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DENTAL CALCULUS IN THE RAT: EFFECT OF DIET AND ERYTHROMYCIN

by

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In recent years dental calculus has been studied in small laboratory animals in order to obtain information concerning the influence of various factors on its deposition. Thus, the influence of animal strain, sex, age (*Baer, Stephan & White, 1961; Stewart & Burnett, 1960*), diet (*King & Glover, 1945; Baer, Stephan & White, 1961; Stewart & Burnett, 1960; Kakehashi, Baer & White, 1962*), and microorganisms (*Baer & Newton, 1959; Fitzgerald, Jordan, Scott & McCann, 1960; Fitzgerald & McDaniel, 1960; Glass & Krasse, 1962; Gustafsson & Krasse, 1962; Theilade, Fitzgerald, Scott & Nylen, 1964*) have been examined.

It has been shown that the consistency of the diet influences the amount of calculus formed. *King (1954)* reported that ferrets fed a diet containing unstripped bones showed no calculus, while animals given the same diet with the bones ground got heavy concretions of calculus. From this he concluded that calculus formation was prevented by the heavy masticatory function required in stripping the meat from the bones. *Baer, Stephan & White (1961)* also observed that diets of the finest consistency resulted in the greatest amount of calculus in rats.

The influence of various nutritional components of the diet upon calculus formation in small laboratory animals has also been investigated. Thus, *Baer, Stephan & White (1961)* found

that a diet containing corn starch produced a greater amount of calculus than diets with equal amounts of mono- or disaccharides, whereas the level of protein did not influence calculus formation. *Stewart & Burnett* (1960) noted that addition to the diet of some types of proteins seemed to accelerate calculus deposition whereas other proteins did not. *Smith, Baer, King & White* (1963) tested 2 different diets, one containing 66 per cent corn starch and the other 57 per cent fat (Crisco), and found no differences in calculus production in rats.

The effects of antibiotics on calculus formation in rats have been studied by *Fitzgerald, Jordan, Scott & McCann* (1960) who found that the broad spectrum antibiotics chloramphenicol or chlortetracycline had no appreciable effect when incorporated in NIH diet 550 at a level of 50 mg per kilogram. Recently, however, *Gressly* (1963) using a modification of the same diet which contained 3.5 per cent KH_2PO_4 reported that a concentration of 100 mg chlortetracycline per kilogram markedly reduced calculus formation. *König & Mühlemann* (1959) reported that topically applied penicillin had no effect in rats. Experiments have also been carried out in humans to test the effect of antibiotics (*Dossenbach & Mühlemann*, 1961). In these experiments penicillin was employed, but no difference was observed between the treated group and controls.

The present experiment was designed in order to obtain more information as to the effect of the consistency of the diet, the composition of the diet, and the addition of antibiotics to the diet on calculus formation in the white rat.

MATERIALS AND METHODS

Weanling female Sprague-Dawley rats were randomly divided into eight experimental groups of 12 animals each and one control group of six animals and housed two in a cage. Their age at the start of the experiment was 26 days. They were all weighed at the onset and twice during the experimental period.

The composition of the diets used is shown in Table 1. Diet Group I received the base diet described as coarse fat-free. This diet contained 20 per cent yellow hominy grits which gave the diet its coarse consistency. For the fine particle diets the hominy

Table 1
Composition of the diets.

<i>Fat-free diet</i>	<i>Grams per kilogram</i>
"Vitamin free" casein*	220
Corn starch	540
Yellow hominy grits**	200
Salt mixture (Wesson)*	40
Vitamin mixture***	q.s.

<i>High fat diet</i>	<i>Grams per kilogram</i>
"Vitamin free" casein*	220
Corn starch	340
Yellow hominy grits**	200
Corn oil	200
Salt mixture (Wesson)*	40
Vitamin mixture***	q.s.

* Nutritional Biochemical Corporation.

