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LOCALIZATION OF ALKALINE PHOSPHATASE IN THE TOOTH-GERMS OF ALBINO RATS

A HISTOCHEMICAL STUDY

by

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The results reported by various authors concerning the activity and localization of alkaline phosphatase in the tooth-germ are contradictory. However, the differences in the results may largely depend on differences in the methods employed. Histochemical studies of this kind always involve a risk of error, which must be taken into account in the interpretation of the results. The most important of these sources of error are "false positive reaction" and "false localization". A false positive reaction may depend on other substances, e.g. "haemosiderin" and calcified matrix, which may give the impression of a real positive reaction in the cobalt technique; therefore, a comparison with inactivated sections must always be made. In the present paper, false localization is defined as a positive reaction appearing at sites other than the primary localization *in vivo*. False localization may depend on either a postmortal diffusion of the enzyme from its original site or a diffusion of the products of enzymic hydrolysis. Hence the fresh tissue must be fixed as soon as possible.

The author has studied the localization of alkaline phosphatase in the tooth-germs of albino rats and has attempted to take the above-mentioned factors into account.

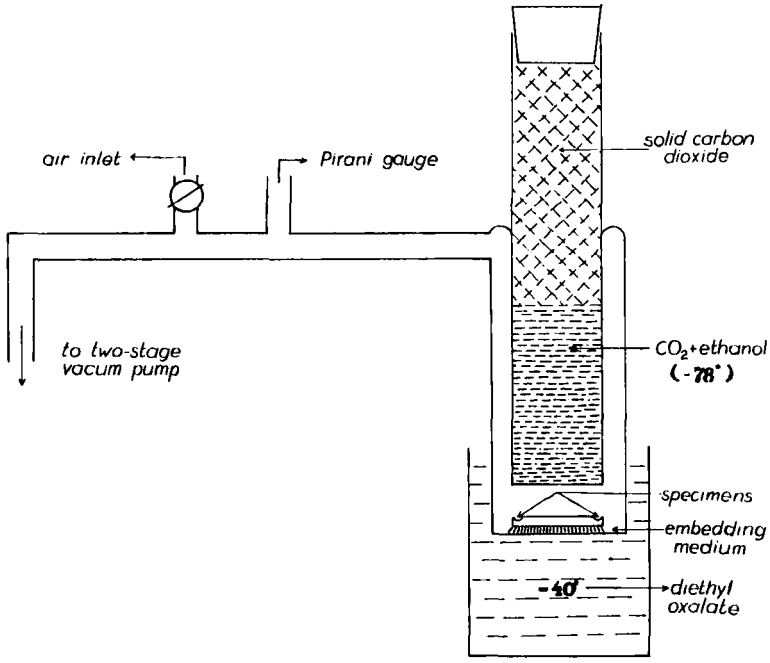

Fig. 1. Sketch of the freeze-drying apparatus used. For explanation see text.

MATERIAL AND METHODS

The material comprises 25 rats aged from a few hours to 3 days. In order to investigate the distribution of the enzyme in the enamel organ during the different phases of its development, the tooth-germs of the first, second and third molars were examined. In each rat, these tooth-germs are at different stages of differentiation, owing to the differences in time of their development.

Twenty of the animals were killed by decapitation. The molar regions of both the lower and the upper jaws were removed and embedded in paraffin after fixation in absolute acetone for 20 hours at +4° C. 5 μ thick sections were treated according to *Fredricsson*'s modification of *Gomori*'s method. The essential difference between *Fredricsson*'s method and that of *Gomori* is that in the former the fixative (acetone) is an ingredient of the dif-

ferent solutions, designed to reduce diffusion artefacts to a minimum. These specimens were compared to specimens taken from 5 other rats of the same age and treated in a freeze-drying apparatus similar to *Freed*'s. (Fig. 1). The tissues were prepared immediately after the animals had been killed, and no specimen was more than 2 mm thick. The specimens were dropped into

