From:
The Institute of Dentistry,
University of Turku,
Turku, Finland.

HISTOCHEMICAL STUDIES ON PHOSPHATASE AND ARYLSULPHATASE ACTIVITY IN HUMAN CARIOUS DENTINE

by

MARKKU LARMAS

INTRODUCTION

Robison's booster theory (1930) proposes that alkaline phosphatase activity may play an important role in the initial mineralization process. Histochemically alkaline phosphatase activity has been described in tooth buds of various species. There are diverging opinions concerning the existence and significance of the alkaline phosphatase activity which occurs in the amelo- and odontoblasts (*Greep et al.*, 1948; *Symons*, 1954; *Sasso & Castro*, 1957; *Burstone*, 1960; *Ten Cate*, 1962; *Kurahashi et al.*, 1967). Histochemically alkaline phosphatase activity can be demonstrated in mineralizing dental tissues. However, little is known about the histochemical localization of alkaline phosphatase activity in adult human teeth.

Several authors have ascribed acid phosphatase activity to osteoclasts, (for reference see *Burstone*, 1960). It is thus thought that acid phosphatase activity may play some role in the resorption of bone. Some activity has been reported also in the distal cytoplasms of odontoblasts, but the dentine was not reactive (*Balogh*, 1965). *Kurahashi et al.* (1967) found some acid phosphatase activity in the dentinal tubules and at the dentinoenamel junc-

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tion in young albino rats during odontogenesis. *Obdyke* (1962) found no acid phosphatase activity in human carious dentine.

This study was undertaken because of the common concept that acid phosphatase activity may play an important role in the destruction processes of highly mineralized tissues, and also because of the working hypothesis that dentine does not differ in this respect. Further, enzymes acting on sulphate groups in mineralized tissues are interesting, for example, because of the important role of mineral binding acid mucopolysaccharides. In the present study arylsulphatase activity was treated as a model of enzymes acting on sulphate groups.

MATERIAL AND METHODS

The same methods and partly the same samples were used as earlier (*Larmas et al.*, 1968) in collecting carious dentine (excavated *in situ* or from freshly extracted teeth) and cutting 10 μ sections in a cryostat. The histochemical demonstration of the alkaline and acid phosphatase was carried out according to *Burstone* (1960). For the demonstration of alkaline phosphatase naphthol AS-MX (Sigma Chemical Company, St. Louis, USA) and AS-TR phosphate (Nutritional Biochemical Corporation, Cleveland, USA) were used. MX phosphate, Red Violet LB salt (diazotized 5-benzamido-4-chloro-2-toluidine) (Sigma Chemical Company) and TR phosphate, Blue BBN salt (diazotized 4-amino-2,5-diethoxybenzanilide, Verona Dyestuffs, Inc., Springfield, USA) were used in coupling the liberated naphtol. For the demonstration of acid phosphatase the substrates used were AS-GR phosphate (Sigma Chemical Company) and AS-TR phosphate (Nutritional Biochemical Corporation). Fast Blue BBN salt was in this case used in coupling the liberated naphthol. The incubation medium was the same as suggested by *Burstone* (1960). In the estimation of arylsulphatase activity the method of *Rutenburg et al.* (1952) was followed. The substrate used was 6-bromo-2-naphthylsulphate (Mann Chemical Laboratories, Inc., New York, USA). Fast Blue B (tetrazotized o-dianisidine, L. Light & Co. Ltd., Colnbrook, England), was used in this case in coupling the liberated naphthol. The effect of the diazonium salts alone on the tissue sections was studied in a similar reaction mixture as above but omitting the substrates. The effect of EDTA and sodium fluoride on the phosphatase and arylsulphatase activity was studied after dissolving the compunds involved in the reaction mixture to a final concentration of 1.0×10^{-2} M or 1.0×10^{-3} M. Unless otherwise stated the chemicals used were purchased from E. Merck AG, Darmstadt, Germany.