** Quaker Oats Company.

*** Gustafsson's (1959) vitamin mixture, used at one third the concentrations recommended for autoclaved diets.

For the fine particle diets the hominy grits were ground in a ball mill for 24 hours. All the material thus treated passed a 40 mesh screen and 30 per cent passed a 60 mesh screen. Ninety-six per cent of the unground grits used in the coarse particle diets were retained by the 40 mesh screen. Erythromycin, when used, was mixed in the diet at a level of 50 mg per kilogram of diet.

grits were ground in a ball mill for 24 hours. These coarse and fine particle diets were further modified by the incorporation of the antibiotic erythromycin at a level of 50 mg per kilogram and by the substitution of corn oil (20 g per hundred grams of diet) at the expense of the starch in the diet. This resulted in eight diet groups as follows: I. coarse fat-free; I E. coarse fat-free plus erythromycin; II. fine fat-free; II E. fine fat-free plus erythromycin; III. coarse high fat; III E. coarse high fat plus erythromycin; IV. fine high fat; IV E. fine high fat plus erythromycin. In this way the various factors of diet consistency, fat content and antibiotic treatment could be compared under conditions permitting the assessment of each variable independently.

One further group (V) of six animals was kept on the stock diet of Purina laboratory pellets. Food and water was available *ad libitum* to all groups.

RIGHT MAXILLARY MOLARS.

Buccal surfaces.

Lingual surfaces.



RIGHT MANDIBULAR MOLARS.

Buccal surfaces.

Lingual surfaces.



Fig. 1. For the recording of the distribution of calculus in the rats, parts of the rat caries form proposed by *Keyes* (1958) was used. The buccal and the lingual surfaces of the maxillary and mandibular teeth were divided into 1, 2, or 3 units which were scored separately according to the amount of calculus present in each unit. The division into units is indicated by the vertical dotted lines.

The experiment was run for 8 weeks at the end of which period the animals were sacrificed. The undefleshed jaws were scored for the amount and distribution of calculus upon the teeth employing the following scoring system: The teeth were examined for deposits of calculus under a dissecting microscope at a magnification of 30 X. The distribution of the deposits in each animal was recorded on a form designed for caries recording in rats by *Keyes* (1958). For computational purposes the buccal and lingual surfaces of the molars were divided into one, two, or three units dependent on the morphology of the particular tooth. Figure 1 shows the parts of the record sheet employed with dotted lines indicating the division of each buccal and lingual surface of the molars in units. In addition to recording the extent of the molar surfaces covered with calculus, each unit received a quantitative score ranging from 0—3, describing the quantity of the calculus on each unit. 0, was assigned to units with no calculus, 0.5, to

units with a trace of calculus; 1, to units with a thin layer of calculus; 2, to units with moderate layers of calculus, and 3, to units with heavy layers of calculus. The values thus obtained are roughly proportional to the amounts of calculus present. For example; a unit given a score of 2.0 would have approximately twice as much calculus as one given a score of 1.0. All recordings and scorings were made independently by each investigator. Since reasonable consistency occurred between the recordings and scores from the two investigators, it was judged satisfactory to use only one investigator's recordings and scores for subsequent computations. In addition, since the distribution of calculus was found to be bilaterally similar, only values from right sides were used for computations.

RESULTS

That all of the diets were nutritionally adequate can be seen from Table 2 showing average daily food consumption for two different periods during the experiment and the average weight gain per group for the entire course of the experiment. The animals did as well or better on the experimental diets as they did on the commercial pelleted ration normally fed stock rats. Table 3 shows that there is a certain pattern of distribution of

Table 2
Average food consumption and weight gains in grams.

Group number	Diet	Food consumption g per rat per day		Weight gain g
I	Coarse fat-free	13.7*	15.0**	145***
I E	Coarse fat-free + erythromycin	14.4	14.9	152.5
II	Fine fat-free	14.6	12.6	136
II E	Fine fat-free + erythromycin	14.5	12.5	138
III	Coarse high fat	9.3	8.6	150
III E	Coarse high fat + erythromycin	8.9	9.1	146
IV	Fine high fat	11.4	11.3	151
IV E	Fine high fat + erythromycin	12.0	10.9	157
V	Purina laboratory pellets	—	—	137

* At the 15th experimental day

** At the 36th experimental day

*** At the 56th (last) experimental day

Table 3

Effects of diet composition and erythromycin on distribution of calculus on rat molar teeth*.

