HISTOCHEMICAL STUDIES OF DECIDUOUS AND PERMANENT TEETH IN VARIOUS STAGES OF DEVELOPMENT

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By

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Introduction

Histochemical studies of glycogen, acid mucopolysaccharide, RNA and alkaline phosphatase in the developing teeth have been made by Engel and Furuta (6), Bevelander and Johnson (1, 2, 3, 4), Morse and Greep (11), Engel (7), Greep et al. (9), Wislocki et al. (15), Bruckner (5) and Wislocki and Sognnaes (16). However, opinions diverge among them, especially with regard to the histochemistry of ameloblasts and odontoblasts. Engel (7) reported that both the ameloblasts and odontoblasts showed no alkaline phosphatase reaction, whereas Bevelander and Johnson (1, 3) maintained that these cells had an intense reaction.

In spite of the literature mentioned above, no histochemical studies on the developing permanent teeth are treated.

In the present investigation, we have dealt with the appearance and localization of glycogen, acid mucopolysaccharide, RNA and alkaline phosphatase in the deciduous and permanent teeth in various stages of development.

Materials and Methods

As materials, 22 rats ranging from the 13th day of pregnancy to the 3rd day after birth, 7 cats from the 2nd to 14th day after birth, and 2 goat embryos measuring 90 mm in body length were used. The rats whose dentition is monophyodont were used to investigate the histochemistry of the teeth during odontogenesis. The cats whose dentition is diphyodont were employed for histochemical studies of the deciduous and permanent teeth.

Complete heads of rat embryos ranging from the 13th to 17th day of pregnancy and jaws of rats and cats after the 18th day of pregnancy were immediately fixed in 95 per cent alcohol after decapitation. To cut the teeth in a later stage of development the tissues were decalcified in an alcohol solution of pH 4.5 for 7 days. All materials were embedded in paraffin or celloidin and

sectioned 5 to 20μ thick. The stages of the developing rat teeth, from the dental lamina stage to the enamel and dentin formation stage, were decided following the description of Lefkowitz et al. (10).

The staining methods were as follows: For the demonstration of polysaccharide, the sections were stained by the PAS method. The identification of glycogen was made by means of the salivary test at 37°C in an incubator. For RNA and acid mucopolysaccharide, thionin was used. For alkaline phosphatase, Gomori's revised method was employed by using sodium glycerophosphate as a substrate. For the histological observation of the teeth, Hematoxylin-Eosin stain was employed.

Results

1. *Result obtained from the teeth in the stage of enamel organ formation*

The histochemical studies were conducted on the developing molar and incisor teeth of the rat ranging from the 13th to 21st day of pregnancy and also on those of the goat measuring 90 mm in body length. The results obtained from the rat are given in Table 1.

Table 1. Histochemical aspects of the rat teeth during odontogenesis.

Tissues		Glycogen	Acid mucopolysaccharide	RNA	Alkaline phosphatase
Oral epithelium		++	−	++ to +	−
Dental lamina		++	−	++ to +	−
Enamel organ	Outer enamel epithelium	−	−	++ to +	−
	Enamel pulp	+ to −[1) 3)], ++	− to +[1)]	++ to ±[1)]	+[*], ++[2)]
	Inner enamel epithelium	−	−	+ to ++	− to +
Odontoblasts		−	−	++	++
Dental papilla		−	− to +	+ to ±	+[*]
Periodontal tissue		++	−	+	++

Notes: 1) ····· Stellate reticulum
2) ····· Stratum intermedium
3) ····· Stellate reticulum near the outer enamel epithelium
* ····· Frequently positive reaction

Glycogen

A large amount of glycogen (Table 1) was always found in the oral epithelium of the embryos through the course of development. In the dental lamina, glycogen appeared abundantly in the surface layer of the epithelium, but not in the basal layer (Fig. 1).