RESULTS

The alkaline phosphatase activity was poor in carious dentine when testing it by AS-MX phosphate as substrate. Marked activity was observed in the predentine layer (Fig. 1). In addition, marked activity was seen in carious dentine when the carious process had reached the predentine. Low activity was occasionally seen in the surface layer of carious dentine. The same phenomenon could be observed with naphthol-AS-TR-phosphate as substrate.

The acid phosphatase activity was more common in carious dientine. It was seen as stripes in the direction of dental tubules occurring n the surface layer of carious dentine up to the decalcified layer, but not in unaffected dentine (Fig. 2). Some activity was occasionally observed in the predentine (Fig. 3). When the carious process had penetrated the predentine and thereafter reached the pulp, marked activity was seen to originate from the site of exposure (Fig. 4).

Very distinct arylsulphatase activity was seen in all sections of various regions of carious dentine. It was more marked in the infected layer, the activity diminishing when approaching the decalcified layer. The activity

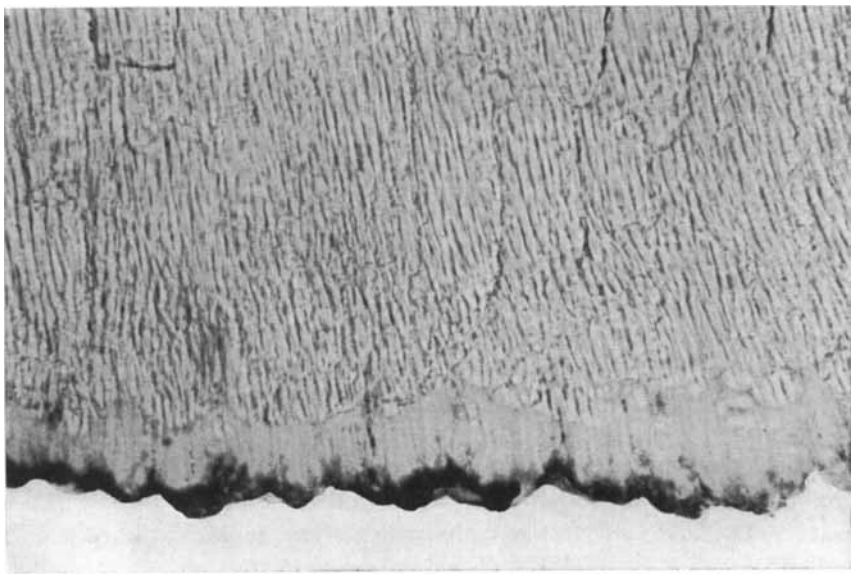

Fig. 1. A peripulpal section of undecalcified dentine from a carious human molar. Note the strong alkaline phosphatase activity involving part of the predentine (the black area in the picture). No enzyme activity could be observed in the advanced occlusal carious lesion, situated above the area shown. Substrate: Naphthol-AS-MX-phosphate. Incubation time: 45 min. (×220).

Fig. 2. A longitudinal section of an advanced carious lesion. The acid phosphatase activity is seen to occur as narrow dark stripes all over the picture in the direction of dentinal tubules. Substrate: Naphthyl-AS-TR-phosphate. Incubation time: 90 min. (×100).

was not homogenous in the dentine but occurred as distinct stripes in the direction of the dental tubules (Fig. 5). No activity was seen in unaffected dentine.

When testing the effect of some chemical compounds, it was seen that NaF at the final concentration of 10^{-2} M in the reaction mixture seemed to have an inhibiting effect of arylsulphatase activity. It also displayed a slight inhibitory effect on the acid phosphatase activity but no effect could be demonstrated on alkaline phosphatase under the circumstances involved. NaF and EDTA at the final concentration of 10^{-3} M, seemed to have no effect on the reactions.