Diet	Jaw	Surface	Tooth Scores			Total Scores		
			M ₁	M ₂	M ₃	Surf.	Jaw	All teeth
I Coarse fat-free	Maxilla	Buccal	2.2	0.6	0.3	3.1	7.4	15.7
		Lingual	2.4	1.6	0.3	4.3		
	Mandible	Buccal	1.5	0	0	1.5	8.3	
		Lingual	4.6	1.7	0.5	6.8		
I E Coarse fat-free + erythromycin	Maxilla	Buccal	1.8	0.1	0	1.9	2.9	3.0
		Lingual	1.0	0	0	1.0		
	Mandible	Buccal	0	0	0	0	0.1	
		Lingual	0.1	0	0	0.1		
II Fine fat-free	Maxilla	Buccal	4.7	1.1	0.2	6.0	12.5	15.1
		Lingual	4.6	1.5	0.4	6.5		
	Mandible	Buccal	0.7	0.1	0.1	0.9	2.6	
		Lingual	1.3	0.4	0	1.7		
II E Fine fat-free + erythromycin	Maxilla	Buccal	2.0	0.1	0	2.1	3.1	3.3
		Lingual	1.0	0	0	1.0		
	Mandible	Buccal	0.1	0	0	0.1	0.2	
		Lingual	0	0.1	0	0.1		
III Coarse high fat	Maxilla	Buccal	1.9	0.5	0.5	2.9	5.6	14.0
		Lingual	1.0	1.0	0.7	2.7		
	Mandible	Buccal	2.2	0	0.1	2.3	8.4	
		Lingual	3.6	1.4	1.1	6.1		
III E Coarse high fat + erythromycin	Maxilla	Buccal	0.9	0	0	0.9	1.6	1.9
		Lingual	0.7	0	0	0.7		
	Mandible	Buccal	0.1	0	0	0.1	0.3	
		Lingual	0.2	0	0	0.2		
IV Fine high fat	Maxilla	Buccal	4.7	1.5	0.7	6.9	17.9	25.7
		Lingual	5.1	3.4	2.5	11.0		
	Mandible	Buccal	1.1	0.2	0	1.3	7.8	
		Lingual	3.4	2.4	0.7	6.5		
IV E Fine high fat + erythromycin	Maxilla	Buccal	1.0	0.1	0	1.1	1.7	1.9
		Lingual	0.6	0	0	0.6		
	Mandible	Buccal	0.1	0	0	0.1	0.2	
		Lingual	0.1	0	0	0.1		
V Pellets	Maxilla	Buccal	1.2	0.1	0.1	1.4	2.2	2.6
		Lingual	0.8	0	0	0.8		
	Mandible	Buccal	0	0	0.1	0.1	0.4	
		Lingual	0.3	0	0	0.3		

* Figures refer to group average scores for the right side.

the calculus in all groups. The teeth most frequently affected with calculus and with the heaviest deposits were the first molars both in the maxilla and the mandible. The incidence of calculus and the calculus score were less on the second and third molars. The deposits seemed to be approximately equally divided between the buccal and lingual surfaces of the maxillary teeth, while on the mandibular teeth the lingual surfaces were more affected and had higher calculus scores than the buccal surfaces. Calculus was not found on the incisors.

The single most effective variable in these experiments was the incorporation of erythromycin in the diets at a level of 50 mg per kilogram of diet. This resulted in a much diminished frequency and severity of calcified deposits in all groups regardless of fat content or physical consistency of the diet (Fig. 2, 3). The amount of calculus in the groups that received erythromycin was approximately of the same order as that found in the group receiving laboratory pellets. The teeth upon which the erythromycin had the least effect on calculus formation were the maxillary first molars.

Comparison of the group receiving a coarse fat-free diet (group I) with the group receiving a fine fat-free diet (group II) showed that the two groups developed approximately the same total amount of calculus. However, the distribution of the calculus differed within the two groups. While group I animals had less calculus on their maxillary teeth than group II animals they had more calculus on their mandibular teeth than the animals in group II. A comparison between the group receiving a coarse high fat diet (group III) and the group receiving a fine high fat diet (group IV) revealed that animals on the latter diet had a much higher calculus score than group III. This was most pronounced for the maxillary teeth.