The cells in the central portion of the enamel organ in the bud stage were rich in glycogen, but those in the basal layer of the organ were not, the former being destined to give rise to the enamel pulp and the latter to the enamel epithelium (Fig. 2).

In the enamel organ, a moderate amount of glycogen was found in the enamel pulp which was derived from the oral epithelium, while none was found in the outer and inner enamel epithelium enclosing the enamel pulp (Fig. 3). Glycogen was not found in the dental papilla of the early stage, but it was abundant in the periodontal tissue. When the enamel organ further developed up to its last stage, a large amount of glycogen was demonstrated in the stellate reticulum near the outer enamel epithelium, but not in the stratum intermedium.

In the highly differentiated tooth germ having a strip of predentin and well developed odontoblasts, glycogen was lacking in the dental papilla, odontoblasts, predentin and in the ameloblasts.

In the goat tooth germ, the stage of which was of enamel organ formation, a large amount of glycogen appeared in the oral epithelium, dental lamina, enamel pulp and in the periodontal tissue, but not in the dental papilla and inner and outer enamel epithelium, nearly coinciding with the results of the rat tooth germs (Fig. 4).

Acid mucopolysaccharide

A small amount of acid mucopolysaccharide (Table 1) appeared in the stellate reticulum of the enamel organ and in the dental papilla of differentiated tooth germ, though none appeared in other tissues.

RNA

At the early stage of odontogenesis, RNA was abundantly found in the oral epithelium, dental lamina, outer enamel epithelium and in the enamel pulp, and moderately in the dental papilla. With the developmental progress of the enamel organ, the RNA decreased gradually and finally only a slight amount of it was found at the stage of highly differentiated tooth germ. The RNA was found moderately in the inner enamel epithelium at the early stage of odontogenesis. It increased with the odontogenic process and finally became abundant in the ameloblasts, the well developed inner enamel epithelial cells. A large amount of RNA was also found in the odontoblasts (Fig. 5).

Alkaline phosphatase

An intense alkaline phosphatase reaction (Table 1) was found in the stratum intermedium, ameloblasts, odontoblasts and in the periodontal tissue. Weak reaction was frequently found in the enamel pulp and in the dental papilla. No alkaline phosphatase reaction was always found in the other tissues.

In the goat enamel organ, RNA was abundantly found in the oral epithelium, dental lamina, outer and inner enamel epithelium, enamel pulp, and

moderately in the dental papilla. Moderate alkaline phosphatase reaction was found in the enamel pulp and an intense reaction in the periodontal tissue. These results nearly coincided with those of the rat tooth germ.

Bevelander and Johnson (2) studied histochemically the localization of glycogen in the developing teeth of the pig and reported that glycogen was abundant in the oral epithelium, dental lamina, enamel epithelium and in the enamel pulp, but none was found in the dental papilla or in the odontoblasts. Engel (7) reported that in the albino rat teeth, glycogen was found in the oral epithelium, dental lamina and frequently in the outer enamel epithelium, stellate reticulum, stratum intermedium and in the dental papilla of the molar. In the early stage of odontogenesis, it appeared in the ameloblasts and in the odontoblasts.

In the present investigation, glycogen was found in the oral epithelium, dental lamina, and in the enamel pulp, agreeing with the reports of Bevelander and Johnson and of Engel. While glycogen was not found in the outer and inner enamel epithelium as well as in the ameloblasts, disagreeing with the reports of Bevelander and Johnson and of Engel. No glycogen was found in the odontoblasts or in the dental papilla, thus agreeing with the report of Bevelander and Johnson, but disagreeing with that of Engel.

Engel and Furuta (6) studied the distribution of alkaline phosphatase in the developing teeth of albino rats and mentioned that alkaline phosphatase reaction was demonstrated in the outer enamel epithelium, stellate reticulum, stratum intermedium and in the dental papilla, but not in the ameloblasts and odontoblasts. Bevelander and Johnson (1, 3), using the pig, reported that alkaline phosphatase reaction was shown in the dental lamina, stratum intermedium, stellate reticulum, ameloblasts and in the odontoblasts.

