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Autoradiography and Histochemistry of Mineralized Tissues by Means of the Ullberg Freeze-sectioning Technique

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ABSTRACT

The present paper is a review of some different applications of whole-body freeze-dried sections to illustrate the usefulness for studie on mineralized tissues. Thus the application of autoradiography fluorescence microscopy and enzyme histochemistry and their combinations is described.

The whole-body freeze sectioning technique was originally developed for studies on the distribution of labelled drugs in small laboratory animals (5). However it has also proven to be very useful for studies on the mineralized tissues since it allows the sectioning of all the tissues of the body, including bone and teeth without decalcification and any other tissue preparation than freezing. This is a review of some results obtained from studies on these tissues.

One of the advantages with the method is that all tissues may be analyzed without any preselection of tissues to be studied and it has lead to many unexpected results. One of the very first findings of this kind was the observation by André (1) that ^{3}H-labelled tetracycline was accumulated in the teeth and bone. Unfortunately very little attention was paid to it at that time. Not until 1962 it was reported that the teeth of children given tetracycline were markedly discolored as well as malformed (6). If Andre's unexpected observation had been more widely known many of these developmental disturbances of the teeth may have been avoided. The affinity of the tetracyclines to the mineralized tissues in combination with their property to be fluorescent in ultraviolet light, have made them useful tools for mor-

phological studies on mineralized tissues and the whole-body sections have also been used for fluorescence microscopy.

The fact that the mineralized tissues could be sectioned without preceding decalcification or any other preparatory procedure other than freezing made it possible to study the distribution of isotops with a very short half-life such as fluoride-18. By means of the whole-body technique Eriksson and Ullberg (3) showed a very selective accumulation and retention of fluoride in the mineralized tissues. Appelgren, Eriksson and Ullberg (2) took advantage of the differences in physical half-life between fluoride-18 and calcium-45 and showed in a double isotope autoradiographic study that the accumulation of fluoride in the mineralized tissues is even more selective than that of calcium. They also noted a difference in the distribution of the two elements in the developing enamel. In a later study I found that the uptake of fluoride in the developing mainly occured during the stage of enamel matrix formation while the main uptake of calcium occurred at a later stage (4). This has been confirmed in other studies and there is still a debate about the mechanism of binding of fluoride to the developing enamel.

The usefulness of the whole-body sectioning technique is illustrated by some enzyme histochemical studies in which the tissue-specific localization of some isoenzymes is demonstrated. The classic enzyme in studies on mineralized tissues is alkaline phosphatase. In a whole-body section of a young rat alkaline phosphatase activity can be demonstrated in a number of tissues such as bone, teeth, intestinal mucosa, kidney, adrenal cortex, lung and brain. If a section is heated to 56° for 60 min. most of the alkaline phosphatase is inactivated except that in intestinal mucosa, bone and teeth.

Fig. 1 Whole-body sections of a young rat incubated for alkaline phosphatase showing all the enzyme activity (A) and that of the heat inactivated enzyme (B).

A detailed study on developing teeth of monkeys showed that the alkaline phosphtase activity in the capillary walls of the pulp was sensitive to vanadate in contrast to the activity in the activity in the cells forming the dental hard tissues.

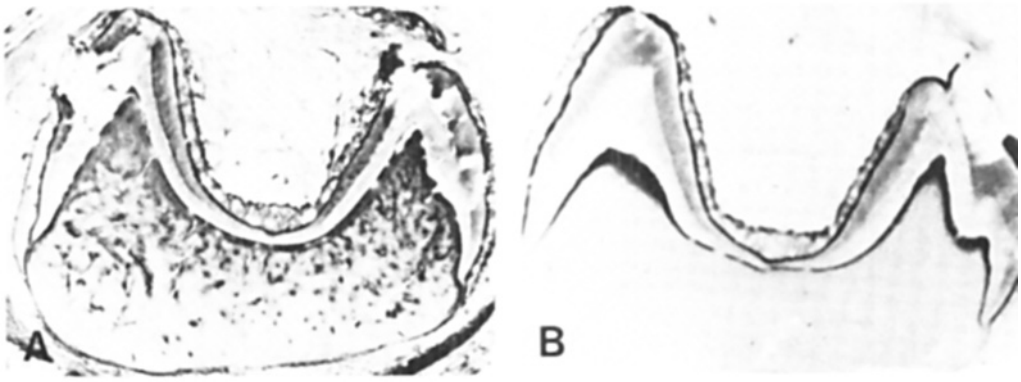

Fig. 2 Frozen sections through the first molar of a young monkey showing the activity of alkaline phosphatase (A) and that of vanadate resistant alkaline phosphatase (B). Not the inhibition of the capillary enzyme activity in the pulp.

Also the tissue specific distribution of isoenzymes of acid phosphatase can be demonstrated by means of the whole-body sections. Incubation of a whole-body section of a young rat for acid phosphatase resulted in a marked staining of a number of tissues such as bone, teeth, salivary glands, keratinized epithelium, adrenal medulla, liver, kidney and intestinal mucosa. When fluoride was added to the incubation medium most of the acid phosphatase activity in the soft tissues was almost completely inhibited, while there was still demonstrable activity in bone and teeth. However, a more detailed analysis revealed that osteoclastic acid phosphatase was inhibited while the osteoblastic activity of the enzyme was still demonstrable. Copper inhibited the activity in the osteoclasts but left the enzyme activity in most soft tissues apparently unaffected. Tartrate inhibited the activity of all the soft tissues, the osteoblasts and the cells of the dental tissues. The only activity that seemed to remain unaffected was the osteoclastic acid phosphatase. We have purified the osteoclastic acid phosphatase, characterized it and produced polyclonal antibodies against it. Immunohistochemistry with polyclonal antibodies showed that the enzyme is present in the ruffled border area of the osteoclasts.