From Table 3 it is further seen that there were no pronounced differences in the amount and distribution of calculus between the group receiving a coarse fat-free diet (group I) and the group receiving a coarse high fat diet (group III). However, the group receiving a fine fat free diet (group II) developed definitely less calculus than the group receiving a fine high fat diet (group IV).

DISCUSSION

Various factors have been considered to play a role in calculus deposition, among them the physical consistency of the diet and the oral microflora. In the present study the physical consistency of the diet was varied by the incorporation of hominy grits of different particle size or the replacement of a portion of the corn starch with corn oil. The incorporation of 50 μg erythromycin per gram of the diet was made to gain some information as to the role of the oral microflora susceptible to this antibiotic. Although specific determinations of the effect of the antibiotic on the oral flora were not performed in this study, concentrations of erythromycin of this magnitude, or less, are known to alter the oral microflora sufficiently to prevent the development of dental caries in rats (*Fitzgerald & Jordan, 1955*). On this basis it is assumed that the marked reduction in calculus deposition observed in the animals receiving erythromycin was also due to the antibacterial effects of this agent.

The inhibition of calculus formation by erythromycin was somewhat unexpected in view of earlier observations that similar concentrations of chlortetracycline or chloramphenicol did not affect calculus deposition in rats (*Fitzgerald, Jordan, Scott & McCann, 1960*). However, *Gressly* (1963) reported that higher levels of chlortetracycline inhibited calculus formation in a high phosphate diet.¹ *Dossenback & Mühlemann* (1961) found penicillin lozenges ineffective in humans and *König & Mühlemann* (1959) reported that topically applied penicillin had no effect in rats. One explanation for the seeming discrepancy might be differences

¹ With high concentrations of chlortetracycline the possibility must also be considered that calculus formation is inhibited as a result of the chelation of calcium by this drug (*Weinberg, 1957*). Neither chloramphenicol nor erythromycin is active in this respect.

Fig. 2. The right molars of a rat from group I (coarse fat — free diet) showing the deposits collected during the experiment (arrows): a) buccal surfaces of maxillary molars; b) lingual surfaces of maxillary molars; c) buccal surfaces of mandibular molars; d) lingual surfaces of mandibular molars.

Fig. 3. The right molars of a rat from group IE (coarse fat — free diet + erythromycin) showing practically no calculus collected during the experimental period: a) buccal surfaces of maxillary molars; b) lingual surfaces of maxillary molars; c) buccal surfaces of mandibular molars; d) lingual surfaces of mandibular molars.