In the present investigation, alkaline phosphatase reaction was always found in the stratum intermedium, ameloblasts, odontoblasts, frequently in the enamel pulp and in the dental papilla, thus nearly agreeing with the reports of Engel and Furuta and of Bevelander and Johnson, except for the ameloblasts and odontoblasts. As just stated, the presence of alkaline phosphatase in the ameloblasts and odontoblasts was demonstrated by Bevelander and Johnson, but not by Engel and Furuta. The present result coincided with that of Bevelander and Johnson.

2. *Result obtained from the teeth in the stage of calcification*

The materials used in this investigation consisted of cats ranging from the 2nd to 14th day after birth and rats ranging from the 1st to 3rd day after birth.

For histochemical studies of the teeth, different kinds of decalcifying solution have been used by many investigators (5, 8). Therefore, as the first

step of this investigation, we have preliminarily selected the most suitable decalcifying solution for the detection of histochemical substances. The results are given in Table 2.

Table 2. Appearance of histochemical substances in tissues after decalcification.

Decalcifying solutions	Composition of decalcifying soln.	Solvent of decalcifying soln.	pH	Glycogen (Liver)	Acid mucopolysaccharide (Cartilage)	RNA (Liver)	Alkaline phosphatase (Duodenum)
Without decalcifying solution (Control)				++++	++++	++++	++++
Bruckner's solution(5)	Acetic acid Sodium acetate Zinc sulfate	Dist. water	4.5	+	++++	++++	+
Modified Bruckner's solution	Same as the above	70% alcohol	4.5	++++	++++	++++	++++
Greep's solution(8)	Acetic acid Formic acid	Dist. water	4.9	+	++++	++++	+
Modified Greep's solution	Same as the above	70% alcohol	4.9	++++	++++	++++	++++
Nitric acid-Formalin solution				−	+	+	−
Nitric acid-Alcohol solution				+	+	+	−

Note : All tissues were fixed in 95 per cent alcohol and put in the solution for 7 days.

As shown in Table 2, both the modified Bruckner's solution and the modified Greep's solution proved highly satisfactory for the detection of glycogen and of alkaline phosphatase. All solutions, with the exception of the nitric acid-formalin solution and the nitric acid-alcohol solution, were suitable for the demonstration of RNA and of acid mucopolysaccharide. Therefore, as the decalcifying solution for the detection of acid mucopolysaccharide and RNA, Bruckner's solution was utilized, while, for detection of glycogen and alkaline phosphatase, modified Bruckner's solution was employed.

The results obtained from the teeth in the stage of calcification are given in Table 3.

Table 3. Histochemical aspects of cat teeth in the stage of calcification.

Tissues	Glycogen	Acid mucopolysaccharide	RNA	Alkaline phosphatase
Ameloblasts	−	−	++++	++++
Enamel	−	++++	−	++++
Dentin	−	++++	−	++++
Odontoblasts	−	−	++++	++++
Dental pulp	−	+	−	++++

Glycogen

At the stage of calcification (Table 3), no glycogen was found in the tooth germ. PAS reactive glycoprotein was abundantly found in the enamel, dentin and in the dental pulp.

Acid mucopolysaccharide

A large amount of acid mucopolysaccharide appeared in the interprismatic substance of enamel and in the Neumann's sheath of dentin. A small amount of it was also found in the ground substance of dental pulp (Fig. 6).

RNA

A large amount of RNA was found in the ameloblasts and in the odontoblasts (Table 3), but not in the enamel and in the dentin. A small amount of it was also demonstrated in the dental pulp.

Alkaline phosphatase

The result obtained from the teeth in the early stage of development has been already described in section 1, showing the presence of alkaline phosphatase in the odontoblasts and ameloblasts. At the stage of calcification, a similar result was obtained in the ameloblasts and in the odontoblasts. An intense reaction also occurred in the dental fiber, in the enamel prism (Fig. 7) and in the border of the dental pulp.

