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THE ABILITY OF VARIOUS MICRO-ORGANISMS TO PRODUCE HISTOCHEMICALLY DETERMINABLE ENZYME ACTIVITY IN HUMAN DENTINE

by
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INTRODUCTION

Several studies using the gnotobiotic technique have shown that dental caries is developed in rats inoculated with streptococci or lactobacilli (*Orland et al.*, 1955; *Fitzgerald, Jordan & Stanley*, 1960; *Gibbons et al.*, 1966; *Rosen et al.*, 1968). Earlier attempts to produce dental caries with lactobacilli have been generally unsuccessful in contrast to the repeated success with a single strain of streptococcus (*Orland*, 1959, *Fitzgerald, Jordan & Stanley*, 1960), or combined with a proteolytic bacillus (*Orland*, 1959). Recently, however, *Rosen, Lenney* and *O'Malley* (1968) were able to produce dental caries in three strains of gnotobiotic rats inoculated with *Lactobacillus casei* (ATCC 4646). Thus lactobacilli and streptococci have been shown to be cariogenic micro-organisms in experimental animal caries.

Other micro-organisms besides lactobacilli and streptococci have been considered from time to time as the causative agents of dental caries (see: *Burnett & Scherp*, 1968). The causal association of micro-organisms with dental caries is difficult to show because a wide variety of micro-organisms have on occasion been isolated from carious lesions. Their presence does not necessarily indicate their importance in the development of dental caries.

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The aim of this paper is to provide information about the ability of various micro-organisms to invade and soften normal human dentine and to produce different types of hydrolytic enzyme activity in the degrading tissue. Consequently, the working hypothesis of this paper, in contrast to the papers mentioned above, considers all micro-organisms of a carious lesion as »cariogenic». Dental caries can be considered as a mixed dental infection with numerous causative steps. The hypothesis that all micro-organisms may be partly cariogenic, *i.e.* that they may contribute to some step in the progress of caries, does not exclude the fact that certain micro-organisms are more potently cariogenic. The hydrolytic enzyme activity in carious dentine reported in previous papers (*Larmas, Mäkinen & Scheinin*, 1968; *Larmas*, 1968; *Larmas & Mäkinen*, 1971) could be considered as an indicator of the development of dental caries.

MATERIALS AND METHODS

Dentine samples. Pieces of normal dentine were prepared from caries-free teeth, extracted mainly for orthodontic reasons. The teeth were stored for three weeks at +4° C before use. Dentine pieces of $2 \times 3 \times 3$ mm were prepared by a diamound burr with a water spray. Two or three pieces of as equal size as possible were prepared from the same tooth. The samples were sterilized by autoclavizing (for 10 min at 121°C) in the first 30 ml growth media, initiating the series of experiments, as described later in more detail.

Growth media. The solid P-agar was composed as described earlier (*Mäkinen*, 1969) and Bacto-Micro Inoculum Broth was made by rehydrating 37 g of the commercial medium in 1000 ml distilled water. The medium was autoclavized for 15 min at 121°C. The TSHGA, GSHT, Liver Meat Extract Broths, the solid blood agar, McLeod agar, and Rogosa agar media were prepared as described earlier (*Mäkinen*, 1969; *Larmas*, 1971).

Cultivation of micro-organisms. For this investigation 13 different micro-organism species or strains were chosen (Table I). The lactobacilli, which were maintained as stab cultures in P-agar, were transferred from this medium to 7 ml of the Bacto-Micro Inoculum Broth and cultivated for 24 hours without aeration. Micro-organisms of the 7 ml tubes were finally transferred aseptically by a slope into 30 ml of pre-warmed TSHGA, GSHT, or Liver Meat Extract Broth. Other test organisms were maintained as slope cultures on P-agar and transferred from these into 30 ml tubes of TSHGA, GSHT, or Liver-Meat Extract Broth.

Before sterilization of the 30 ml growth media, a piece of normal dentine was immersed in 13 different bottles and autoclavized for 10 min at 121°C.

Table I.

Summary of the procedures used to obtain soft dentine pieces for cryostat sectioning. Histochemical enzyme determinations were performed with samples obtained after the procedures designated as Ia, IIa and IIIa

Designation of the group in which the cryostat sections could be made	Total length of the experimental period from the beginning of the first inoculation (days)*	Procedures (performed subsequently with one and the same sample)
Ia	40	Daily transfers to fresh media until cryostat sections could be made (no decalcification was performed)
Ib	41—47	Decalcification was performed: incubation in media the pH of which was adjusted to 4.0 with lactic acid
IIa	48—54	Daily transfers into fresh media until cryostat sections could be made
IIb	55—61	Second decalcification was performed as above
IIIa	62—68	Daily transfers to fresh media

*) The range of the total length of the experimental period shown indicates that in group Ia all samples were incubated for 40 days, and that in other groups the samples were found to be sufficiently softened within the observation period of seven days forming each group.

