rats and the liver hemorrhage noted by Weichselbaum in rats with cystine deficiency[13] even though we have not observed the clinical symptoms which he described.

The observations recorded here do not indicate whether or not it is possible to vary the incidence of the hemorrhage and the incidence of the necrosis independently of each other.

The conclusions which seem to be justified by these experimental results are that *under these experimental conditions* (1) choline (and methionine, a choline precursor) have a preventive action on the development of this hepatic cirrhosis and (2) the sulfur-containing amino acids, cystine and methionine, have a preventive action on the development of the hepatic hemorrhage and necrosis. This suggests that the cirrhosis and the hemorrhage and necrosis are separate and distinct entities.

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Histochemical Studies of Phosphatase Distribution in Developing Teeth of Albino Rat.*

MILTON B. ENGEL AND WILLIAM FURUTA. (Introduced by I. Schour.)

From the Departments of Orthodontia and Histology, College of Dentistry, University of Illinois, Chicago.

The presence of phosphatase in teeth was first demonstrated by Robison and Soames[1] and confirmed by other studies made on extracts of the dental tissues.[2-7] It has been shown grossly that enzyme

[13] Weichselbaum, T. D., *Quart. J. Exp. Physiol.*, 1935, **25**, 363.

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[6] Shafer, Z. M., *Northwestern U. Bull. of Dent. Res., Grad. Study Quart.*, 1937, **37**, 26.

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activity is higher in young growing teeth than in calcified adult teeth.[2, 7]

Gomori[8, 9] and Takamatsu[10] developed a technic for the histologic localization of phosphatase and reported on its distribution in various tissues. An illustration of a tooth germ of a mouse embryo showing widespread enzyme activity with no apparent specific localization was included in a study of normal and neoplastic tissues published by Kabat and Furth.[11]

The purpose of this paper is to report the results of qualitative histochemical studies made on the phosphatase distribution in developing teeth of young rats.

Material and Method. The studies were conducted on the developing molar and incisor teeth of 12 young white rats, one and 4 days old (Wistar stock). Immediately after the animals were killed the heads were halved in the mid-sagittal plane and fixed in 95% alcohol. The tissue was then dehydrated without decalcification, embedded in celloidin and sectioned at 12 to 14 μ. The sections were first stained for calcium and then incubated in a solution of calcium nitrate, β sodium glycerol phosphate and sodium barbital, following the technic of Gomori.[8] The tissues were then stained for precipitated phosphate, with the same technic and other modifications. A few sections were stained with hematoxylin and eosin after sectioning.

Controls were also studied by treating the dental tissues as follows: (1) Heating to 70°C for 15 minutes before incubating, to destroy

TABLE I.
Distribution of Phosphatase in Developing Tooth Germs of 1- and 4-day-old Rats.

	1 day			4 days		
	Incisor	First Molar	Second Molar	Incisor	First Molar	Second Molar
Enamel Organ						
Ameloblasts	—	—	—	—	—	—
Stratum Int.	+	+	—	+	+	+
Outer layers	+	—	—	+	Stellate reticulum + in some areas	—
Undifferentiated portion (odontogenic organ)	—	—	—	—	—	—
Pulp						
Odontoblasts	—	—	—	—	—	—
Tissue subjacent to odontoblasts	+	—	—	+	+	—

8 Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 23.

9 Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

10 Takamatsu, H., *Trans. Jap. Path. Soc.*, 1939, **29**, 492.

11 Kabat, E. A., and Furth, L., *Am. J. Path.*, 1941, **17**, 303.

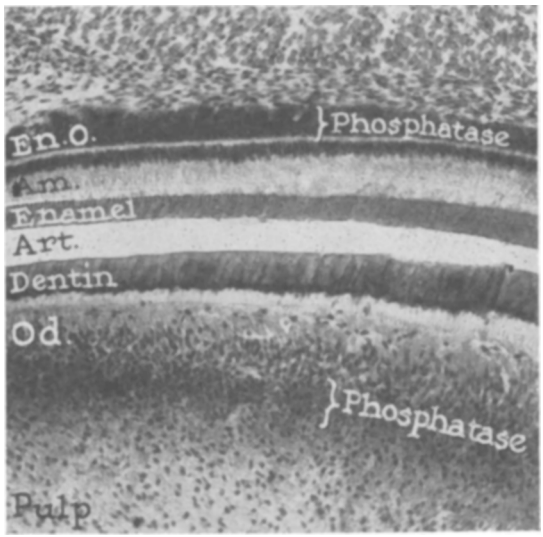

FIG. 1.

