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THE CARIOGENIC EFFECT OF VARIATIONS IN THE SALT MIXTURE IN A SYNTHETIC CARIOGENIC DIET

EXPERIMENTAL DENTAL CARIES IN GOLDEN HAMSTERS. XI.

by

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Experiments carried out on rats and hamsters during recent years have indicated that certain phosphates, when added to a cariogenic diet, may cause a decrease in caries incidence. Thus, *McClure* in 1958 observed a marked caries-reducing effect of Na_2HPO_4, and this observation was confirmed by *McClure & Muller* in 1959. *McClure* found in 1960 that bread in a bread-glucose diet prepared with a flour containing 1 % Na_2HPO_4 reduced the caries incidence, on an average, by 51 %. If used in the same way $(NH_4)_2HPO_4$ was even more effective. A caries-reducing effect was also achieved by additions of $Ca(H_2PO_4)_2$ and $Ca_3(PO_4)_2$.

In 1961 *Strålfors* published results from investigations with $Ca_3(PO_4)_2$ in bread, and $CaHPO_4$ and $Ca(H_2PO_4)_2$ mixed in the diet. His results are in accordance with those mentioned above.

Harris & Nizel (1959) found that the cariogenic effect of a natural diet was reduced by 60 % when equivalent amounts of the diet were ashed and added to the diet, thus doubling the amount of salts.

König, Marthaler & Mühlemann (1961) have studied the effect of various phosphates both separately and in combination with fluorides. They found a significant caries reduction by adding sodium meta-, pyro- and orthophosphates to a cariogenic diet.

Even if it has been stated that additions of certain sodium and calcium phosphates may reduce the caries incidence, the mechanism is in no way clear. It has not been possible to show that this increased supply of minerals results in an increased content of Ca and P in the teeth.

That some changes, however, take place in the enamel surface layer, which may be attributed to the action of caries-inhibiting phosphates is evident from the experiments by *König et al.* (1961) already mentioned. They have compared the enamel surface solubility, by measuring the phosphate removal by phthalate buffer of pH 4.0, in a control group with that in the groups with caries-inhibiting phosphates in the diet. The phosphate removal was significantly lower in these latter groups.

Studies on the Ca: P ratio of the diet (e.g. by *Wynn et al.*, 1956, *Haldi et al.*, 1959 and *Harris & Nizel*, 1959) have not afforded any explanation as to the reduced caries incidence. In one of his experiments *Strålfors* (1961) suggested the possibility of an exogenous effect with calcium phosphates.

In this connection also the buffering capacity of the phosphates has been mentioned as a factor. *Gustafson, Stelling, Abramson & Brunius* found in 1955 that addition of buffering substances to a cariogenic diet decreased the caries incidence significantly.

Several other experiments with minerals have been carried out showing varying results. As too small a number of animals has been used in these experiments, they will not be mentioned here.

In the experiments now presented (carried out 1960—1961), the aim was primarily to study the effect of a large reduction of the salt mixture in one of our synthetic cariogenic diets, and secondarily to study the effect of omitting the phosphates only, replacing them by other salts.

To prevent mobilization of the body phosphates through the vitamin D in the diet, this vitamin was excluded from the experimental diets.

EXPERIMENT A

Experimental

At weaning 102 male hamsters were distributed into 4 groups, (60 A, 60 B, 60 C and 60 D), as far as possible with regard to litters. The experimental time was 150 days, after which time the animals were sacrificed and examined for caries. The numbers of the animals were 34, 31, 28 and 29, respectively. Twenty-four of the animals in group 60 A had littermates in both groups 60 B and 60 C, and 23 of the animals in group 60 B had littermates in group 60 D.

Group 60 A was fed the synthetic cariogenic diet 328, the composition of which is given in Table 1 together with the other diets used. It served as a control diet.

Group 60 B was fed diet 329 which was identical with diet 328 except that vitamin D was excluded.*

Group 60 C received diet 330 which was different from diet 328 in so far as vitamin D was excluded and the amount of the salt mixture reduced to ¼.

Group 60 D received diet 331 which differed from diet 328 in that vitamin D and the phosphates of the salt mixture were excluded.

All groups received distilled water and food *ad libitum.*

Table 1

Diets	*328*	*329*	*330*	*331*
Casein	400	400	400	400
Sucrose flour	1300	1300	1300	1300
Arachis oil ADE	140			
Arachis oil AE		140	140	140
Cellulose flour	60	60	60	60
Salt mixture "6"	80			
Salt mixture "8"		80	20	
Salt mixture "9"				75
Wheat starch			60	5
Vitamin mixture "20"	16	16	16	16
Vitamin mixture "22"	4	4	4	4
	2000	2000	2000	2000

* We are aware that salt mixtures "6" and "8" are not quite identical, the Cu and Mn sulphates in "6" being replaced by citrates in "8". As the concentration of Cu and Mn is the same in the two mixtures, however, the difference should not have any relevance.