Each medium was then inoculated by one micro-organism species or strain. The micro-organisms were cultivated for 20—28 hours at 37°C. The infected pieces of dentine were then transferred into a new sterile medium of the same type. The final turbidity reading of all media was measured with a Klett-Summerson colorimeter (filter no. 62). The final pH of the media was measured at 25° C with glass and calomel electrodes. The above procedure was repeated daily after 20—28 hours growth until the pieces of dentine were soft enough for sectioning in the cryostat. In other cases the micro-organisms were cultivated for at least 40 days.

Sufficient softening of some dentine pieces incubated in the presence of certain micro-organisms was not detectable in 40 days. Such pieces of dentine were demineralized with lactic acid in the following way: The dentine pieces were transferred to the same fresh medium as previously and the pH of the medium was adjusted to pH 4.0 with DL-lactic acid (The British Drug Houses Ltd, Poole, England), as described earlier (*Larmas*, 1971). Transfers to these acidic media were repeated daily for one week. Thereafter the transfers were

again made into the original medium (without added lactic acid) and inoculated with the original micro-organism. These daily transfers were then made until cryostat sections could be prepared, but not longer than for one week.

The pieces of dentine which even after the above procedures were too hard for sectioning were transferred to the above mentioned medium of pH 4.0 for another week (with daily transfers to a fresh medium). After this the original medium was inoculated with the micro-organism involved. After these procedures cryostat sections could be made from all pieces of dentine. The experimental system is summarized in Table I.

As a control, pieces of normal dentine were incubated after sterilization in the 30 ml of sterile media, transferring the dentine pieces daily to fresh media exactly as in the actual experiments, but omitting the inoculation. No softening of these dentine samples was observed in 68 days. Pieces of normal dentine were also incubated in the acidic media (with a lactic acid addition as described above) with daily transfers to a fresh solution as above, until cryostat sections could be made.

During the entire experiments subcultivations were made on solid media to check the purity of the organisms. The purity was also confirmed by Gram staining. In case of subinfection, the experiment involved was stopped and repeated from the very beginning.

A few micro-organisms were studied in more detail in order to elucidate their growth pattern and the change of pH of their culture media. For these experiments *Candida albicans* was chosen to represent oral yeasts, *Streptococcus salivarius* to represent oral cocci, and *Escherichia coli* 113 and *Lactobacillus casei* ssp. *rhamnosus* (ATCC 7469) to represent typical gram-negative and gram-positive rods. In these cases the inoculations were made into 300 ml growth media with a piece of normal dentine. Samples of 10 ml were taken at suitable time intervals. The turbidity and pH of the samples were measured as earlier mentioned.

Histochemical demonstration of enzyme activity. The softened pieces of dentine were attached with distilled water to a specimen holder plane of a microtome, and sections of 10 μ were cut out in a cryostat (Model CTI, International Equipment Company, Needham Heights, Mass., USA), as described earlier (*Larmas*, 1971). The histochemical demonstration of aminopeptidase was carried out according to *Nachlas et al.* (1957), with N-L-leucyl-2-naphthylamine as substrate (Mann Research Laboratories Inc., New York, N.Y., USA). Acid and alkaline phosphatase activity were determined according to *Burstone* (1960) using naphthol AS-MX and AS-GR phosphate (Sigma Chemical Company, St. Louis, Mo., USA) and naphthol AS-

TR-phosphate (Nutritional Biochemical Corporation, Cleveland, Ohio, USA). The method used in determination of arylsulphatase activity was that of *Rutenburg et al.* (1952), but the substrate used was 6-bromo-2-naphthyl sulphate. The methods used have been described in more detail in other publications (*Larmas, Mäkinen & Scheinin,* 1968; *Larmas,* 1968; 1971). The effect of the azo dyes alone on the tissue sections was studied in a similar reaction mixture as above, but omitting the substrates.

The semiquantitative estimation of enzyme activity on tissue sections was based on the evaluation of the activity density of the sections, not on that of the intensity of the activity, because, in the microscopic examination, only coarse differences of intensity could be observed (Table III).

Unless otherwise stated, all chemicals used in this study were purchased from E. Merck AG, Darmstadt, Germany.

RESULTS

Table II lists the micro-organisms and certain details concerning their cultivation and growth. In Fig. 1 the final pH values of the TSHGA medium, determined at the point of stopping of the growth, are shown. Table II indicates that most streptococci, when grown in TSHGA medium, softened the dentine samples in 40 days. This also applies to *L. casei* and *Candida albicans*. Fig. 2 shows typical examples of the daily growth curves of four micro-organisms cultivated in TSHGA medium, and further that the pH of the media fell close to 5.0 with *E. coli* (113) and *Candida albicans*, and below 4.0 with *L. casei* (ATCC 7469) and *Str. salivarius*. In TSHGA medium the pH was maintained above 6.0 for at least 6 hours growth. In other media the corresponding time was even longer. With *Candida albicans* an increase in pH of the TSHGA growth medium could first be observed after 20 hours growth. All these organisms reached their stationary phase of growth between daily transfers.