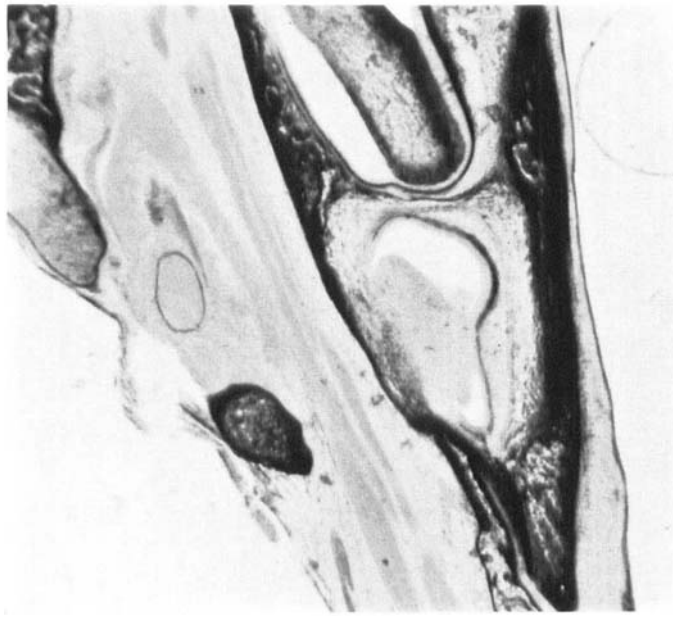

Fig. 2. Photomicrograph through the third molar of a 12 hour-old rat. Magnif. 10×3. Note the staining reaction in the stratum intermedium, and that this is gradually reduced towards the junction of the outer and inner enamel epithelium.

isopentane, which was cooled by liquid oxygen to —160° C. The tissues were dried in the freeze-drying apparatus for at least 15 hours under vacuum (10^{-3} mm Hg), at a temperature of —40° C. They were embedded in paraffin in the apparatus without breaking vacuum. After deparaffination the sections were fixed in absolute acetone and stained.

Control sections were obtained from both animal groups by inactivation of the enzyme in ultraviolet light for about one hour before incubation.

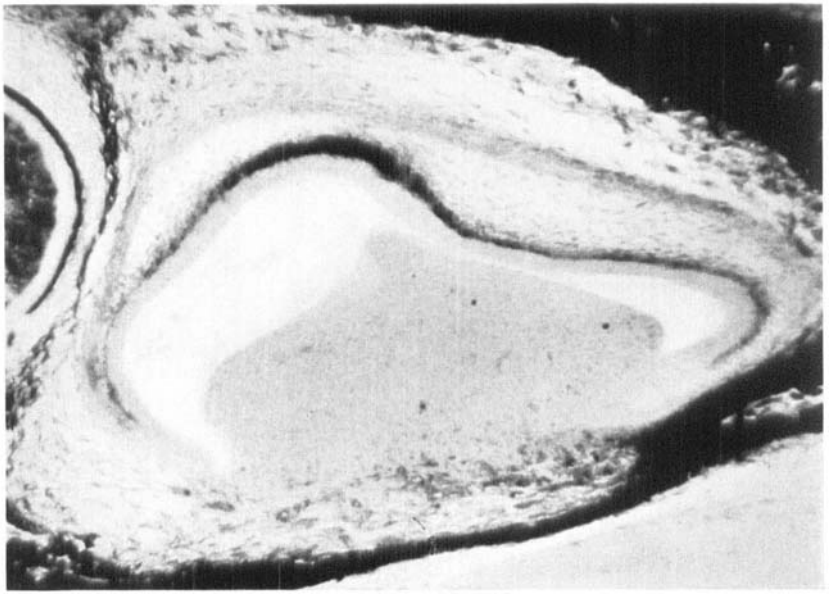

Fig. 3. Higher magnification of fig. 2. Magnif. 10 × 10.

RESULTS

A dark-brown to black precipitate was found at the site of the alkaline phosphatase activity, the activity being indicated by the intensity of the black colour in the preparation. Artefacts were found in the form of spaces due to the fixing medium, acetone. In the freeze-dried sections, artefacts were also found, owing to the formation of tissue-disrupting ice crystals.

Before the differentiation of the odontoblasts takes place, an activity occurs in the enamel organ. This activity is represented by a strong staining reaction that is strictly localized to the *stratum intermedium*. The staining is, however, gradually reduced towards the junction of the outer and inner enamel epithelium, i. e. in the proliferating parts of the enamel epithelium. No alkaline phosphatase is present in the ameloblasts, the *stratum reticulare*, the outer enamel epithelium, or the mesenchymal papilla.

