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SUBJECT: Abstract of Final Report of Research
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This investigation was undertaken because enzymes are vitally important protein biologic materials which control biochemical reactions in the healing incisor alveolus. It was, therefore, deemed of considerable importance to investigate the histochemical distribution of alkaline phosphatase in the healing incisor alveolus and in the mandible.

It has been well established by this investigation that alkaline phosphatase is present in relatively high concentration in the healing incisor alveolus after beta-aminopropionitrile anorganic bone implants.

A chelating method has been presented which enables one to remove mineral salts from calcified tissues (teeth and bone) and still leave the alkaline phosphatase intact. By this method thin sections of bones and teeth were prepared irrespective of their initial hardness, and alkaline phosphatase was localized in sections incubated with naphthol AS-MX phosphate substrate at pH 8.3 for 24 hours. The chelating fluid (0.2M ethylenediaminetetraacetate at pH 7.0) was changed several times weekly until demineralization was complete. Reactivation of alkaline phosphatase was a necessity after chelation with EDTA.
Definitive study of alkaline phosphatase in the healing incisor alveolus of the albino rabbit after implantation of DAPH-anorganic bone chips revealed that the greatest concentration and greatest activity was limited to the osteoblasts, periosteum, endosteum, connective tissue of incisor alveolus around anorganic bone chips and in the marrow spaces of the alveolar process and mandible. Alkaline phosphatase activity was lacking in osteocytes, anorganic bone chips and the matrix of newly formed bone.

Definitive study of alkaline phosphatase in the mandible of the albino rabbit revealed that the greatest concentration and greatest activity was limited to the incisor and molar stratum intermedium, subodontoblastic layer of the incisor pulp, gingival and oral mucosal capillaries, salivary gland acini, capillaries in skeletal muscle, labial alveolar periosteum and molar pulp. Alkaline phosphatase activity was lacking in the periodontal ligament, dentin and predentin, skeletal muscle, cementum and cementoid, enamel matrix, ameloblasts, central zone of the pulp, odontoblasts, keratin and in the stratified squamous epithelium.

The most important observation resulting from this investigation is the fact that alkaline phosphatase activity was present in the connective tissue around the DAPH-anorganic bone implants and in the periosteum around newly deposited bone in the mandible. The pathogenesis or the precise role played by the DAPH-anorganic bone chips is obscure. The rate of chemical reactions in the mineralized tissues of the incisor alveolus are probably enhanced as a result of the activity of alkaline phosphatase.

**CONCLUSION**

The evidence in this investigation indicates that sharp histochemical localization of the alkaline phosphatases are significant in a study of the healing of an incisor alveolus even though there is an overall cytoplasmic activity as evident by red staining. The sharp localization indicates that the products of enzymatic hydrolysis have remained in a relatively fixed position.
Up to the present time enzyme research has been directed toward alkaline phosphatase after decalcification of mineralized tissues with various acids. As a result of this investigation, it is apparent that excellent possibilities exist for future studies of alkaline phosphatase in mineralized tissues following chelation with EDTA. Further studies dealing with alkaline phosphatase utilizing the technique described in this investigation are obviously desired. An important conclusion resulting from this investigation is that alkaline phosphatase survives chelation with ethylenediaminetetraacetate.