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DIETARY CALCIUM AND CARIES EXPERIMENTAL DENTAL CARIES IN GOLDEN HAMSTERS. XII.

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In an earlier experiment, *Gustafson, Stelling & Brunius* (1963) found that a decrease in the quantity of a salt mixture in a synthetic cariogenic diet to one fourth of that in the basal diet was followed by a five-fold increase in the amount of caries which developed. The mineral content of the basal diet had been calculated to be just adequate for health. On analysis, the Ca contents of the respective diets were found to be 0.113 % and 0.316 % and their P contents 0.307 % and 0.609 %.

The present investigations were planned to study the effect of increasing the Ca content of the above-mentioned low-Ca diet to that of the basal diet, i.e. from 0.113 % to 0.316 %, without changing the P content (0.307 %).

It has been shown that addition of Ca, as CaCl_2 , to hamsters' drinking water may have a caries-reducing effect (*Strålfors*, 1961 IV). Supplements of CaCO_3 , however, have given controversial results. *Dalderup* in 1959 did not find any caries-reducing effect of diets containing 3 % CaCO_3 , while *Limbasuta & Johansen*

(1960) found an increase in caries with a similar diet. *Strålfors* (1961, VI) found a 46 % caries reduction when the Ca content of the diet was increased from 0.40 % in the control group to 1.2 % by adding CaCO_3 .

The present experiments were carried out in 1962 and 1963.

EXPERIMENT I

As the buffering capacity of the diet may influence its cariogenicity, this capacity must, as far as possible, be kept unchanged when Ca is added to the diet. For this reason CaCl_2 was chosen as a suitable additive.

Only male hamsters were used. At weaning they were distributed into 3 groups on a litter-mate basis as far as possible. The content of salt mixture "8" in the experimental diets (62A, 62B and 62C) was only one fourth of that of the basal diet. (Table 1).

Table 1

Diets	Basal diet	62A	62B	62C
Casein	400	400	400	400
Sucrose flour	1300	1300	1300	1300
Wheat starch	—	60	—	—
Wheat starch- CaCl_2 mix. 1	—	—	60	—
" " " " 2	—	—	—	60
Arachis oil ADE	140	140	140	140
Cellulose flour	60	60	60	60
Salt mixture "8"	80	20	20	20
Vitamin mixture "20"	16	16	16	16
" " "22"	4	4	4	4
	2000 g	2000 g	2000 g	2000 g
<i>Wheat starch-CaCl₂ mixture 1.</i>		<i>Wheat starch-CaCl₂ mixture 2.</i>		
Wheat starch	61.8 g	Wheat starch		53.6 g
CaCl_2 , anhydrous, 20 mesh.	4.2	CaCl_2		12.4
<i>Salt mixture "8"</i>				
NaCl	43.3	Ca-lactate		325.0
$\text{MgSO}_4 \cdot \text{H}_2\text{O}$	76.5	Fe(3)-citrate		29.5
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	86.8	Mn(2)-citrate		5.49
K_2HPO_4	238.5	Cu(2)-citrate		0.902
$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	135.0	KJO_3		0.161

*Arachis oil ADE.*DL α -Tocopherol 1 g

Vitamin D: 1,600,000 I.U. = 16 g V 20712, Synth. A-acetate 100.000 I.U./g

Arachis oil to 1,400 g

Vitamin mixture "22".

Thiamine mononitrate	1.5 g	Ascorbic acid	6.0
Riboflavin	1.5 g	Wheat starch	40.64
Nicotinamide	2.25 g		
Pyridoxine hydrochloride	1.5 g	<i>Vitamin mixture "20"</i>	
Calcium-D-pantothenate	1.5 g	Choline chloride	100
Pteroylglutaminic acid	1.2 g	d-Cystine	25
Biotin	0.01 g	Inositol	50
Cykobemin 1:1000	3.0 g	p-Aminobenzoic acid	25
Menadiol phosphate	0.9 g		

Diet 62A contained 0.113 % Ca. The contents of CaCl₂ in diets 62B and 62C were 0.191 % and 0.564 %, which were equivalent to 0.069 % and 0.204 % of Ca. The total contents of Ca in diets 62B and 62C were thus 0.182 % and 0.317 %.

All animals received food and distilled water *ad libitum* for the experimental period of 125 days. After this time, the animals were sacrificed, their jaws dissected out and examined and caries recorded according to our usual method (*Gustafson, Stelling & Brunius, 1952*) which gives the percentage of the area of the teeth which is carious, expressed in units.

Results

The number of animals in groups 62A and 62B was 30 and 32, respectively. Of these, 27 in each group were litter-mates. The animals in these groups grew at the same rate.