Wislocki et al. (15) and Wislocki and Sognnaes (16) studied histochemically the teeth of human, rhesus monkey, guinea pig and rat, and reported that acid mucopolysaccharide was demonstrable in the dental tubules (Neumann's sheath), in the interprismatic substance and in the ground substance of the dental pulp. Wislocki and Sognnaes (16) further reported that a large amount of RNA was found in the odontoblasts and in the ameloblasts.

Greep et al. (9) studied the appearance of alkaline phosphatase in the teeth of the adult rat and stated that an intense reaction was found in the dental fiber and in the pulp border. Wislocki and Sognnaes (16), who conducted the teeth of adult animals, reported that alkaline phosphatase reaction appeared in the enamel prism and in the dental pulp.

In the present investigation, acid mucopolysaccharide was found in the enamel, dentin and in the dental pulp; RNA appeared in the ameloblasts, odontoblasts and in the dental pulp; and alkaline phosphatase reaction was shown in the ameloblasts, enamel, dentin, odontoblasts and in the dental pulp. These results nearly coincided with the reports of Wislocki et al. and of Greep et al.

3. *Result obtained from the developing permanent teeth of the cat*

The developing permanent tooth germs of the incisor of the cat ranging from the 2nd to 14th day after birth, the stage of which was of enamel organ

formation, were used as materials. They were seen on the lingual side of each deciduous tooth. The appearance of glycogen, acid mucopolysaccharide, RNA and alkaline phosphatase in these tooth germs was determined histochemically by the methods as already stated. The results are given in Table 4.

Table 4. Histochemical aspects of permanent teeth of the cat in the stage of enamel organ formation.

Tissues	Glycogen	Acid mucopoly-saccharide	RNA	Alkaline phosphatase
Dental lamina	++	−	++	−
Outer enamel epithelium	−	−	++	−
Enamel pulp	+	−	+ to +	−
Inner enamel epithelium	−	−	++	−
Dental papilla	−	−	+ to +	−

Glycogen

A large amount of glycogen (Table 4) was found in the dental lamina, and a moderate amount in the enamel pulp. The outer and inner enamel epithelium and dental papilla had no glycogen.

Acid mucopolysaccharide

No acid mucopolysaccharide was found in any tissues of the tooth germ.

RNA

A large amount of RNA was shown in the dental lamina, outer enamel epithelium and in the inner enamel epithelium. A moderate amount of it was also found in both the enamel pulp and dental papilla (Fig. 6).

Alkaline phosphatase

No alkaline phosphatase reaction was found in any tissues of the tooth germ.

The distribution pattern of glycogen, acid mucopolysaccharide, RNA and of alkaline phosphatase in the enamel organ of the permanent teeth was not essentially different from that in the corresponding organ of the deciduous teeth.

Discussion

As to the role of glycogen in the developing teeth, Bevelander and Johnson (2) stated that glycogen plays a role in the development and metabolism of the tooth until calcification takes place. The present results seem to support their consideration from the fact that glycogen was demonstrated abundantly in the enamel pulp at the stage of enamel organ formation, but it disappeared at the stage of calcification.

As already mentioned, a large amount of RNA was found in both the ameloblasts and the odontoblasts, indicating a high metabolic activity during the formation of enamel and dentin. This, together with the presence of phosphatase in these cells as has been already stated, suggests that the cells are concerned with the synthesis of the protein of the tooth matrix.