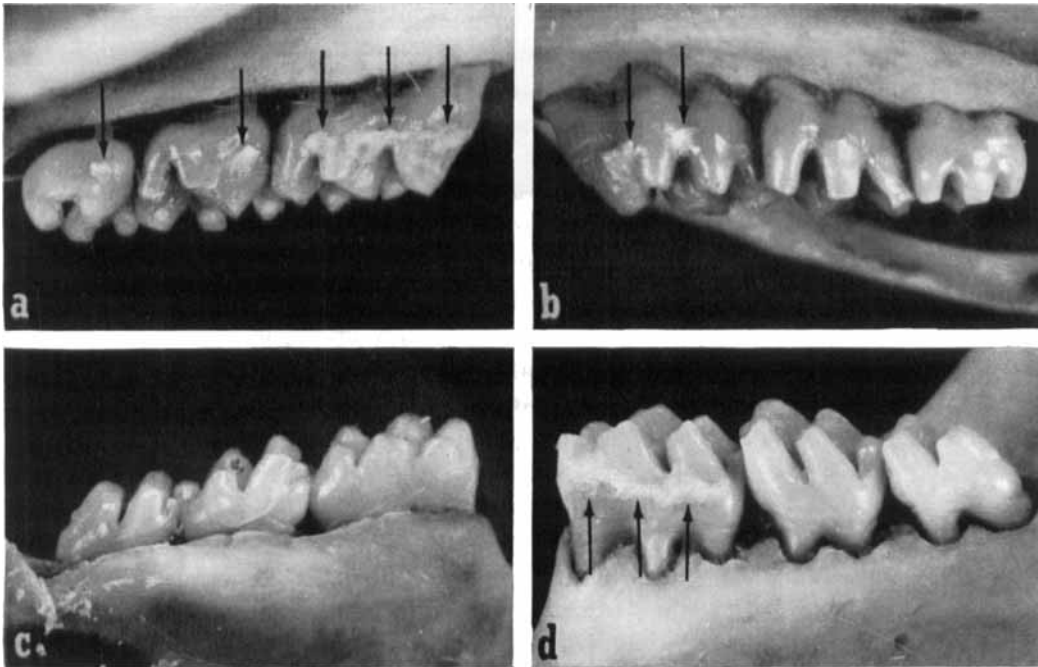


Fig. 2.

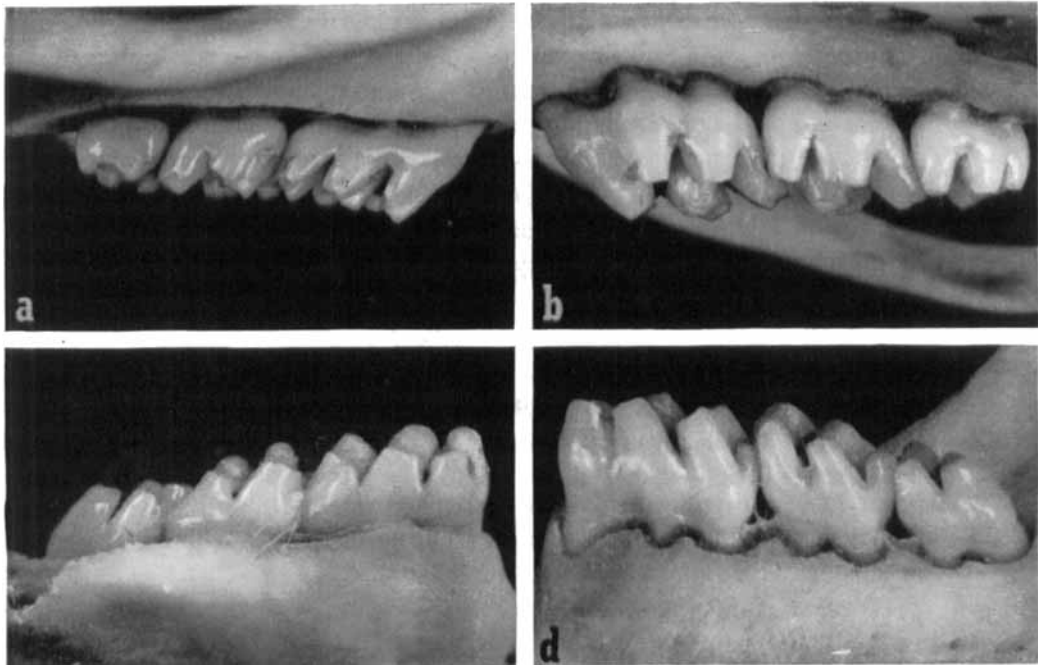


Fig. 3.

in experimental diets and procedures in the various studies. The most obvious inference is that erythromycin selectively inhibited a microorganism which played a role in calculus deposition and which was not inhibited by penicillin, or low levels of chlorotetracycline or chloramphenicol. However, when one considers the antibacterial spectra of the antibiotics in question, it is difficult to hypothesize such an organism since the antibacterial spectrum of erythromycin is intermediate between that of penicillin and that of chlortetracycline and chloramphenicol. This suggests still another alternative, namely that erythromycin may have influenced the balance of the oral microflora to permit the growth of species of microorganisms whose presence is unfavorable to the deposition of calculus. Further studies are required to determine which, if any, of these possibilities is the correct explanation.