The histochemical experiments are illustrated in Plate I, where Fig. 3 shows aminopeptidase activity in a longitudinal section of dentine, produced by *L. casei* (ATCC 7569) in TSHGA medium. Fig. 5 (Plate I) shows the same enzyme activity produced by *Candida albicans* in GSHT medium. In Fig. 7 moderate aminopeptidase activity is seen to be produced by *Str. pyogenes* (ATCC 6636) in Liver-Meat Extract Broth. Fig. 4 demonstrates that rich arylsulphatase activity is seen in the section, produced by the oral *Candida albicans* when grown in TSHGA medium. Fig. 6 shows moderate arylsulphatase activity produced by *L. casei* (ATCC 10306) in Liver-Meat Extract

Table II.

List of the micro-organisms used in the study and some details on their cultivation. The range of the pH and turbidity values shown represent extreme values observed

Test organisms	Growth Media	Final pH of the growth media	Turbidity reading reached	Time required for softening of dentine (cf. Table I)
Candida albicans	TSHGA	4.4—6.1	230—450	Ia
	GSHT	4.65—5.3	160—410	Ia
	Liver-meat	4.6—6.3	180—480	Ia
E. coli	TSHGA	4.6—4.9	140—240	Ia
113	GSHT	5.1—5.35	135—210	IIIa
	Liver-meat	5.3.—6.1	185—250	IIa
E. coli 154	TSHGA	4.6—4.85	120—225	IIa
	GSHT	4.6—5.2	130—205	IIa
	Liver-meat	4.8—5.1	140—230	IIIa
Corynebacterium bovis	TSHGA	4.55—5.3	110—195	IIIa
NCTC 3224	GSHT	4.9—5.6	105—180	IIa
	Liver-meat	4.75—6.3	120—190	IIIa
Lactobacillus casei ssp.	TSHGA	3.6—4.1	140—220	Ia
rhamnosus ATCC 7469	GSHT	4.3—5.0	90—170	Ia
	Liver-meat	4.3—4.75	145—210	Ia
L. casei NCTC 10302	TSHGA	3.7—4.3	125—210	Ia
	GSHT	4.3—4.8	110—195	Ia
	Liver-meat	4.3—5.0	130—205	Ia
L. fermenti ATCC 9338	TSHGA	3.9—4.8	110—190	IIa
	GSHT	4.3—5.35	90—185	IIa
	Liver-meat	4.3—6.4	80—190	IIIa
L. lactis ATCC 8000	TSHGA	3.9—4.2	105—170	IIa
	GSHT	4.5—4.7	80—140	Ia
	Liver-meat	4.3—4.9	95—160	IIa
Streptococcus	TSHGA	4.3—4.6	140—210	Ia
sp ATCC 9854	GSHT	4.5—4.75	125—200	Ia
	Liver-meat	4.3—5.0	140—210	Ia
Streptococcus pyogenes	TSHGA	4.0—4.4	130—205	Ia
ATCC	GSHT	4.4—4.6	130—190	Ia
	Liver-meat	4.3—4.8	140—180	Ia
Streptococcus pyogenes (haemolyticus)	TSHGA	3.8—4.3	160—220	Ia
ATCC 9342	GSHT	4.0—4.4	135—190	Ia
	Liver-meat	4.2—4.7	140—210	IIIa

Table II

Streptococcus sp Viridans type NTS 3165	TSHGA	3.6—4.1	170—225	Ia
	GSHT	3.9—4.4	160—220	Ia
	Liver-meat	4.0—4.9	165—215	Ia
Streptococcus salivarius	TSHGA	3.6—4.2	110—180	Ia
	GSHT	3.9—4.7	100—170	Ia
	Liver-meat	4.2—4.7	110—165	IIa
Sterile media:				
Lactic acid	TSHGA	4.0	—	Ia
	GSHT	4.0	—	Ia
	Liver-meat	4.0	—	Ia
Controls	TSHGA	6.7	—	—
	GSHT	6.7	—	—
	Liver-meat	7.2	—	—

Broth, and Fig. 8 shows no enzyme activity, the dentine piece having been incubated in Liver-Meat Extract Broth inoculated with *Corynebacterium bovis* (NCTC 3224).

Table III summarizes the results. Of all micro-organisms studied, the oral *Candida albicans* produced most abundant aminopeptidase and arylsulphatase activity, even more than *L. casei* (ATCC 7469 and NCTC 1032) cultivated in carbohydrate-rich media. In general streptococci produced poor aminopeptidase activity in the dentine samples, as was the case with *L. fermenti* (ATCC 9338) and *L. lactis* (ATCC 8000). On the other hand, arylsulphatase activity was produced more abundantly by these micro-organisms, particularly by *L. lactis* (ATCC 8000) and *Str. pyogenes* (ATCC 6636 and ATCC 9342). *Corynebacterium bovis* (NCTC 3224) produced no histochemically demonstrable aminopeptidase activity in the samples, but moderate arylsulphatase activity could be observed in dentine sections incubated with this organism. *E. coli* (113 and 154) formed moderate aminopeptidase and arylsulphatase activity. None of the organisms produced histochemically demonstrable acid or alkaline phosphatase.