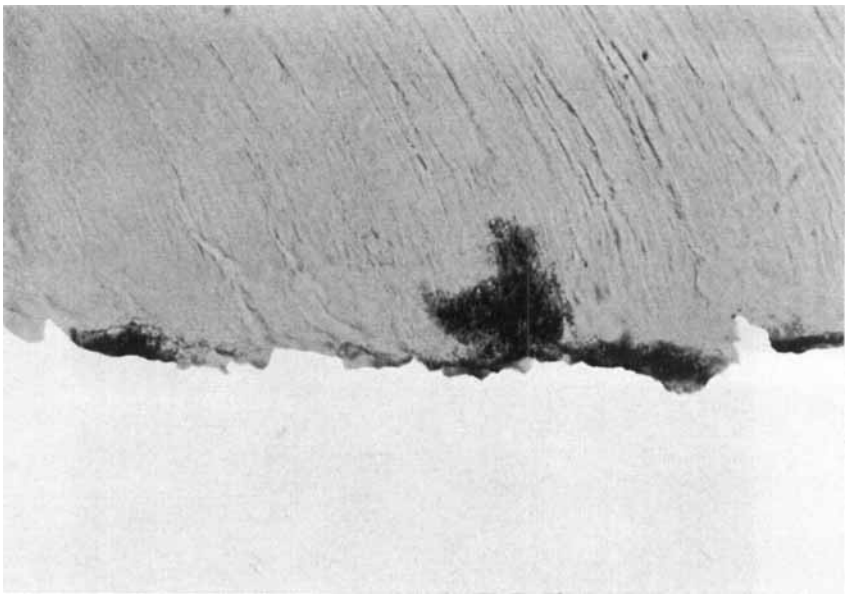

Fig. 3. A peripulpal section from the tooth seen in Fig. 1. Note the acid phosphatase activity (as black area) in the predentine layer. Substrate: Napthyl-AS-GR-phosphate. Incubation time: 90 min. (×220).

DISCUSSION

Alkaline phosphatase activity has been described histochemically in osteoblasts and chondroblasts (*Burstone*, 1960). The localization of alkaline phosphatase in the predentine layer in adult human teeth below the carious dentine showed that even under an advanced carious process the odontoblasts seem to maintain their ability to produce enzymes with alkaline phosphatase activity.

It is well known that the acid phosphatase activity of most tissues is rather low when testing it histochemically. It may be due to that the original technique (*Gomori*, 1952) may give erratic results. For instance *Chanqus* (1957) claimed that even mild decalcification procedures may completely inhibit acid phosphatase activity. In reference to the use of frozen sections, acid phosphatase is known to be readily solubilized by freezing and thawing (*Appelmans et al.*, 1955; *Gianetto & DeDuve*, 1955). Thus it is extremely interesting to note that when using frozen sections of carious and sound dentine, marked activity can be observed in the carious process. The present results differ from those of earlier (*Obdyke*, 1962) as to alkaline and acid phosphatases.

Fig. 4. Carious dentine extends to the pulp. Strong acid phosphatase activity occurs as black stages in the vicinity of the pulp (P). Weak activity is seen as grey stripes in the superficial layers of the cavity (C). Substrate: Napthyl-AS-TR-phosphate. Incubation time: 45 min. (×40).

On the basis of the results obtained with NaF it may be concluded that it in the high concentration used merely displayed some unspecific anion or salt effect on the enzyme reaction studied. Consequently, such low fluoride concentrations which occur in the oral cavity evidently have no effect on the enzymes involved.

Arylsulphatase activity is thought to originate from the bacterial plaque, in which *Mäkinen* (1966) has shown considerably high enzyme activity on