Robison and Soames (13) stated that the ossification is related to the presence of phosphatase. Sylvén (14) studied the ossification of the bone and reported that the normal ossification process is characterized by a rapid disappearance of metachromatic chondroitin sulfate, concurrently with a great increase in alkaline phosphatase. Nakajima (12) suggested that during the ossification, PO_4 ion is separated from the phosphate ester in the blood and from glucose-monophosphate formed by the phosphorylase from the glycogen contained in the cartilage cells by the action of phosphatase. He considered further that the PO_4 ion thus prepared and that in the blood combine with Ca ion in the blood, resulting in the precipitation of calcium phosphate in the bone. Thus, phosphatase and polysaccharide such as glycogen and chondroitin sulfite are closely associated with the formation of the bone. If a similar relation is accepted in the formation of the tooth, it is highly probable that both the acid mucopolysaccharide and alkaline phosphatase play an important role during calcification of the tooth. Indeed, in the developing teeth of the rat, goat and cat, acid mucopolysaccharide and alkaline phosphatase always occurred together in the stellate reticulum of the enamel organ, cells of the dental pulp, enamel matrix and in the dentin matrix during the process of calcification.

Summary

The results obtained from this investigation, using the rat, cat and goat tooth as materials, are summarized as follows:

1. A large amount of glycogen was found in the oral epithelium, dental lamina and in the periodontal tissue through the course of development. A moderate amount of it was demonstrated in the enamel pulp at the stage of enamel organ formation. It disappeared at the stage of calcification.

2. A large amount of acid mucopolysaccharide was found in the interprismatic substance of enamel and in the Neumann's sheath of dentin, and a small amount in the stellate reticulum.

3. A large amount of RNA was demonstrated in the oral epithelium, dental lamina, enamel epithelium, enamel pulp, ameloblasts and in the odontoblasts; a moderate amount in the dental papilla; and a small amount in the stellate reticulum and dental pulp.

4. An intense alkaline phosphatase reaction occurred in the stratum intermedium, ameloblasts, odontoblasts, enamel prism and in the dental fiber; a

moderate reaction in the dental pulp; and a weak reaction frequently in the enamel pulp and in the dental papilla.

5. The distribution pattern of glycogen, acid mucopolysaccharide, RNA and alkaline phosphatase in the enamel organ of permanent teeth was not essentially different from that in the corresponding organ of deciduous teeth.

6. It appears probable that glycogen plays an important role in the development of the tooth at the stage prior to the calcification, and that acid mucopolysaccharide and alkaline phosphatase in the calcification process.

7. RNA in the ameloblasts and odontoblasts indicates a high metabolic activity during the formation of enamel and dentin.

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Explanation of Figures

Fig. 1. Dental lamina from an incisor of a rat of the 14th day of pregnancy. PAS stain. ×100.
A large amount of glycogen is seen in the surface layer of the dental lamina.

Fig. 2. Enamel organ in the early stage of development from a molar of a rat of the 16th day of pregnancy. PAS stain. ×100.
A large amount of glycogen is seen in the enamel pulp, but not in the basal layer.