When the differentiation of the odontoblasts has started, there is only slight enzyme activity in these cells and in the cell-layers adjacent to the odontoblasts in the mesenchymal papilla. The

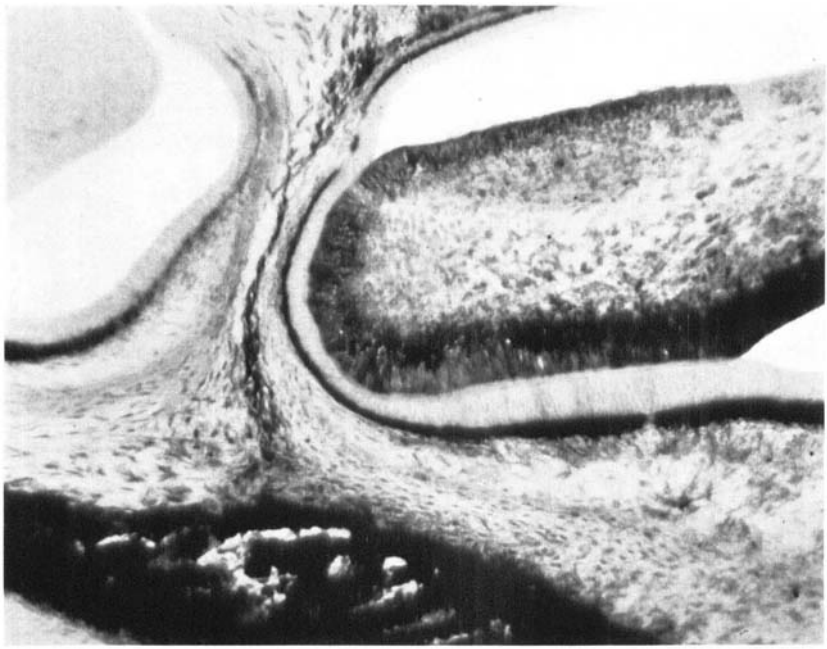

Fig. 4. Photomicrograph through the second molar of a 12 hour-old rat. Magnif. 10 × 10. Note the distinct localization of the activity in the stratum intermedium and the staining reaction in the outer cell-layers of the mesenchymal papilla.

phosphatase content is increased in the *stratum intermedium* and presents the same distinct localization as mentioned above. The ameloblasts show no activity whatever. The outer epithelium and the *stratum recticulare* show very weak activity or none at all.

At the beginning of the formation of the dentine, the activity of the enzyme in the odontoblasts is great. When a thin layer of dentine (the mantle predentine) has been calcified *the greatest activity shifts, however, to the layers of cells in the pulp just inside the odontoblasts.* The distribution of alkaline phosphatase in the *stratum intermedium* is still as distinct as before, and the greatest activity of the enamel organ is found here. The ameloblasts are now only slightly stained, and the staining is localized chiefly to the nuclear membrane. The *stratum reticulare* and the outer enamel epithelium have only a very slight positive reaction.

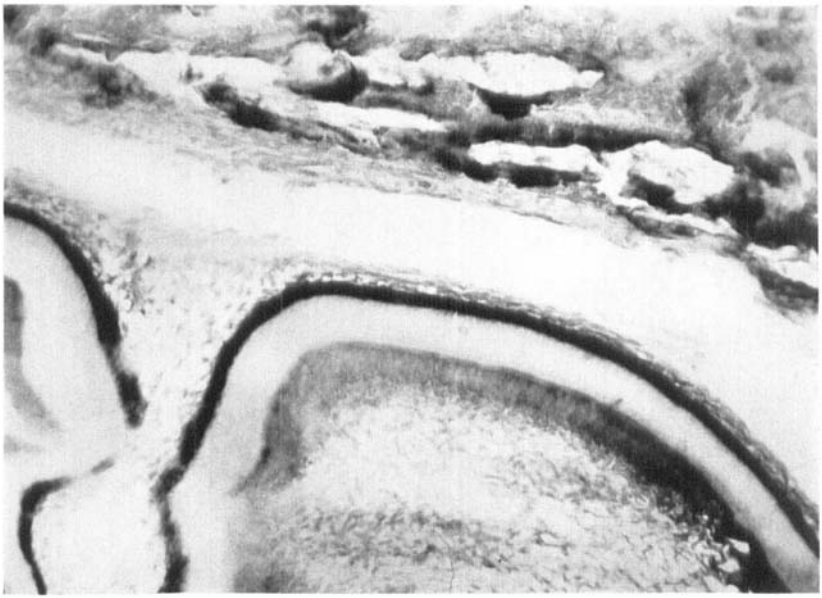

Fig. 5. Photomicrograph through a freeze-dried second molar of a 24 hour-old rat. Magnif. 10×10. Note the activity distinctly localized to the stratum intermedium and the activity in the odontoblasts. Only a weak staining reaction in the cell-layers just inside the odontoblasts.