Arachis oil ADE:

DLα-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.

Arachis oil AE:

DLα-Tocopherol 1 g
Vitamin A: 1,600,000 I.U. Synth. A-acetate
Arachis oil to 1,400 g.

Salt mixture "6":

	g		g
NaCl	43.3	Ca-lactate	325.0
$MgSO_4 \cdot H_2O$	76.5	Fe-citrate	29.5
$NaH_2PO_4 \cdot H_2O$	86.8	KJ	0.125
K_2HPO_4	238.5	$CuSO_4 \cdot 5H_2O$	1.25
$Ca(H_2PO_4)_2 \cdot H_2O$	135.0	$MnSO_4 \cdot H_2O$	3.79

Salt mixture "8":

	g		g
NACl	43.3	Ca-lactate	325.0
$MgSO_4 \cdot H_2O$	76.5	Fe(3)-citrate	29.5
$NaH_2PO_4 \cdot H_2O$	86.8	Mn(2)-citrate	5.49
K_2HPO_4	238.5	Cu(2)-citrate	0.902
$Ca(H_2PO_4)_2 \cdot H_2O$	135.0	KJO_3	0.161

Salt mixture "9":

	g
NaCl	80.1
KCl	204.1
$MgSO_4 \cdot H_2O$	76.5
Ca-lactate	490.0
Fe(3)-citrate	29.5
Mn(2)-citrate	5.49
Cu(2)-citrate	0.902
KJO_3	0.161

Vitamin mixture "20" with cystine:

	g
Choline chloride	100
d-Cystine	25
Inositol	50
p-Aminobenzoic acid	25

Vitamin mixture "22":

	g		g
Thiamine mononitrate	1.5	Biotin	0.01
Riboflavin	1.5	Cykobemin 1 : 1000	3.0
Nicotinamide	2.25	Menadiol phosphate	0.9
Pyridoxine hydrochloride	1.5	Ascorbic acid	6.0
Calcium-D-pantothenate	1.5	Wheat starch	40.64
Pteroylglutaminic acid	1.2		

An analysis of the casein and the starch used showed that the casein contained 225 mg Ca and 1020 mg P per 100 g, and the starch 19 mg Ca and 59 mg P per 100 g.

Thus the contents of Ca and P in the diets were found to be:

Diet	% Ca	% P
328	0.316	0.609
329	0.316	0.609
330	0.113	0.307
331	0.314	0.204

The buffering capacity of diets 328, 330, and 331 was determined in the following way: Twenty grams of each diet were mixed with 20 g distilled water, increasing amounts of (a) 1M lactic acid, (b) 1N HCl, and (c) 1N NaOH were added to the various mixtures and the pH measured with a glass electrode. The amounts of lactic acid or HCl which were needed to decrease the pH from its original value to 3.00 were measured (Tables 2 and 3). Likewise, the amounts of NaOH required to increase the pH to 7.00 (Table 4) were determined.

Table 2

pH	ml 1M lactic acid		
	diet 328	diet 330	diet 331
4.73	0.0	—	—
4.65	0.1	—	0.0
4.57	0.2	0.0	0.1
4.50	0.4	0.1	0.2
4.25	1.1	0.4	0.65
4.00	2.15	1.0	1.35
3.75	3.65	1.95	2.55
3.50	6.35	3.65	4.65
3.25	10.6	6.0	8.2
3.00	17.0	10.4	14.0

Table 3

pH	ml 1N HCl diet 328	diet 330	diet 331
4.85	0 0	—	—
4.65	0.2	—	0.0
4.55	0.28	0.0	0.18
4.50	0.3	0.05	0.2
4.25	0.62	0 3	0.5
4.00	1.1	0.65	1.0
3.75	1.7	1.0	1.6
3.50	2.3	1.4	2.3
3.25	2.9	1.9	2 9
3.00	3.5	2.35	3.5

Table 4

pH	ml 1N NaOH diet 328	diet 330	diet 331
4.55	—	0.0	—
4.65	—	0.05	0.0
4.85	0.0	0.2	0.15
5.00	0.2	0.35	0.35
5.25	0.6	0.55	0.65
5.50	1.2	0.85	0.95
5.75	1.9	1.2	1.3
6.00	2.4	1.55	1.6
6.25	2.9	2.0	1.8
6.50	3.5	2.5	2.1
6.75	4.2	3.1	2.35
7.00	4.7	3.6	2.5

Tables 2 and 3 show that the buffering capacity of the diets is definitely different, diet 328 having the highest capacity and 330 the lowest. The buffering capacity up to 7.00 (Table 4) is of interest with regard to the fact that the pH of hamster saliva is reported to be 8 or more, and it is not known to what degree this is able to increase the pH of the originally acid diet.