In 62C, with the highest addition of CaCl₂, only 9 animals survived the experimental period. Twelve animals died after 100 to 125 days on the diet, the rest earlier. After about 70 days on the diet, the animals' weight definitely decreased. The cause of this, and the death of the animals in this group is still unknown. It may be that the animals refused to eat because of the taste of the CaCl₂. It is also possible that acidosis developed. In a fourth group, which was not included in this experiment but which had a still higher content of CaCl₂ in the diet, all animals died after a

short time. It was thus evident that CaCl_2 is not a suitable agent for experiments of this kind, when higher doses are to be used.

The caries incidence was found to be:

Group 62A (30 animals)	64.3 \pm 5.1 carious units
Group 62B (32 animals)	43.6 \pm 3.8 „ „
Group 62C (9 + 12 animals)	40.0 „ „

The high caries value of 64.3 units on Diet 62A corroborates our previous results with this diet. When the diet was mixed with CaCl_2 (62B) to produce a Ca-content of 0.183 %, its cariogenicity was reduced by 32 % to 43.6 units. It would have been of interest had Group 62C, with its further increase in Ca-content, been suitable for comparison. The value, 40.0 units, obtained in this group is too unreliable to be used. A further comparison between groups 62A and 62B shows that 68 % of the recorded teeth in 62A had a carious area of 50 % and more, and that only 32 % of the recorded teeth in 62B had lesions of this size.

EXPERIMENT II

In this experiment calcium gluconate was added to the basal diet 62A. This substance is known to be harmless and has no pronounced taste. Its buffering effect was thought to be negligible under the conditions of this experiment.

At weaning, male hamsters were distributed into three groups, on a litter-mate basis as far as possible. Group 63A received our basal cariogenic diet, the composition of which is given in Table 2 together with that of the two experimental diets.

Group 63B received the same diet but with the content of salt mixture reduced to one fourth.

Group 62C received the same diet as Group 63B but with Ca gluconate added to comprise 2.27 % of the diet.

The contents of Ca in the various diets were 0.316 %, 0.113 % and 0.314 %, respectively.

Distilled water and food were available to the animals *ad libitum*.

Table 2

Diets	63A		
	(Basal diet)	63B	63C
Casein	400	400	400
Sucrose flour	1300	1300	1300
Wheat starch	—	60	14.5
Arachis oil ADE	140	140	140
Cellulose flour	60	60	60
Salt mixture "8"	80	20	20
Vitamin mixture "20"	16	16	16
" " "22"	4	4	4
Calcium gluconate	—	—	45.5
	2000	2000	2000

The composition of Arachis oil, Salt mixture "8", and vitamin mixtures is given in Table 1.

After an experimental period of 100 days, the animals were sacrificed and then examined as in Experiment I. The number of animals in each group was 22, 22, and 23, respectively. In Group 63A 18 animals had litter-mates in both 63B and 63C. In Group 63B, 22 animals had litter-mates in 63C.

Results

The average weights of the animals in the various groups at the start of the experiment were 25, 25, and 25 g, respectively, and the average weight gain was practically the same in all groups (57, 56, and 58 g). The caries incidence, expressed as above, was found to be:

Group 63A	1.5 ± 0.5 carious units
Group 63B	28.7 ± 4.6 " "
Group 63C	6.4 ± 2.4 " "

The caries incidence after 100 days on our basal cariogenic diet is very low, viz. 1.5 units. When the amount of the salt mixture in this diet is reduced to one fourth (Group 63B), there is a considerable increase in caries incidence with an average caries area of as much as 28.7 units, an effect which confirms our previous results. When the diet with this high cariogenicity is mixed with

Ca gluconate to match the control diet in Ca-content, its cariogenic effect is reduced to 6.4 units.

The experiment thus shows that, under these conditions, Ca gluconate had a strong caries-reducing action. This inhibitory effect was, however, not strong enough to reduce the caries incidence to that of animals on the control diet. This was not to be expected, however, as it is unlikely that the large decrease in the amounts of the other constituents of the salt mixture would have no effect. In our previous study (*Gustafson et al.* 1963) mentioned above, it was shown that the exclusion of the phosphates from the salt mixture, with a consequent two-third's reduction in the P content of the diet, did not change the cariogenic effect of the diet. From the present experiment and from Experiment I it appears that a deficiency of Ca is of much greater importance for the development of caries than a deficiency of P.

DISCUSSION

Supplements of Ca gluconate have been tried by *McClure* (1960) but he was unable to find any caries-reducing effect when using a diet consisting of 18 % glucose and 79 % bread baked of flour containing Ca gluconate. The Ca content of this diet was relatively high at 0.46 %, but this was largely due to an addition of CaCO_3 . This may be the reason why no caries-reduction was achieved.