No enzyme activity could be observed in any of the control sections of dentine incubated in the media. The media themselves were not able to soften the dentine samples (except for those containing added lactic acid) and no trace of enzyme activity could be observed in these sections.

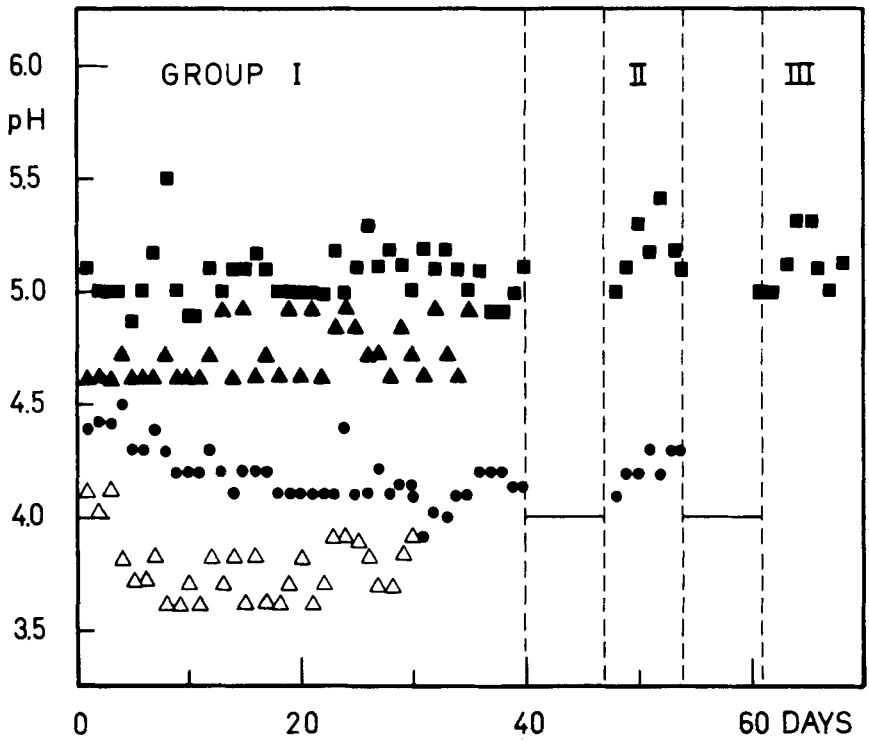


Fig. 1. Changes of the pII values of the TSHGA medium after the 24 hours incubation. Only the final pH values are seen in the figure, not the values of the sterile medium of which the pH value fell daily. The decalcification period, when the pH was 4.0, is seen between the dotted lines. (■ Corynebacterium bovis; ▲ E. col'i (113); • L. fermenti; △ Sreptococcus viridans).

DISCUSSION

The experimental system used in this investigation, *i.e.* the study of the *in vitro* softening of dentine and of the occurrence of histochemically demonstrable enzyme activity, most likely suffers from some limitations when compared to conditions applied *in vivo*. For example, the method used to measure the softening of dentine was coarse and subject to certain errors. Individual differences in the degree of mineralization of the dentine samples could not be assayed and the autoclavization of the samples may have occasioned changes in their chemical structure. Furthermore, it was difficult to prepare dentine pieces of exactly identical size and with identical surfaces. Some of these limitations have also been discussed earlier (*Larmas*, 1971). However, the results of this paper can be considered indicative.

Table III

The ability of some micro-organisms to produce histochemically demonstrable enzyme activity in dentine

Micro-organism	Culture Media	Amino-peptidase	Acid phospha-tase	Alkaline phospha-tase	Aryl-sulphatase
Candida albicans	TSHGA	+++++	—	—	++++
	GSHT	+++++	—	—	+++++
	Liver-meat	+++++	—	—	+++++
Escherichia coli 113	TSHGA	++	—	—	++
	GSHT	++	—	—	+++
	Liver-meat	+++	—	—	—
Corynebacterium bovis	TSHGA	—	—	—	++
	GSHT	—	—	—	+++
	Liver-meat	—	—	—	+++
Lactobacillus casei ssp. rhamnosus ATCC 7469	TSHGA	++++++	—	—	
	GSHT	++++	—	—	+++
	Liver-meat	++	—	—	++
L. casei NCTC 10302	TSHGA	++++	—	—	+++++
	GSHT	+++++	—	—	+++++
	Liver-meat	+	—	—	+++
L. fermenti ATCC 9338	TSHGA	+	—	—	++
	GSHT	—	—	—	+++
	Liver-meat	—	—	—	+
L. lactis ATCC 8000	TSHGA	+	—	—	+++
	GSHT	+	—	—	++++
	Liver-meat	++	—	—	++
Streptococcus sp ATCC 9854	TSHGA	+	—	—	+++
	GSHT	—	—	—	++
	Liver-meat	—	—	—	+
Streptococcus pyogenes ATCC 6636	TSHGA	+	—	—	++
	GSHT	+	—	—	+++++
	Liver-meat	+	—	—	+++
Streptococcus pyogenes (haemolyticus) ATCC 9342	TSHGA	++	—	—	++
	GSHT	++	—	—	+++
	Liver-meat	++	—	—	+++
Streptococcus sp Viridans type NCTC 3165	TSHGA	+	—	—	++
	GSHT	—	—	—	++
	Liver-meat	+	—	—	+++
Streptococcus salivarius	TSHGA	++	—	—	++
	GSHT	+	—	—	++
	Liver-meat	+	—	—	+

