on smooth surface caries than protamine, whereas protamine gave the highest reduction in occlusal caries.

The compounds did not interfere with the general health or the gain of body-weight of the animals.

RESUME

EFFET CHEZ LE HAMSTER DE LA NARINGENINE ET DE LA PROTAMINE COMME REDUCTEURS DE LA CARIE

L'addition de 0,1 % de sulfate de protamine ou de 0,2 % de naringénine à un régime cariogène à donné chez le hamster une réduction significative de l'incidence totale de la carie. Le naringénine à un plus grand effet réducteur de la carie sur la carie des surfaces lisses que la protamine, tandis que la protamine donne la plus grande réduction de la carie dans les caries occlusales.

Les produits employés n'ont eu aucun effet nuisible sur l'état général ni sur l'accroissement de poids des animaux.

ZUSAMMENFASSUNG

DIE KARIESHERABSETZENDE WIRKUNG VON NARINGENIN UND PROTAMIN BEIM HAMSTER

Ein Zusatz von 0,2 % Naringenin oder 0,1 % Protamin zu einer kariogenen Nahrung erzeugte bei Hamstern eine deutliche Herabsetzung der Karieshäufigkeit.

Naringenin hatte auf Glattflächenkaries eine grössere kariesherabsetzende Wirkung als das Protamin, wohingegen ein Zusatz von Protamin bei Kauflächenkaries die grösste Herabsetzung ergab.

Die Präparate hatten weder auf den Allgemeinzustand noch auf die Gewichtszunahme der Tiere irgendwelchen Einfluss.

REFERENCES

1. *Gustafsson, B. E., C.-E. Quensel, L. Swenander Lanke, C. Lundqvist, H. Grahnén, B. E. Bonow* and *B. Krasse.* Acta Odontol. Scand., *11:* 232 (1954).
2. *Gustafsson, B. E.:* Svensk Tandläkaretidskrift, suppl., p. 341 (1952).
3. —»— Svensk Tandläkaretidskrift, suppl., p. 349 (1952).
4. *Krasse, B.:* Acta Odontol. *Scand., 12:* 173 (1954).
5. *Keyes, P. H.:* J. Dental Research., *25:* 341 (1946).
6. *Strålfors, A.:* Svensk Tandläkaretidskrift, *49:* 108 (1956).
7. *Fosdick, L. S., J. C. Calandra, R. Q. Blackwell* and *J. H. Burrill:* J. Dental Research, *32:* 486 (1953).

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