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THE UREOLYTIC ACTIVITY OF DENTAL CALCULUS

by

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In a previous paper (*Frostell,* 1960) the ureolytic activity of dental plaque material was determined. *Bliss* (1937) found that dental calculus rapidly decomposes urea. Since it was found that dental plaque material is sometimes incrustrated by mineral salts, it was thought that the decomposition of urea by dental plaque material might be influenced by the presence of dental calculus. Further, it was thought to be of interest to determine the ureolytic activity of dental calculus quantitatively in order to compare it with the activity exerted by the soft dental coatings.

MATERIAL AND EXPERIMENTS

First series

Dental calculus was removed by means of a scaler from the teeth of people with heavy deposits. Both supragingival calculus and subgingival calculus were collected. The material from each patient was studied separately. Only large pieces of the material were used for further study. These were thoroughly rinsed with Ringer solution in order to remove soft debris, then with a pincette they were placed on a watch glass in a stream of

air. After about four minutes they were dry. They were then immediately moved to a glass mortar and ground to a very fine powder. About one half of the amount of powder obtained from a person was weighed to the nearest tenth of a milligramme and then suspended in 15 ml of a Ringer solution (NaCl, 8.0, KCl, 0.2, $CaCl_2$, 0.2 g/l). This powder weight was called wet weight in spite of the fact that the powder appeared dry. The rest of the powder was also weighed, dried for 18 hours at 105° C, and then weighed again. Thus both the wet weight and the dry weight of a given amount of calculus powder were obtained. The relation between wet weight and dry weight was calculated and was used for the calculation of the activity per milligramme dry weight.

In the suspension of calculus in Ringer solution the powder sank slowly to the bottom. The suspension was vigorously stirred and 10.0 ml were taken with a pipette to a glass beaker belonging to an apparatus previously described (*Frostell*, 1959). In the same way 5.0 ml were taken to another beaker for the determination of any ammonia formed from sources other than the urea added in the experiment. The alkali and the ammonia production activities were determined as described earlier (*Frostell*, 1960) when experiments were performed with dental plaque material. The total volume was 11.0 ml and thus the final urea concentration was 0.06 M in contrast to the experiments with dental plaque material in which the corresponding figures were 7.0 ml and 0.1 M. The experiment was started as soon as possible and in any case not later than 45 minutes after the material had been removed from the teeth.

In most experiments the activity was low. It was assumed, too, that some of the material slowly dissolved in the Ringer solution, causing an alkalization of the calculus suspension. For that reason the alkali-production values — which were calculated from the amount of 0.01 N HCl required to keep the pH constant — are not considered reliable. The ammonia-production activity was determined from the amount of ammonia produced during the experiment as previously described.

Stained smears were made from some of the suspensions. In some experiments a drop of the suspension was transferred to blood agar for aerobic and anaerobic incubation.

Results

The results of the determination of the alkali-production activity, *i.e.* the production of monovalent alkali per milligramme material per minute, and the ammonia-production activity, *i.e.* the production of ammonia per milligramme material per minute, in the presence of 0.06 M of urea are given in Table I. For reasons given above, the alkali-production values are considered unreliable. They are, however, given as a fair control of the results of the determinations of the ammonia-production activities.

Table 1.
Ureolytic activity of dental calculus
Calculus powder, urea concentration 0.06 M

Patient	Wet weight/Dry weight	Activity in 10^{-9}E/min.			
		per mg w.w.		per mg d.w.	
		HCl	NH_3	HCl	NH_3
N. N.	1.006	(2.34)	0.17	(2.35)	0.17
K. K.	1.036	(2.45)	3.79	(2.53)	3.93
G. J. L.	1.079	(2.00)	0.21	(2.20)	0.23
B. I.*	1.075	(6.05)	10.20	(6.50)	10.96
L. K.	1.019	(4.06)	0	(4.14)	0
A. J.*	1.007	(1.19)	3 15	(1.20)	3.17
U. W.*	1.051	(1.79)	0	(1.88)	0
A. L. J.*	1.077	(3.39	2.52	(3 65)	2.71
S. N.	1.010	(5.34)	7.12	(5.39)	7.19
S. E.*	1.035	(4.06)	5.24	(4.20)	5.42
	Mean: 1.040	(3.27)	3.24	(3.40)	3.38

* Female

The mean alkali-production activity of powdered dental calculus of 10 persons in the presence of 0.06 M urea was 3.27×10^{-9} E per mg wet weight per minute and 3.40×10^{-9} E per mg dry weight per minute. The mean ammonia-production activity was 3.24×10^{-9} E per mg wet weight per minute and 3.38×10^{-9} E per mg dry weight per minute. The differences between wet weights and dry weights were not very great, as may be seen in Table 1.