Explanations: +++++, very abundant enzyme activity with each substrate in all five sections, obtained from different layers of dentine: ++++, as previous, but the activity was approximately 25 % lower: +++, abundant enzyme activity in some (two or three) sections, moderate in others: ++, moderate enzyme activity in some (two or three) sections: +, poor activity in some sections (two or three) only: —, no enzyme activity in any of the sections. Abundant activity density indicates that enzyme activity was seen all over the section and poor activity density indicates activity in certain restricted areas only. Moderate enzyme activity indicates a situation intermediate between the two first mentioned. None of the controls revealed any enzyme activity.

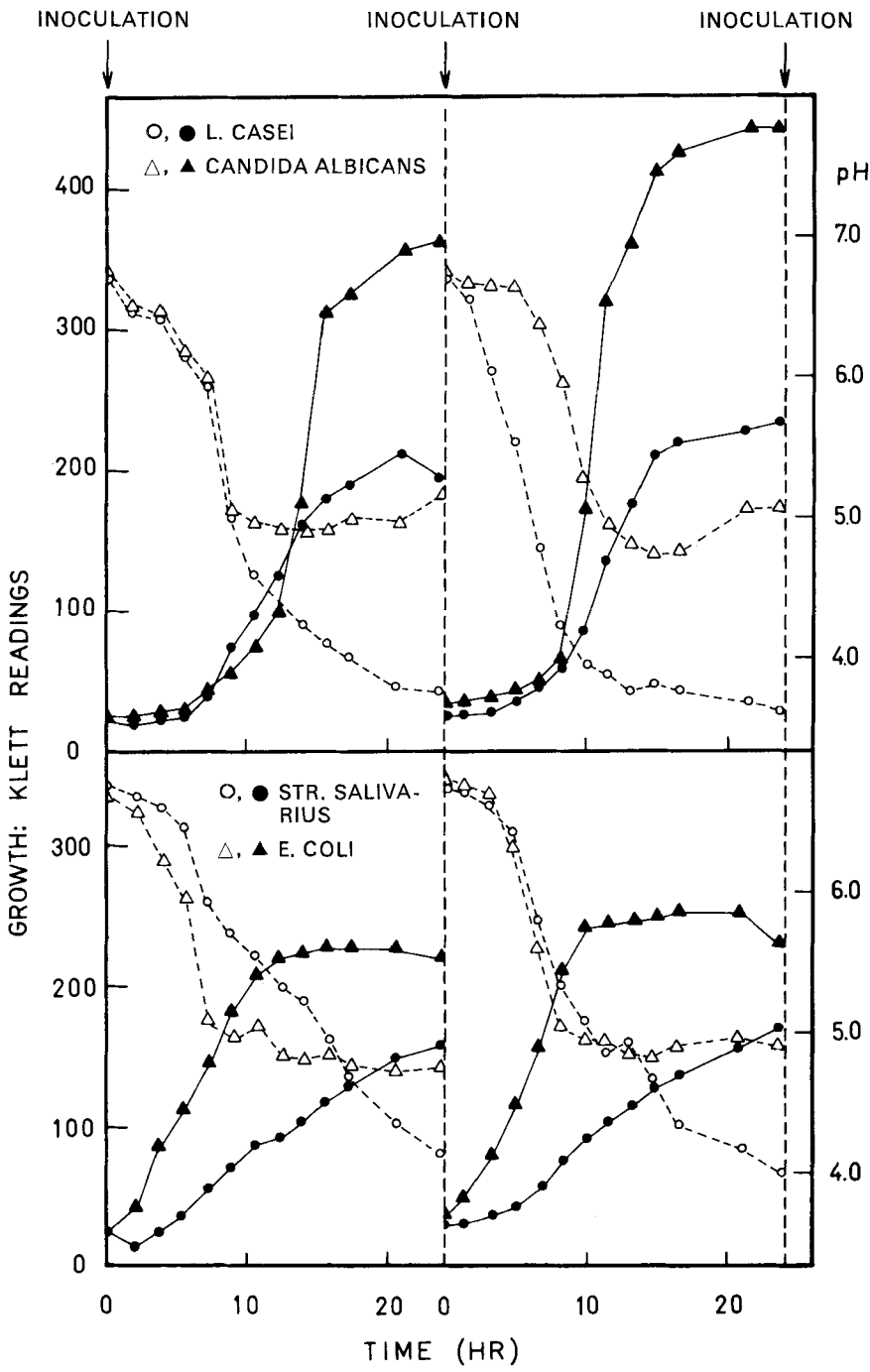
Evidently the hydrolytic enzymes studied, excluding phosphatases, are of microbial origin, because no enzyme activity could be observed in samples incubated in the absence of micro-organisms. Enzymes possibly present in the samples can be considered to have been destroyed in the autoclavization.