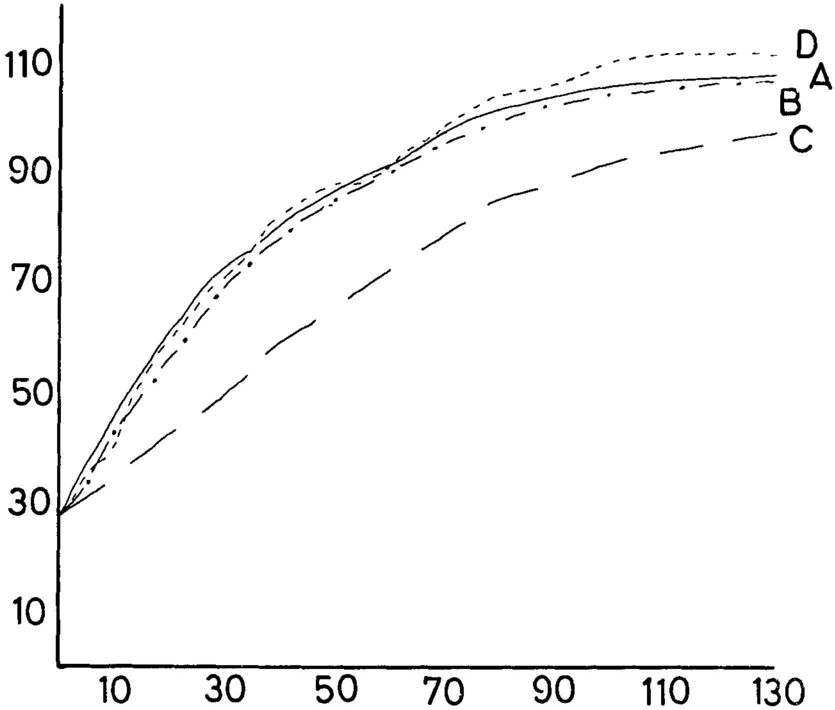

Fig. 1.

After sacrificing the animals and dissecting out the jaws, the examination, recording and scoring were performed by our usual method, (*Gustafson et al.*, 1952), which gives the percentage carious area of the teeth. Only three pairs of teeth are included in the calculation of the caries incidence.

Results

The growth

The growth was the same on diets 328 (A), 329 (B), and 331 (D); on diet 330 (C), however, it was much less (Fig. 1).

In the animals on diet 330 the jaw bone seemed to be more brittle than in the animals on the other diets. In spite of the absence of vitamin D, however, no signs of rickets could be observed in roentgenograms of the long bones. A histological investigation of the long bones failed to show extensive changes which could be referred to a vitamin deficiency.

Caries examination

Table 5

Group	Carious units	No. of carious teeth	No. of animals with caries
60 A (328)	14.0 ± 2.7	2.9 ± 0.3	82 %
60 B (329)	18.6 ± 2.8	3.7 ± 0.3	100 %
60 C (330)	79.1 ± 3.0	5.9 ± 0.1	100 %
60 D (331)	19.0 ± 3.0	3.7 ± 0.3	90 %

From the caries examination (Table 5) it is evident that,

(a) the exclusion of vitamin D from the diet did not significantly influence the caries incidence, (Groups 60 B/60 A),

(b) the exclusion of both vitamin D and the phosphates from the salt mixture did not significantly change the caries incidence, (Groups 60 D/60 A), and

(c) the decrease in the quantity of salt mixture in the diet caused an increase in the development of caries of more than five times that of the control group, (Groups 60 C/60 A).

Discussion

In the light of the experiments described above, which indicate a caries-decreasing effect of the addition of phosphates to diets, one would be inclined to ascribe the high cariogenicity of diet 330 to the decrease in phosphates which has taken place with the reduction of the amount of salt mixture in this diet. This idea, however, has to be abandoned as the amount of P in diet 330 (0.307 %) is much higher than that of diet 331 (0.204 %). Diet 331 did not show any increase in cariogenicity.

The extremely low content of Ca in diet 330 (0.113 %) may be a possible caries-producing factor, and this factor will be the subject of coming experiments.