The study of Gram-stained smears of the suspensions revealed large amounts of predominantly Gram-positive microorganisms lying singly or in groups. Most of the organisms were Gram-positive branched threads resembling actinomyces, but non-branched threads (leptotrichia, *Tjøtta, Bøe*) were also seen. Gram-positive cocci and rods were occasionally found, as were fusobacteria, and curved Gram-negative rods.

From the suspensions were cultivated aerobic and anaerobic streptococci, neisseria, diphtheroids, veillonella, bacteroids, actinomyces, leptotrichia and anaerobic Gram-negative curved mobile rods (vibrio, selenomonas?).

Second series

Since it was thought that the urease activity was due to microorganisms in the calculus and that these organisms might be destroyed by the grinding, and since the urea concentration in the first series was different from that used when dental plaque material was studied, a second series of experiments with 10 new persons was performed with calculus that was crushed into small pieces instead of being ground in a mortar. The volume in these experiments was only 7 ml and the urea concentration at the start of the experiment was 0.1 M (0.098 M) as in the previous experiments with plaque material. The dry weights were not determined in this series. This made it possible to use more calculus for the actual urease-activity experiment than in the first series.

A weighed amount of crushed calculus, which was prepared as previously described, was suspended in 6.0 ml of Ringer solution. A control containing calculus in 6.0 ml Ringer solution without urea was also run in each experiment.

Results

The results of the experiments of the second series are given in Table 2. The mean alkali-production activity of crushed calculus from 10 persons in the presence of 0.1 M urea was 2.14×10^{-9} M (monovalent) per mg wet weight per minute and the mean ammonia-production activity was 3.04×10^{-9} E per mg wet weight per minute.

Table 2.

The ureolytic activity of dental calculus

Crushed calculus. Urea concentration 0.1 M

Patient	Activity in 10^{-9} E/min/mg w. w.	
	HCl	NH_3
S. Z.	(1.24)	1.06
I. T.*	(6.67)	11.97
A. R.	(2.82)	8.61
K. K.	(0.51)	1.56
U. K.*	(1.47)	1.90
B. P.	(1.99)	1.24
N. N.	(0.90)	0.10
E. H.*	(0.75)	0
I. G.*	(2.89)	3.40
A. S.*	(2.15)	0.55
Mean:	(2.14)	3.04

* Female

DISCUSSION

Two series of experiments were performed with small experimental differences between them. The two series cannot be directly compared since more than one factor was varied. The second series, however, can be directly compared with the experiments previously performed with dental plaque material.

It may be observed, however, that in the two series the values of the ammonia-production activity vary within wide limits (Table 1, 0—10.2; Table 2, 0—11.97), but that the mean activity is of the same order of magnitude in the two series. It is perhaps justifiable to believe that the small differences in technique between the two series were of little importance for the results of the determinations of the activities, *i.e.* that, for example, the grinding of the calculus did not destroy the active principle to any significant degree. The author believes that newly deposited calculus is more active than old calculus and that differences in the age of the calculus to some degree account for the differences in activities found in this investigation.

Under similar experimental conditions and at the same urea concentration the ureolytic activity of dental calculus was much lower than that of dental plaque material. Thus it is not probable that the ureolytic activity of dental plaque material is caused by the presence of dental calculus in such material, as one might suppose when reading the paper of *Bliss* (1937).

It is very important to realize that the activities given in this paper refer to the experimental conditions maintained during the experiment and not to conditions in the oral cavity, where, for example, the urea concentration is very low (about 0.002 M, calculated after *Albrechtsen & Thaysen,* 1955). It has been found that the urease activity of dental plaque material is dependent on the substrate concentration in a typical manner (*Frostell,* in print). Thus, the values of alkali- and ammonia-production activities refer only to the urea concentration and the other experimental conditions maintained during this study and during the previous one in which dental plaque material was studied.