That oral *Candida* produced most aminopeptidase and arylsulphatase activity in dentine could be expected on the basis of preliminary experiments (*Larmas*, 1971). This study also confirms the observation of *Mäkinen* (1969) that oral streptococci are not very effective formers of enzymes displaying iminopeptidase and arylaminopeptidase activity and that cells of *L. casei* were effective formers of enzymes showing arylaminopeptidase activity. No alkaline or acid phosphatase activity could be demonstrated in the softened dentine, although carious dentine displays considerable acid phosphatase activity, demonstrated histochemically (*Larmas*, 1968) or biochemically (*Mäkinen, Larmas & Scheinin*, 1969; *Mäkinen*, 1970). This may be due to the fact that histochemically the localization of phosphatase activity seemed to be normally in the odontoblast layer.

Mäkinen (1969) has earlier found biochemically that the highest arylaminopeptidase activity in the cells of *L. casei* was not found in any restricted growth phase but rather during the whole life cycle, excluding, however, the lag phase of growth. The present results showed that after the active growth phase of many micro-organisms, even histochemically demonstrable enzyme activity could be demonstrated in dentine although there were not any cells to be seen in the sections. The time required for the softening of dentine may partly depend on the hydrolysis of the organic stroma by enzymes, because in this system acid-producing streptococci did not soften the dentine faster than candida, for example, which produced less acid but more hydrolytic enzymes (Figs. 1 and 2, Tables II and III). It was of interest that *L. fermenti*, decreasing the pH of the growth medium almost identically to streptococci, did not soften the dentine as rapidly (Fig. 1). Evidently there are some unknown factors, besides the acid production and the formation of hydrolytic enzymes, which partly contribute to the destruction of dentine. The conditions discussed here concern the organic stroma of dentine only, not that of enamel.

Evidently the working hypothesis that all or most micro-organisms of carious dentine may contribute to some step in the progress of dentine caries,

Fig. 2. The growth patterns (left-hand scale) and the pH of the TSHGA medium during the growth of the cells: *Lactobacillus casei* and *Candida* (A), *Streptococcus salivarius* and *E. coli* (B). The figure represents a two days period of the whole experiment and similar changes in pH and growth could be observed during the entire experiment. (Growth: solid line; pH: hatched line).


INOCULATION
INOCULATION
INOCULATION
O, ● L. CASEI
△, ▲ CANDIDA ALBICANS
O, ● STR. SALIVA-RIUS
△, ▲ E. COLI
GROWTH: KLETT READINGS
pH
400
300
200
100
0
7.0
6.0
5.0
4.0
0
10
20
TIME (HR)