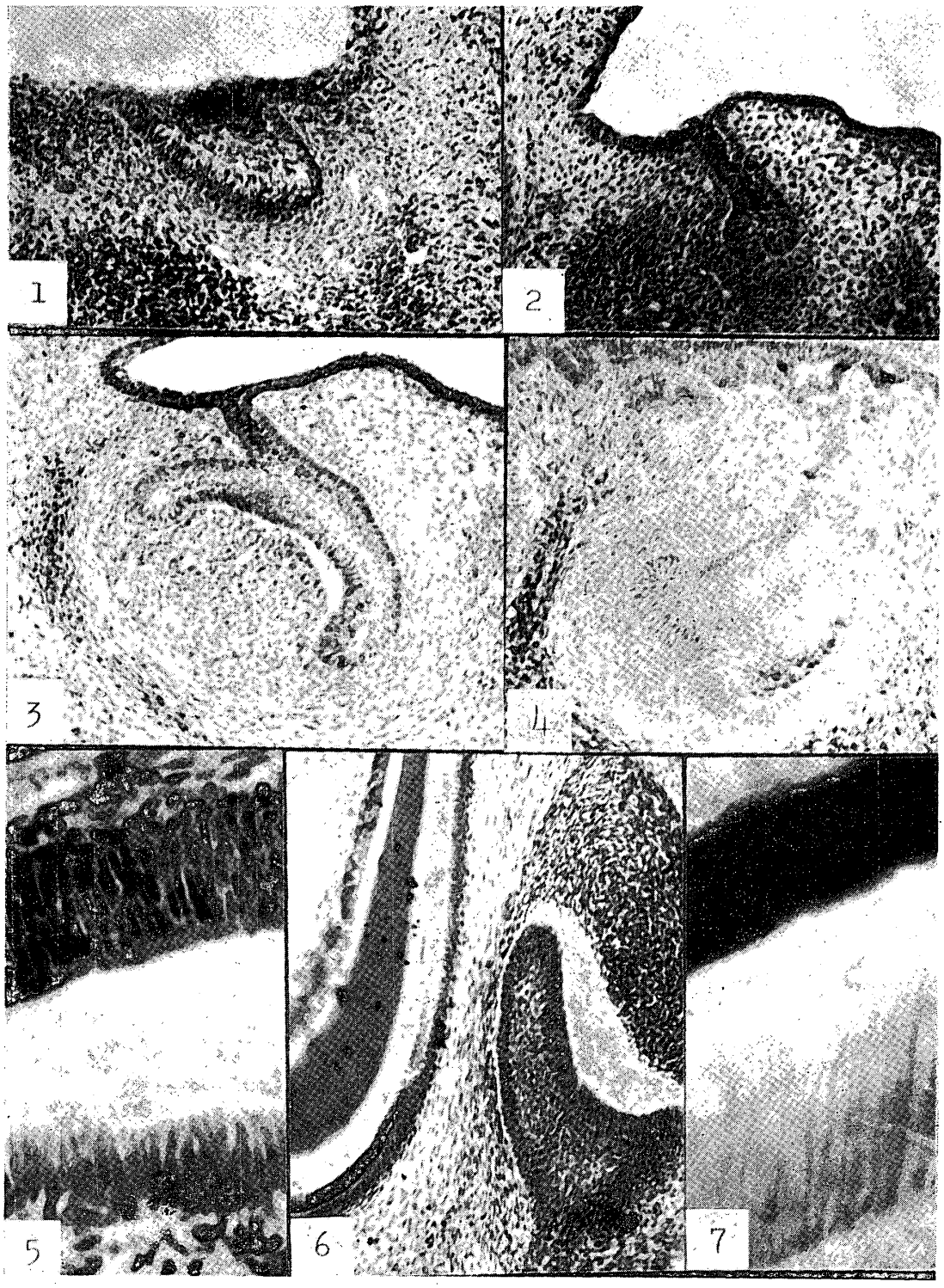
Fig. 3. Enamel organ from a molar of a rat of the 17th day of pregnancy. PAS stain. ×100.
A moderate amount of glycogen is found in the enamel pulp and a large amount in the periodontal tissue.

Fig. 4. Enamel organ from an incisor of a goat of 90mm in body length. PAS stain. ×100.
A large amount of glycogen is found in the enamel pulp and in the periodontal tissue.

Fig. 5. Ameloblasts and odontoblasts from a molar of a rat of the 20th day of pregnancy. Thionin. ×400.
A large amount of RNA is shown in the ameloblasts and in the odontoblasts.

Fig. 6. Deciduous and permanent tooth from an incisor of a cat of the 3rd day after birth. Thionin stain. ×100.
Acid mucopolysaccharide is seen in the enamel and in the dentin of deciduous tooth. A large amount of RNA is shown in the ameloblasts and in the odontoblasts of a deciduous tooth. A large amount of RNA is also seen in the enamel epithelium and a moderate amount in the enamel pulp and dental papilla of the permanent tooth.

Fig. 7. Deciduous tooth from an incisor of a cat of the 3rd day after birth. Gomori's alkaline phosphatase stain. ×400.
An intense alkaline phosphatase reaction is seen in the dental fiber and in the enamel prism.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