DISCUSSION

The results of the previously published investigations concerning the histochemical distribution of alkaline phosphatase during tooth development are, as mentioned above, contradictory. Several studies have established the fact that there is great activity in the *stratum intermedium* (4, 5, 12, 14, 17, 18, 19) and in the pulp (1, 5, 12, 13, 14, 15, 17, 18, 19). The results disagree, however, as to the reactions in the ameloblasts and the odontoblasts. A few authors (5, 11, 17) observed no activity at all in these two layers, or only a weak nuclear staining. *Sasso & Castro,* in their recently published study, are of the opinion that the positive staining found in the ameloblasts and the odontoblasts is due to a diffusion artefact, as an intense positive reaction is found in the adjacent *stratum intermedium* and Weil's zone. It seems improbable, however, that such a diffusion should have taken place,

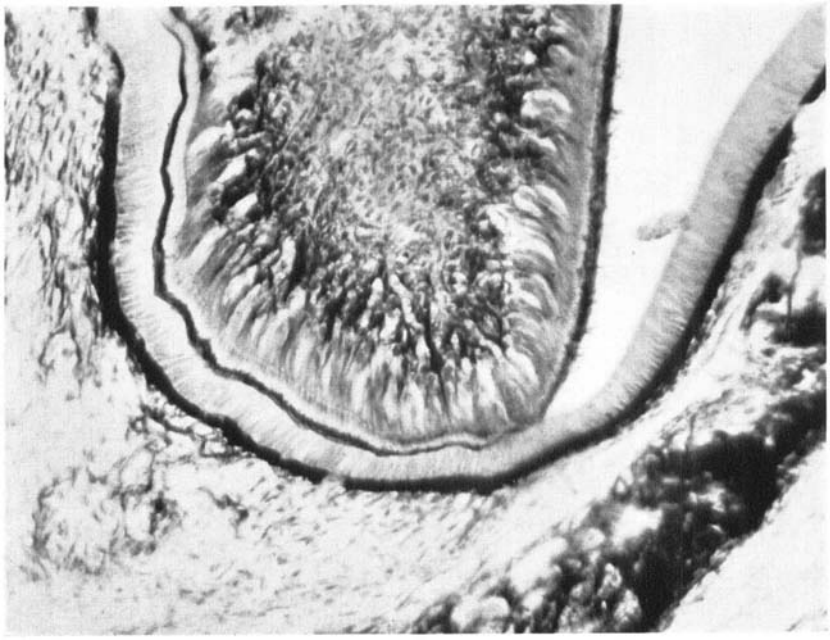

Fig. 6. Photomicrograph through the first molar of a 48 hour-old rat. Magnif. 10 × 10. Note that when a thin layer of dentine (the mantle predentine) has been mineralized the greatest activity shifts from the odontoblasts to the layers of cells in the pulp just inside the odontoblasts. The staining reaction in the stratum intermedium is still as distinct as before.

and the findings in this study of enzymatic activity in the odontoblasts agree with most of the investigations concerning the distribution of the enzyme during tooth development. One observation in the present study deserves emphasis, *viz.* the shifting of the greatest activity in the pulp from the odontoblasts to the adjacent layers when the mantle predentine has been formed and the formation of the circumpulpal predentine has started. In his survey of phosphatase and bone, *Bourne* suggested that some findings indicate that phosphatase has an influence on the production and maturation of the protein matrix in which the bone salts are received. This change in activity during the different phases of dentinogenesis may also indicate a difference in the histogenesis of these two layers of the dentine.

SUMMARY

A study of the alkaline phosphatase activity in the tooth-germ of the albino rat is presented. The distribution and activity of the enzyme in the different parts of the tooth-germ is described, and it is emphasized that the greatest activity of the pulp tissue shifts from the odontoblasts to the adjacent cell layers after the formation of the mantle predentine, when the formation of the circumpulpal predentine is beginning. This change in activity may indicate a difference in the histogenesis of these two layers of the dentine.