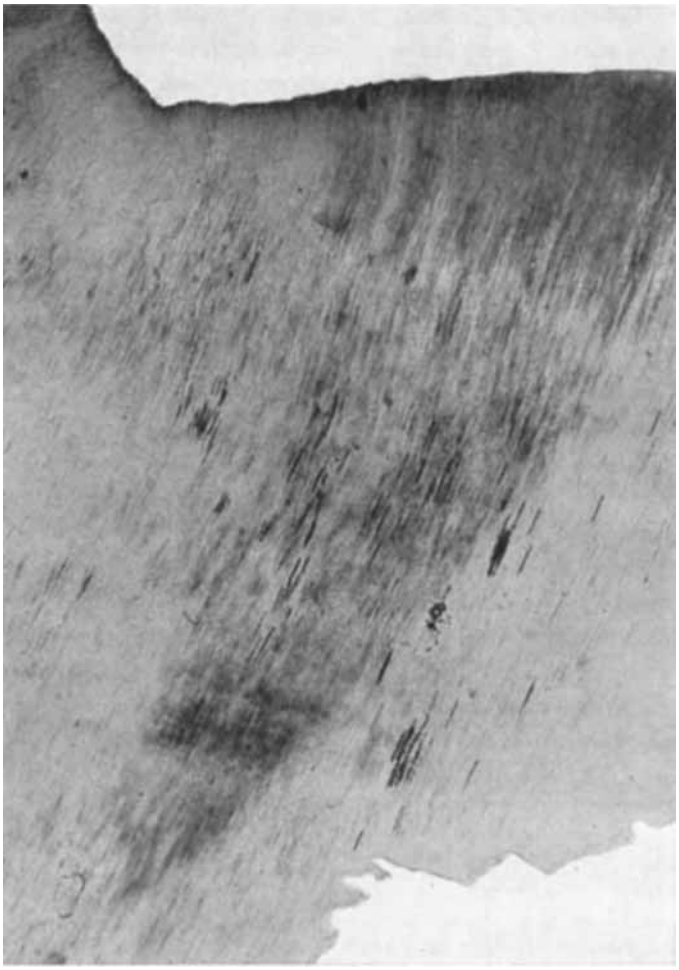

Fig. 5. A longitudinal section of human carious dentine excavated *in situ* from a human molar. The arylsulphatase activity is demonstrated as dark narrow stripes along the dentinal tubules over the whole section. In the superficial parts (top) of the carious dentine the activity is homogenous while occasional very strong (black) activity can be distinguished in the deeper part of the lesion. Substrate: 6-bromo-2-naphthylsulphate. Incubation time: 5 hours. (×40).

6-bromo-2-naphthylsulphate. No enzyme activity could be observed in the histologically unaffected dentine. Thus it seems to be in connection with the caries process, as was the situation with arylaminopeptidases (*Larmas et al.*, 1968). The right to use the term unaffected dentine has been discussed earlier (*Larmas et al.*, 1968).

Earlier histochemical studies on carious dentine (*Takuma et al.*, 1967) have shown that the peritubular matrix in dentine reduces its acid mucopolysaccharide reactions during the carious process. *Toto* (1966) has proposed that the softening of dentine in caries may be caused by a loss of the mineral binding acid mucopolysaccharides. This may be interpreted as a result of an enzyme hydrolysis of bacterial origin acting on sulphate groups in dentine. The role of the described arylsulphatase activity in this hydrolysis remains speculative. It must be born in mind, however, that the histochemical method for arylsulphatase activity does not give distinct localization and weak activity may disappear by diffunsion, because of the post-coupling azo dye technique.

It is of interest that phagocytic cells, which presumably include osteoclasts, show considerably acid phosphatase activity. On the other hand, micro-organisms in dental plaque may produce acid phosphatases (*Mäkinen*, 1966). Such activity, probably of microbiological origin, also occurs in carious dentine. This indicates that pathological dissolution of calcified tissue probably needs acid phosphatase activity either from osteoclasts or micro-organisms. These enzyme activities are considered to indicate a portion of the metabolic events which occur in carious dentine (in addition to acid dissolution and proteolysis) after the formation of adherent microbial plaques on the teeth. Which processes are primary remains still speculative. The mechanisms of the enzyme action require further study.

SUMMARY

The occurrence of alkaline and acid phosphatase as well as arylsulphatase activity was studied histochemically in undecalcified frozen sections from human carious and unaffected dentine. The method was based on the use of various 2-naphthol esters of phosphoric and sulphuric acid as model substrate. Alkaline phosphatase activity was observed in the predentine layer of adult human teeth even under advanced carious lesions. No activity was normally seen in carious and unaffected dentine. When the carious process had reached the predentine, marked alkaline phosphatase activity was observed. This activity was found to be spreading from the exposure area into the carious dentine. The acid phosphatase activity was generally seen in the carious dentine along the dentinal tubules. When the carious process reached the pulp the same phenomenon could be observed as with alkaline phosphatase activity, i.e. a strong acid phosphatase activity seemingly originating

from the affectec predentine layer. Marked arylsulphatase activity was also observed in carious dentine. The activity decreased gradually when approaching the pulp. These observations support the hypothesis that the same enzyme actions are active in the dissolution of dentine as well as in the resorption of bone.