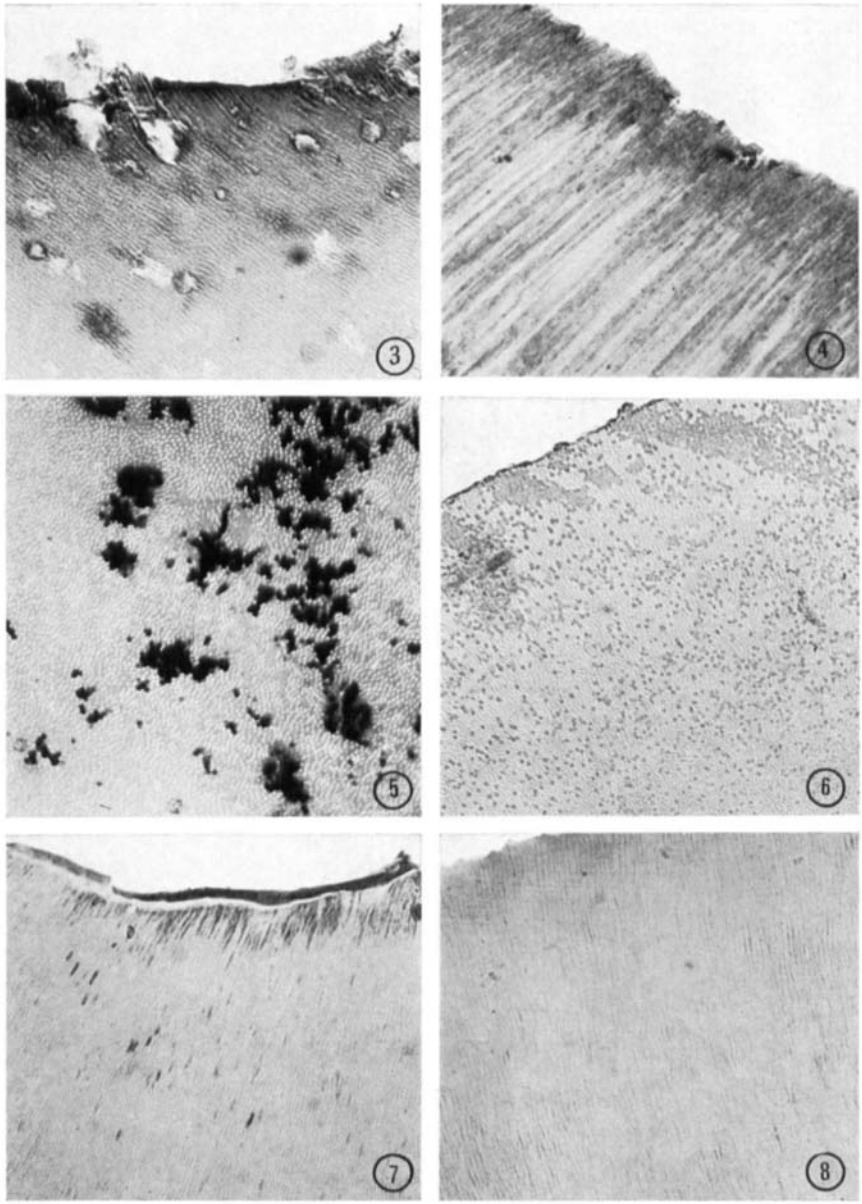


PLATE I

Fig. 3. Abundant (++++) arylaminopeptidase activity density in a longitudinal section of dentine, hydrolyzed by incubation with Lactobacillus casei (ATCC 7469) in the TSHGA medium. The activity is seen as dark grey stripes in the dentinal tubules of the section. Substrate: N-L-leucyl-2-naphthylamine, incubation time: 30 min. (× 30).

is not totally wrong. This study suggests that the occurrence of arylaminopeptidases and arylsulphatases in dentine produced by micro-organisms is an indication of the destruction rate of dentine.

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SUMMARY

The ability of 13 different micro-organisms to produce histochemically demonstrable aminopeptidase, arylsulphatase, acid and alkaline phosphatase activity in human dentine was studied. $2 \times 3 \times 3$ mm pieces of normal dentine were incubated for 28—68 days at 37°C in various culture media which were infected by one micro-organism species or strain at a time. The dentine samples were transferred daily to fresh media. Before inoculation the dentine samples were autoclavized in the medium initiating the experimental period. In 40 days some of the micro-organisms were able to soften the dentine samples enough for cryostat sectioning. The dentine pieces which were not softened were decalcified with lactic acid (pH 4.0) for a week. Even after this period a few samples required another week for decalcification before cryostat sections could be prepared. In general streptococci, *Candida albicans*

Fig. 4. Very abundant (++++) arylsulphatase activity in a longitudinal section of dentine, produced by oral candida when grown in the TSHGA medium. The activity occurs as dark stripes along the dentinal tubules in the probe section. Substrate: 6-bromo-2-naphthylsulphate, incubation time: 30 min. (× 30).

Fig. 5. Abundant (++++) arylaminopeptidase activity (++++) in a transversal section of dentine, produced by *Candida* grown in the GSHT medium. The enzyme activity is seen as dark stripes in the lumen of dentinal tubules. Substrate: N-L-leucyl-2-naphthylamine, incubation time: 30 min. (× 40).

Fig. 6. Moderate (++) arylsulphatase activity in a transversal section of dentine, hydrolyzed by *Lactobacillus casei* (ATCC 1036) in the liver-meat extract broth. The enzyme activity occurs as dark spots (not the light grey spots) in some of the dentinal tubules. Substrate: 6-bromo-2-naphthylsulphate, incubation time: 30 min. (× 40).

Fig. 7. Moderate (++) arylaminopeptidase activity in a longitudinal section of dentine, produced by *Streptococcus pyogenes* (ATCC 6636) grown in the liver-meat extract broth. Note the activity density concentrated (dark stripes) in the outer parts of the dentine. Substrate: N-L-leucyl-2-naphthylamine, incubation time: 30 min. (× 30).

Fig. 8. No arylsulphatase activity is seen in the transversal section of dentine, incubated with *Corynebacterium bovis* (NCTC 3224). The slide was selected as an example of sections without enzyme activity. Substrate: 6-bromo-2-naphthysulphate, incubation time: 30 min. (× 30).

and *Lactobacillus casei* (ATCC 7469 and NCTC 10302) were able to soften the dentine most rapidly. *L. fermenti* (ATCC 9338) did not soften the dentine as rapidly, as was the case with *Corynebacterium bovis* (NCTC 3224) and *E. coli*, when cultivated in certain media. These samples required an acid decalcification period of one or two weeks. *Candida albicans* and *L. casei* formed most abundant aminopeptidase and arylsulphatase activity in the sections. Streptococci produced only slight histochemically demonstrable aminopeptidase activity but moderate arylsulphatase activity. *E. coli* produced moderate aminopeptidase and arylsulphatase activity. No phosphatase activity could be demonstrated in any of the sections. The formation of these enzymes in the progress of dentine caries is briefly discussed.