It has not been possible, so far, to go further into the rôle of the differing buffering capacities of the three diets. It may, however, be mentioned that diet 331, when measured up to pH 7.0 by titration with NaOH, was found to have only about half the buffering capacity of diet 329. This decrease in buffer action, which is due to the absence of phosphates in the salt mixture, did not lead to an increase in the cariogenicity of the diet. This fact is obviously not in harmony with the view, held by several authors, that the mechanism of the caries-reducing effect of phosphates may be related to the buffering effect of the phosphates. Further investigations of this fact are planned.

EXPERIMENT B

Experimental

In Experiment A the second and third molars of the animals on diet 330 (Group 60 C) were almost completely destroyed by caries. The first molars too were often broken down, but apparently this was not due to caries only. They seemed to have crumbled off under the chewing pressure, possibly because of a high brittleness of the enamel. This could be a result of the low mineral supplies which apparently resulted in the high brittleness of the jaw bone observed when the jaws were dissected out.

It would be of interest to find out if a shorter experimental time would give some information as to the role of fractures for the large lesions, and if a non-cariogenic diet with the same mineral content would produce teeth with signs of fractures or with enamel which was in any other way macroscopically abnormal. The following experiment was therefore carried out:

Fifty-four male hamsters were, at weaning, distributed into two groups, 61 E and 61 F.

Group 61 E consisted of 26 animals which were fed diet 330, the composition of which is shown in Table 1.

Group 61 F consisted of 28 animals on diet 335 which was identical with diet 330 except that the amount of sucrose was replaced by a similar amount of wheat starch.

The experimental time was now restricted to 100 days, after which time the animals were sacrificed. Examination, recording, and scoring was carried out as in Experiment A.

Results

Only the animals on diet 330 (Group 61 E) developed dental caries with an average of caries units of 54.1 ± 4.9. This value is to be compared with that of 79.1 after 150 days in Experiment A. Out of the recorded 6 teeth in each animal 55 % had a carious surface of 50 % or more after 100 days' experimental time, which corresponds to 85 % after 150 days. An increased predisposition toward fractures cannot be deduced from these figures.

The teeth of the animals in the non-cariogenic diet group (61 F) showed no fractures, and the surface of the enamel was of normal appearance. If the enamel on this low mineral diet were of inferior quality this is apparently not demonstrable. The latter experiment also shows that even under the conditions of the experiment the starch is not caries-conducive.

SUMMARY

The effect of a large reduction in the salt mixture in a synthetic cariogenic diet was initially studied. Subsequently, the effect of omitting the phosphates, these being replaced by other salts, together with total deprivation of vitamin D in the diet, was investigated.

Neither the exclusion solely of vitamin D, nor of both this vitamin and the phosphates from the salt mixture significantly altered the caries incidence.

The reduction in the quantity of the salt mixture in the diet caused marked increase in the development of caries as compared with that of a control group.

RÉSUMÉ

L'ACTION CARIOGÈNE DES MODIFICATIONS APPORTÉES DANS LE MÉLANGE SALIN D'UN RÉGIME CARIOGÈNE SYNTHÉTIQUE

CARIE DENTAIRE EXPÉRIMENTALE CHEZ LES HAMSTERS DORÉS. XI.

L'action d'une réduction importante de la quantité de mélange salin dans un régime cariogène synthétique a tout d'abord été étudiée. L'action de l'exclusion des phosphates, remplacés par d'autres sels, jointe à l'absence totale de vitamine D dans le régime

a ensuite été examinée. Ni l'exclusion de la seule vitamine D, ni l'exclusion de la vitamine D et des phosphates du mélange salin n'ont apporté de modifications significatives à l'incidence de la carie.

La réduction de la quantité de mélange salin dans le régime a provoqué une accentuation marquée du progrès de la carie par comparaison avec un groupe de contrôle.

ZUSAMMENFASSUNG

DIE WIRKUNG VON VARIATIONEN DER SALZMISCHUNG IN EINER SYNTETISCHEN KARIOGENEN KOST

KARIESVERSUCHE AN GOLDHAMSTERN. XI.

Zuerst wurde eine Untersuchung betreffend des Effektes einer kräftigen Reduktion der Salzmenge in einer synthetischen kariogenen Kost durchgeführt. Danach wurde der Effekt des Ausschlusses von Phosphaten, die durch andere Salze ersetzt wurden, untersucht. Dieses geschah unter gleichzeitiger Reduktion von D-Vitamin in der Kost.

Weder die Ausschliessung von D-Vitamin noch die Ausschliessung von sowohl D-Vitamin als auch Phosphaten änderte die Kariesfrequenz.

Die Reduktion der Salzmenge in der Kost hatte eine starke Steigerung in der Kariesentwicklung im Vergleich zur Kontrollgruppe zur Folge.

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