RÉSUMÉ

LOCALISATION DE LA PHOSPHATASE ALCALINE DANS LE GERME DENTAIRE DU RAT ALBINOS

Étude histochimique

Le présent travail concerne une étude de l'activité phosphatase alcaline dans le germe dentaire du rat albinos. L'auteur décrit la répartition et l'activité de l'enzyme dans les différentes parties du germe dentaire, et souligne que l'activité la plus grande du tissu pulpaire passe des odontoblastes à la couche de cellules adjacente après la formation de la prédentine périphérique, lorsque la formation de la prédentine circumpulpaire commence. Ce changement dans l'activité peut indiquer une différence dans l'histogenèse de ces deux couches de dentine.

ZUSAMMENFASSUNG

LOKALISATION DER ALKALISCHER PHOSPHATASE IN DEN ZAHNKEIMEN WEISSER RATTEN

In der vorliegenden Untersuchung wird die Phosphataseaktivität im Zahnkeim weisser Ratten und die Aktivität dieses Enzymes in den verschiedenen Abschnitten des Zahnkeimes beschrieben. Es wird besonders hervorgehoben, dass die ausgeprägteste Aktivität in den Mesenchymalpapillen von der Odontoblastenschicht in die dieser Schicht anliegenden Zellen übergeht, sobald das Manteldentin gebildet und das zirkumpulpale Dentin

im Entstehen begriffen ist. Eine solche Aktivitätsveränderung kann auf einen histogenetischen Unterschied zwischen diesen beiden Dentinschichten deuten.

REFERENCES

1. *Bevelander, G. & P. L. Johnson,* 1945: The histochemical localization of alkaline phosphatase in the developing tooth. J. Cell. & Comp. Physiol. *26:* 25—34.
2. —»— 1949: Alkaline phosphatase in amelogenesis. Anat. Rec. *104:* 125—134.
3. *Bourne, G. H.,* 1956: Phosphatase and Bone. The Biochemistry and Physiology of Bone. Edit. G. H. Bourne. Academic Press Inc. New York.
4. *Cederberg, I.,* 1951: The occurrence of chromotrope substances in the toothgerm of albino rats. Acta anat. *12:* 30—34.
5. *Engel, M. B. & W. Furuta,* 1942: Histochemical studies of phosphatase distribution in developing teeth of albino rat. Proc. Soc. Exper. Biol. & Med. *50:* 5—9.
6. *Freed, J. J.,* 1955: Freeze-drying technics in cytology and cytochemistry. Lab. Invest. *4:* 106—122.
7. *Fredriesson, B.,* 1952: A modification of the histochemical method for demonstration of alkaline phosphatase in which the nonspecific reactions and the diffusion phenomena are reduced. Anat. Anz. *99:* 97—106.
8. —»— 1956: The distribution of alkaline phosphatase in the rat lung. Acta anat. *26:* 246—256.
9. *Gomori, G.,* 1939: Microtechnical demonstration of phosphatase in tissue sections. Proc. Soc. Exper. Biol. & Med. *42:* 23—26.
10. —»— 1941: The distribution of phosphatase in normal organs and tissues. J. Cell. & Comp. Physiol. *17:* 71—83.
11. —»— 1943: Calcification and phosphatase. Amer. J. Path. *19:* 197—209.
12. *Greep, R. O., C. J. Fisher & A. Morse,* 1948: Alkaline phosphatase in odontogenesis and osteogenesis and its histochemical demonstration after demineralization. J. Amer. dent. Ass. *36:* 427—442.
13. *Harris, E. S.,* 1950: The sites of alkaline phosphatase in dentinogenesis. Anat. Rec. *107:* 105—120.
14. *Horowitz, N. H.,* 1942: Histochemical study of phosphatase and glycogen in fetal heads. J. dent. Res. *21:* 519—527.
15. *Johnson, P. L. & G. Bevelander,* 1954: The localization and interrelation of nucleic acids and alkaline phosphatase in the developing tooth. J. dent. Res. *33:* 128—135.
16. *Morse, A. & R. O. Greep,* 1947: Alkaline glycerophosphatase in the developing teeth of the rat: Its localization and activity characteristics as influenced by pH of the substrate and length of incubation time. Anat. Rec. *99:* 379—396.

17. *Sasso, W. S. & N. M. Castro,* 1957: Histochemical study of amelogenesis and dentinogenesis. Oral Surg. Oral Med. Oral Path. *10:* 1323—1329.
18. *Symons, N. B. B.,* 1956: Ribonucleic acid-alkaline phosphatase distribution in the developing teeth of the rat. J. Anat. *90:* 117—122.
19. *Wislocki, G. & R. F. Sognnaes,* 1950: Histochemical reactions of normal teeth. Amer. J. Anat. *87:* 239—275.

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