RÉSUMÉ

APTITUDE DE DIFFÉRENTS MICRO-ORGANISMES À PROVOQUER DANS LA DENTINE HUMAINE UNE ACTIVITÉ ENZYMATIQUE DÉCELABLE PAR PROCÉDÉS HISTOCHIMIQUES

Cette étude a porté sur l'aptitude de 13 micro-organismes différents à provoquer dans la dentine humaine une activité aminopeptidase, arylsulfatase, phosphatase acide et alcaline susceptible d'être mise en évidence par procédés histochimiques. Des fragments de dentine normale de $2\times3\times3$ mm ont été incubés pendant 28—68 jours à 37°C dans différents milieux de culture infectés par une seule espèce ou souche de micro-organismes à la fois. Les échantillons de dentine étaient transférés tous les jours dans des milieux frais. Avant l'inoculation, les échantillons de dentine étaient stérilisés à l'autoclave dans le milieu débutant la période expérimentale. En l'espace de 40 jours, certains des micro-organismes étaient en état de ramollir les échantillons de dentine suffisamment pour permettre d'exécuter les coupes au cryostat. Les fragments de dentine qui n'avaient pas été ramollis ont été décalcifiés à l'acide lactique (pH 4,0) pendant une semaine. Après cette période, il restait quelques échantillons demandant encore une semaine de décalcification avant que les coupes au cryostat puissent être exécutées. Dans l'ensemble, les streptocoques, *Candida albicans* et *Lactobacillus casei* (ATCC 7469 et NCTC 10302) étaient en état de ramollir la dentine le plus rapidement. *L. fermenti* (ATCC 9338) ne ramollissait pas la dentine aussi rapidement que dans le cas de *Corynebacterium bovis* (NCTC 3224) et d' *E. coli*, lorsque la culture avait lieu dans certains milieux particuliers. Ces échantillons exigeaient une période de décalcification d'une ou deux semaines dans l'acide. *Candida albicans* et *L. casei* produisaient dans les coupes l'activité aminopeptidase et arylsulfatase la plus

abondante. Les streptocoques ne produisaient qu'une faible activité aminopeptidase décelable par procédés histochimiques, mais ils provoquaient une activité arylsulfatase modérée. *E. coli* provoquait une activité aminopeptidase et arylsulfatase modérée. L'activité phosphatase n'a pu être décelée dans aucune des coupes. La formation de ces enzymes pendent l'évolution de la carie de la dentine fait l'objet d'une brève discussion.

ZUSAMMENFASSUNG

DIE FÄHIGKEIT VERSCHIEDENER MIKROORGANISMEN HISTOCHEMISCH ZU DEMONSTRIERENDE ENZYMAKTIVITÄT IM MENSCHLICHEN DENTIN ZU PRODUZIEREN

Es wurde die Fähigkeit 13 verschiedener Mikroorganismen untersucht, histochemisch zu demonstrierende Aminopeptidase, Arylsulphatase, saure und alkalische Phosphataseaktivität im menschlichen Dentin zu produzieren. In verschiedenen, mit nur einem Mikrobenstamm oder einer Mikrobengattung infektierten Nährböden wurden Stückchen vom normalen Dentin ($2 \times 3 \times 3$ mm) von 28 bis 68 Tage inkubiert. Täglich wurden die Stückchen in einen frischen Nährboden eingesenkt. Vor der Inokulation wurden die Dentinstückchen in den benutzten Nährböden autoklavisiert. Schon 40 Tage reichten aus, damit einige der Mikroben fähig waren die Dentinstückchen so weich zu machen, dass dieselben mit Kryostat geschnitten werden konnten. Die Stückchen, die nicht weich genug waren, wurden mit Milchsäure (pH 4.0) eine Woche lang dekalzifiziert. Auch nach diesem Verfahren war für einige Dentinstückchen eine zusätzliche einwöchige Dekalzifikation nötig, ehe man die Kryostatschnitte machen konnte. Die rascheste Weichung des Dentins wurde mit Streptokokken, *Candida albicans* und *Lactobacillus casei* (ATCC 7469 und NCTC 10302) erreicht. *L. fermenti* (ATCC 9338) *Corynebacterium bovis* und *E. coli* zeigten nicht ebenso rasche Fähigkeit, wenn die Kultur in gewissen Nährböden durchgeführt wurde. Den mit diesartigen Bakterien gezüchteten Dentinstückchen war eine Dekalzifikationszeit von einer oder zwei Wochen erforderlich. *Candida albicans* und *Lactobacillus casei* verursachten die stärkste Aminopeptidase- und Arylsulphatase-Aktivität in den Schnitten. Die Streptokokken waren fähig, nur eine unbedeutende histochemisch erkennbare Aminopeptidase-Aktivität aber dagegen eine ziemlich starke Arylsulphatase-Aktivität zu produzieren. Von *E. coli* wurde eine ziemlich starke Aminopeptidase- und Arylsulphatase-Aktivität hervorgerufen. Phosphatase-Aktivität konnte in keinem der Schnitten demonstriert werden. Die Einwirkung jener Enzyme auf dem Erscheinen der Dentinkaries wurde kurz besprochen.

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