The caries-reducing effect in the present experiment was achieved by increasing the dietary Ca by 0.20 % through the addition of Ca gluconate. In Experiment I, in which CaCl_2 was added to the diet, an increase in dietary Ca of only 0.07 % was significantly caries-reducing. Even when considering that the experimental periods are not quite the same in the two experiments, it appears evident that CaCl_2 is more effective as a caries-reducing agent than Ca gluconate. This greater effect may be due, at least in part, to CaCl_2 being much more soluble than Ca gluconate.

As already mentioned, it is very doubtful if CaCO_3 has any caries-inhibiting effect. *McClure* showed in two other experiments, run simultaneously with the gluconate experiment, that bread diets with 1.37 % CaCO_3 (Ca content of the diets 0.58 % and 0.61 %, respectively) were highly cariogenic.

It is not possible at present to explain how caries is inhibited

by increasing the Ca-content of a low-mineral diet. Several factors, such as the degree of mineralization of the teeth, the effect of trace elements and microbiological conditions, may be significant in this connection.

CONCLUSIONS

From our experiments the following conclusions may be drawn,

1. The strongly cariogenic effect of a decrease in the content of minerals in a semi-synthetic diet, observed in previous experiments, has been further confirmed.
2. The addition of CaCl_2 to a diet low in minerals, by which its Ca-content is increased from 0.113 % to 0.183 %, is followed by a 32 % decrease in caries incidence.
3. The addition of Ca gluconate to the same diet, by which the Ca-content of the diet is increased from 0.113 % to 0.314 %, is followed by a 78 % reduction in caries.
4. In diets with a low mineral content, the level of Ca has a significant effect on the cariogenicity.

It is evident from these experiments, and from the work of others, that the caries-inhibiting effect of Ca supplements to diets depends on the source of the Ca. Not all Ca supplements appear to exert a caries-reducing action.

SUMMARY

A study of the effect of increasing the Ca content of a semi-synthetic cariogenic diet low in minerals (having one fourth of the mineral content of an ordinary diet) was carried out in two experiments.

In the first experiment the Ca-content of the diets was increased from 0.113 % to 0.183 % by adding CaCl_2 to the diet. A 32 % reduction in caries incidence was observed.

In the second experiment, the diet was supplemented with Ca gluconate, by which the Ca-content of the diet was increased from 0.113 % to 0.314 %. In this case a 78 % reduction in caries was observed.

It is concluded that, in cariogenic diets with low mineral content, the level of Ca is a determining factor in the development of caries.

RÉSUMÉ

CALCIUM ALIMENTAIRE ET CARIE

CARIE DENTAIRE EXPÉRIMENTALE CHEZ LES HAMSTERS DORÉS. XII.

Une étude de l'action d'une augmentation de la teneur en Ca d'un régime cariogène semi-synthétique pauvre en minéraux (ne contenant qu'un quart de la teneur en minéraux d'un régime ordinaire) a été menée en deux expériences.

Dans la première expérience, la teneur en Ca du régime a été élevée de 0,113 % à 0,183 % par l'addition de CaCl_2 au régime. Une réduction de 32 % a été observée dans l'incidence de la carie.

Dans la deuxième expérience, le régime fut complété par du gluconate de Ca, grâce auquel la teneur en Ca du régime était élevée de 0,113 % à 0,314 %. Dans ce cas, une réduction de 78 % a été observée dans l'incidence de la carie.

Les auteurs concluent que, dans les régimes cariogènes pauvres en minéraux, la proportion de Ca est un facteur déterminant dans le développement de la carie.

ZUSAMMENFASSUNG

KALIZUM DER KOST UND KARIES

KARIESVERSUCHE AN GOLDDHAMSTERN. XII.

Eine Untersuchung betreffend des kariogenen Effektes einer Vermehrung des Ca-Gehaltes in einer semi-synthetischen, kariogenen Kost mit reduzierter Salzmenge (ein Viertel des normalen Salzgehaltes) wurde in zwei Experimenten ausgeführt.

In dem ersten Experiment wurde der Ca-Gehalt durch Zusatz von CaCl_2 von 0,113 % auf 0,183 % erhöht. Eine Kariesreduktion von 32 % wurde beobachtet.

In dem zweiten Experiment wurde der Ca-Gehalt durch Zusatz von Ca-Gluconat von 0,113 % auf 0,314 % erhöht. In diesem Falle konnte eine Kariesreduktion von 78 % festgestellt werden.

Die Experimente zeigen, dass in einer kariogenen Kost von niedrigem Mineralgehalt der Ca-Gehalt von entscheidender Bedeutung für die Kariesentwicklung ist.

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