RÉSUMÉ

ETUDES HISTOCHIMIQUES SUR L'SCTIVITÉ PHOSPHATASE ET SUR L'ACTIVITÉ ARYLSULFATASE DANS LA DENTINE CARIEUSE HUMAINE

L'existence d'une activité phosphatase alcaline, d'une activité phosphatase acide ainsi que d'une activité arylsulfatase a fait l'object d'une étude histochimique sur des coupes par congélation, non décalcifiées, de dentine humaine, carieuse et saine. La méthode employée reposait sur l'utilisatior comme substrats-modèles de différents 2-naphtol-esters de l'acide phosphorique et de l'acide sulfurique. Une activité phosphatase alcaline a été observée dans la couche de prédentine de la dent humaine adulte, même dans les lésions de carie à un stade avancé. On ne constatait normalement pas d'activité dans la dentine saine ni dans la dentine cariée. Lorsque le processus de la carie avait atteint la prédentine, on observait ici une activité phosphatase alcaline marquée. Cette activité se développait à partir de la zone de la dénudation vers la dentine carieuse. L'activité phosphatase acide se recontrait généralement dans la dentine carieuse le long des canalicules dentinaires. Quand le processus de la carie atteignait la pulpe, on observait le même phénomène que pour l'activité phosphatase alcaline, c'est-à-dire une forte activité phosphatase acide semblant provenir de la couche de prédentine touchée. On a aussi observé une activité arylsulfatase marquée dans la dentine carieuse. Cette activité diminuait progressivement en s'approchant de la pulpe. Ces observations confirment l'hypothèse selon laquelle ce sont les mêmes actions enzymatiques qui entrent en jeu dans la désintégration de la dentine et dans la résorption osseuse.

ZUSAMMENFASSUNG

HISTOCHEMISCHE UNTERSUCHUNGEN ÜBER DIE PHOSPHATASE- UND ARYLSULFATASE-AKTIVITÄT IM MENSCHLICHEN KARIÖSEN DENTIN

Die Aktivität sowohl der alkalischen und sauren Phosphatase als der Arylsulfatase wurde histochemisch in nicht dekalzifizierten Gefrierschnitten untersucht, die aus menschlichen kariösen und unaffizierten Zähnen präpariert waren.

Das Verfahren erfolgte durch Anwendung verschiedener 2-Naphtol Ester der Phosphor- und Schwefelsäuren als Modellsubstrate. Alkalische Phosphatase-Aktivität wurde an der Prädentinschicht des Zahnes bei Erwachsenen auch unter einer kräftig entwickelten Karies festgestellt. Am kariösen oder gesunden Dentin wurde keine Aktivität festgestellt. Nachdem die Kariesentwicklung das Prädentin erreicht hatte, wurde am kariösen Dentin eine erhebliche alkalische Phosphatase-Aktivität beobachtet. Es wurde, für durchaus möglich gehalten, diese Aktivität habe sich aus dem Perforationsgebiet bis in das kariöse Dentin ausgedehnt. Die saure Phosphatase-Aktivität wurde am kariösen Dentin regelmässig in Dentinkanälchen festgestellt.

Als die Kariesentwicklung die Pulpa erreichte, konnte das gleiche Phänomen wahrgenommen werden wie bei der alkalischen Phosphatase, d.h. eine starke, saure Phosphatase-Aktivität, die ihren Ursprung offensichtlich von dem affizierten Prädentin hat. Eine starke Arylsulfatase-Aktivität wurde auch am kariösen Dentin beobachtet. Die Aktivität verminderte sich stufenweise in der Richtung der Pulpa. Die Beobachtungen bekräftigen die Annahme, bei der Dissolution des Dentins seien gleiche Enzymtätigkeiten aktiv, wie bei der Knochenresorbtion.

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Adress:

Institute of Dentistry,
University of Turku,
Turku 3,
Finland