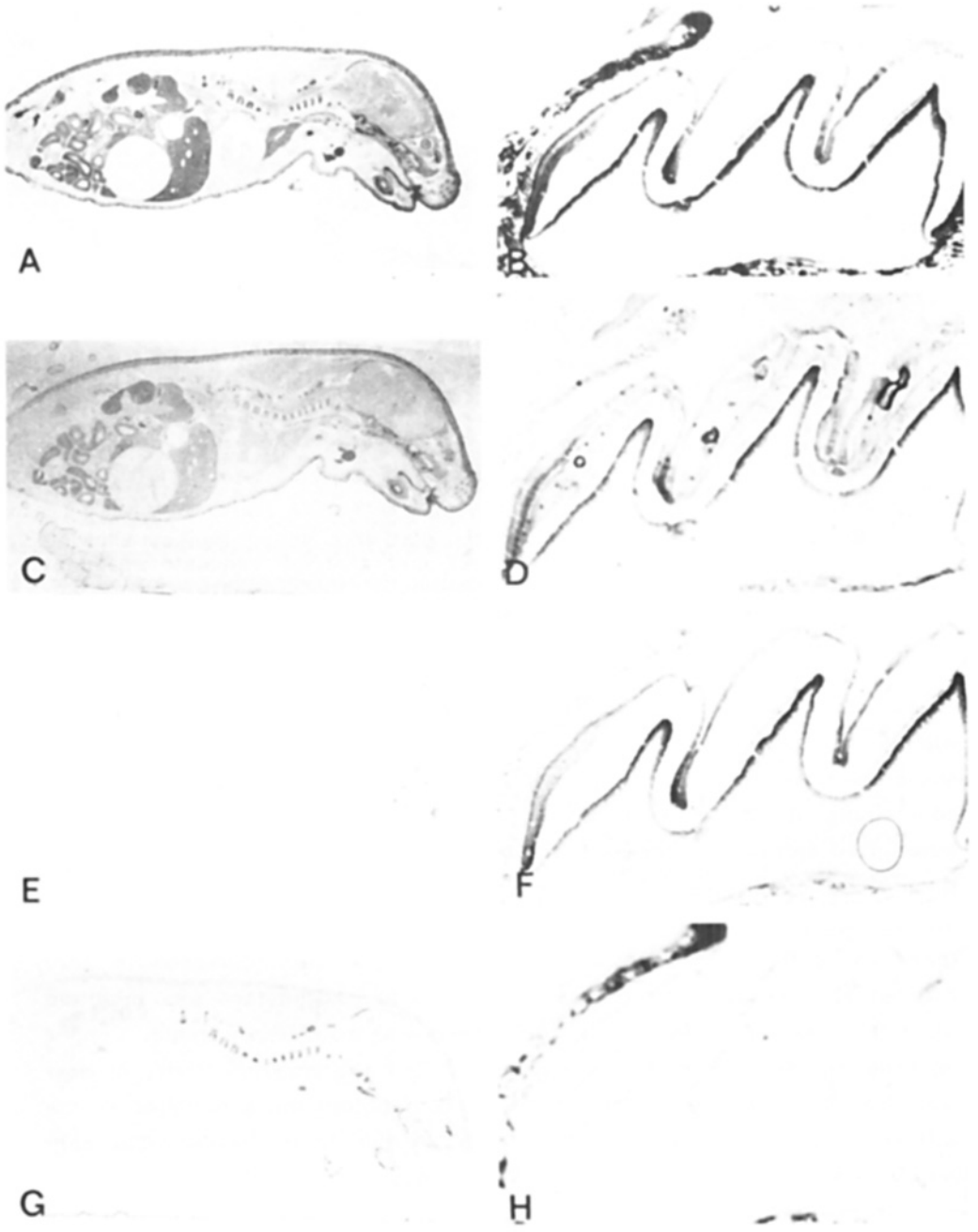

Fig. 3 The activity of acid phosphtase in young rats. In A and B the sections have been incubated without any inhibitory agent. In C and D copper (10 mM), in E and F fluoride (100 mM), in G and H tartrate (100 mM) have been added to the incubation medium. The results are described in the text.

Isoenzymes of acid phosphtase with inhibitor characteristics similar to the ones found in the cells of bone and teeth have also been found in the bacteria that are involved in the degradation dental tissues during the caries process. An interesting observation was that fluoride inhibits acid phosphtase in bacteria at the surface but not the acid phosphatase activity of the bacteria invading the dentinal tubules. There are clinical reports saying that teeth in fluoridated areas have small entrance cavities but wide cavities underneath the surface. Histochemical studies on frozen sections through dental calculus have shown that alkaline phosphatase in the bacteria located in a very similar to the formative cells of the mineralized tissues, suggesting an enzymatic process rather than a passive precipitation of mineral.

CONCLUSIONS

Enzyme histochemical methods for the demonstration of alkaline and acid phosphatase have been applied to the tape-carried freeze-dried whole-body sections. By means of inhibitors tissue specific localization of isoenzymes has been demonstrated.

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