SUMMARY

The ureolytic activity of dental calculus was determined by the method previously used for the study of dental plaque material. Under similar experimental conditions the mean ureolytic activity of dental calculus from 10 persons expressed as ammonia-production was 3.04×10^{-9} E per mg wet weight per minute in the presence of 0.1 M of urea. Thus the ureolytic activity of dental calculus was much lower (approximately one tenth) than that of dental plaque material under similar experimental conditions.

The results indicate that the ureolytic activity of dental plaque material is not caused by contamination with dental calculus, as might be supposed if dental calculus had a very high urease activity. The author believes that the urease activity of dental plaque material and of dental calculus is due to the presence of urease producing micro-organisms.

RÉSUMÉ

ETUDES SUR L'ACTIVITÉ URÉOLYTIQUE DE TARTRE

L'activité uréolytique du tartre a été déterminée selon la méthode déjà utilisée en étudiant la substance de plaques dentaires. Dans des conditions identiques d'expérimentation la valeur moyenne de l'activité uréolytique du tartre de 10 personnes, exprimée en forme de production ammoniacale, s'est élevée à $3{,}04\times10^{-9}$ E par mg de poids mouillé par minute dans la présence de 0,1 M d'urée. Donc, la valeur de l'activité uréolytique s'est montrée beaucoup plus basse (approximativement une dixième) que celle du tartre dans des conditions d'expérimentation similaires.

Au cours de l'argumentation l'auteur a souligné que les résultats indiquent que l'activité uréolytique de la substance des plaques dentaires n'est pas occasionnée par la présence de tartre, ce qu'on pourrait être disposé à supposer si la valeur de l'activité uréase du tartre avait été très haute. L'auteur est d'avis que l'activité de l'uréase de la substance des plaques dentaires et du tartre est à attribuer à la présence de micro-organismes producteurs d'uréase.

ZUSAMMENFASSUNG

UNTERSUCHUNG ÜBER DIE UREOLYTISCHE AKTIVITÄT VON ZAHNSTEIN

Die ureolytische Aktivität von Zahnstein wurde durch die früher angewandte Methode für das Studium von dentalem Plaquematerial bestimmt. Unter den gleichartigen Versuchsbedingungen war die durchschnittliche ureolytische Aktivität des Zahnsteins von 10 Personen, in Ammoniakproduktion ausgedrückt, $3{,}04\times10^{-9}$ E pro mg Nassgewicht pro Minute beim Vorhandensein von 0,1 M Harnstoff. Die ureolytische Aktivität des Zahnsteins war also viel niedriger (annähernd ein Zehntel) als die des dentalen Plaquematerials unter den gleichartigen Versuchsbedingungen.

Es wird hervorgehoben, dass die Resultate erkennen lassen, dass die ureolytische Aktivität des dentalen Plaquematerials

nicht durch das Vorhandensein von Zahnstein verursacht wird, was man hätte vermuten können, falls der Zahnstein eine sehr hohe Ureasenaktivität aufgewiesen hätte. Der Verfasser meint, die Ureasenaktivität des dentalen Plaquematerials und des Zahnsteins sei dem Vorhandensein von ureasenproduzierenden Mikro-Organismen zuzuschreiben.

REFERENCES

Albrechtsen, S. R. & J. H. Thaysen, 1955: The excretion of urea by the human parotid gland. Scand. J. clin. Lab. Invest. *7:* 231.

Bliss, S., 1937: The cause of sore mouth in nephritis. J. biol. Chem. *121:* 425.

Bøe, J., 1941: *Fusobacterium. Studies on its Bacteriology, Serology and Pathogeneity.* Norske Videnskaps-Akad. i Oslo, I Naturv. Klasse No. 9.

Frostell, G., 1959: A titration method for the determination of acid and alkali production by micro-organisms. Acta odont. scand. *17:* 44.

—»— 1960: Studies on the ammonia production and the ureolytic activity of dental plaque material. Acta odont. scand. *18:* 29.

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