Aside from the effect of erythromycin, the physical consistency of the diet was found to play a role both in the distribution and in the severity of calculus deposition. The fine fat-free diet (II) caused heavier deposits on the maxillary molars and lighter deposits on the mandibular surfaces than the coarse fat-free diet (I). These results were qualitatively similar to those for the fine high fat diet (IV) and coarse high fat diet (III) although the calculus scores were far greater for the fine high fat diet. This increase was primarily due to the severity of the accumulations on both the buccal and lingual surfaces of the maxillary molars.

It has been suggested (*McClure, Folk & Rust, 1956*) that reduction of the cariogenicity of a diet by the addition of fats was due to alterations in the physical properties of the diet which reduce the amount retained on the tooth surfaces. It might therefore be expected that similar reductions in calculus deposition would be seen in the high fat diets employed in the present study. In fact, however, the substitution of 20 per cent of corn oil for starch in the present experiments had no effect in the group receiving the coarse particle diet (I vs. III) and increased the amount of calculus in the group receiving the fine particle diet (II vs. IV). The reason for these findings is not known, but chemical analyses of human dental calculus have revealed the presence of small amounts of fat in the matrix (*Mandel & Levy, 1957*) and *Baer & Burstone (1959)* noted esterase activity in the

calculus plaque of humans and suggested that saponification of fats may play a role in the process of calcification. In the present study it seems more likely to us that the addition of the fat to the fine particle diet actually made it more cohesive and thus favored the accumulation of a calcifiable matrix about the teeth.

SUMMARY

Dental calculus formation was studied in groups of Sprague-Dawley rats fed a series of diets modified in particle size, in fat content and by the incorporation of erythromycin at a level of 50 mg per kilogram of diet. Animals receiving a fine particle diet containing 20 per cent corn oil showed the heaviest concretions on the molar teeth. The effects of varying the particle size of non-fat diets were confined mainly to differences in the severity of the deposits at specific sites rather than to changes in total calculus scores. The single most effective factor was the incorporation of erythromycin in the diets. This resulted in markedly decreased calculus scores in all dietary groups.

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RÉSUMÉ

TARTRE DENTAIRE CHEZ LE RAT: ACTION DU RÉGIME ET DE L'ÉRYTHROMYCINE.

La formation du tartre dentaire a été étudiée sur des groupes de rats Sprague-Dawley soumis à une série de régimes modifiés en ce qui concerne la grosseur des particules, la teneur en matières grasses, et par incorporation d'érythromycine dans la proportion de 50 mg par kilogramme d'aliment. Les animaux soumis à un régime à particules fines et contenant 20 % d'huile de maïs

présentaient les dépôts tartriques les plus abondants sur les molaires. L'effet de modifications de la grosseur des particules des régimes non gras se limitait principalement à des différences dans l'abondance des dépôts en certains points particuliers, plutôt qu' à des modifications de la quantité totale de tartre. Le facteur isolé le plus efficace était l'incorporation d'érythromycine aux régimes. Cette incorporation résultait dans tous les groupes de régimes en une diminution marquée de la quantité de tartre observée.

ZUSAMMENFASSUNG

ZAHNSTEIN BEI DER RATTE: EFFEKT VON FUTTER UND ERYTHROMYZIN

Zahnsteinbildung wurde in Gruppen von Sprague-Dawley-Ratten studiert, die mit Futter mit verschiedener Partikelgrösse und verschiedenem Fettinhalt und mit Futter mit Zutat von Erythromyzin (50 mg pro kg Nahrung) gefüttert wurden.

Tiere, die Feinpartikelfutter mit 20 % Maisöl bekamen, zeigten die schwersten Ansammlungen auf den Molaren.

Der Effekt einer Änderung der Partikelgrössen in fettfreien Futtern beschränkte sich hauptsächlich auf Unterschiede im Ausmass der Ansammlungen an besonderen Stellen eher als auf Veränderungen der Gesamtbildung von Zahnstein.

Der einzige massgebende Faktor war die Zutat von Erythromyzin zu den Futtern, welches zu ausgesprochener Abnahme der Zahnsteinbildung in allen Futtergruppen führte.

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