Photomicrograph of a longitudinal section from the mid-region of the labial of an incisor of a one-day-old rat. ×130. The tissue has been stained for phosphatase which appears as dark layers subjacent to the odontoblasts and ameloblasts. Am., ameloblasts; Art., artefact; En.O., fused layers of the stratum intermedium, stellate reticulum and outer enamel epithelium; Od., odontoblasts.

the enzyme; (2) incubation in distilled H_2O; (3) incubation in 2% sodium barbital, in 2% calcium nitrate, and in 2% sodium glycerol phosphate. The controls were then stained according to the Gomori technic.

Findings. All findings are summarized in the accompanying chart (Table I). All control sections were negative with respect to phosphatase.

The histology of the developing tooth germ is illustrated in Fig. 1, which shows the 4 cellular layers of the enamel organ, *i. e.,* the outer enamel epithelium and the stellate reticulum which are fused here, the stratum intermedium and the inner enamel epithelium or ameloblastic layer. The pulp with its connective tissue and odontoblastic layer is encased within the cap of calcified tissues (enamel and dentin).

One-day-old Rats. Molars. At one day the crowns of the first and second molars have attained their definitive pattern. The degree of differentiation of the ameloblasts and odontoblasts is graded from an undifferentiated cuboidal cell in the rapidly proliferating odontogenic area to a specialized columnar cell in the cusp region. The first molar is in a stage just prior to dentin formation. The phosphatase is localized in the stratum intermedium about the cusps.

There is no enzyme activity in the odontogenic area, the ameloblasts, odontoblasts or pulp cells.

Sections of the second molar show complete absence of the enzyme with the exception of a trace seen in a few cases in the stratum intermedium about the mesial cusp.

Incisors. (Fig. 1.) The gradation of cellular differentiation from basal to incisal is also noted in the incisor. At one day, calcification of the newly formed dentin is already proceeding, while the formation of the newly formed enamel has just begun. There is no evidence of phosphatase in the basal portion of the tooth (odontogenic epithelium). However, there is a high degree of enzyme activity in the stratum intermedium and in the continguous cells of the outer enamel epithelium anterior to this region. The pulpal tissue subjacent to the odontoblasts in the area of dentin calcification is a zone of high enzyme activity. The ameloblastic and odontoblastic layers are negative.†

Four-day-old Rats. Molars. Apposition of enamel and calcification of dentin are already proceeding in the first molar. There is a trace of uncalcified dentin in the second molar. This tooth is approximately at the same level of differentiation as the first molar of one-day-old rats.

The stratum intermedium about the crowns of first molars shows a high degree of enzyme activity. In addition, there is activity in some of the adjacent stellate reticulum, particularly about the cuspal-pit areas. In the pulp, those zones subjacent to the odontoblasts in the regions of dentin formation are positive.

The enzyme distribution in the second molar is similar to that found in the first molar of one-day-old rats.

Incisors. At 4 days the incisors have thicker layers of enamel and dentin. The phosphatase distribution is similar to that observed in the incisor of the one-day-old animal.

The problem of bone phosphatase was not investigated in this study. However, the connective tissue surrounding the crypt bone of the teeth showed extensive enzyme activity.

Discussion. Williams[12] expressed the belief that the stratum in-

† While the negative reaction of odontoblastic and ameloblastic layers was clearly evident, in some of the incisor sections a slight staining resembling a positive phosphatase reaction was noted. However, in view of the fact that this was found only occasionally and in a very irregular way, and never in the molars, there is good reason to believe that it was an artefact, *i.e.*, a result of a diffusion of the stain or of the overlapping of positive and negative layers rather than an indication of the actual presence of phosphatase.

12 Williams, J. L., *Dent. Cosmos*, 1896, **38**, 101, 453.

termedium played a rôle in the transfer of mineral salts from the blood to the enamel-forming cells. This study appears to support his view. The high phosphatase concentration in the stratum intermedium may accelerate the rate of hydrolysis of some tissue organic phosphates. This liberated inorganic phosphate may then be actively transported through the ameloblasts and precipitated in the enamel as the solubility product of the calcium salt (apatite) is exceeded. A similar relationship may exist between the pulpal cells and the odontoblasts. Undoubtedly the value of these findings would be enhanced if they could be supplemented by micro-incineration studies such as those of Hampp[13] in which the mineral distribution in the various layers of the tooth germ was demonstrated.

A correlation has been demonstrated between the degree of cellular organization and differentiation, and phosphatase activity in the developing tooth. This is of significance aside from its relation to the specific tissue; the tooth merely serves as an excellent test object for the study of phosphatase relations to the calcification process because in its development the calcification stages are sharply partitioned.

This absence of the enzyme from undifferentiated areas when calcification is not occurring and its appearance at levels of higher differentiation where calcification is about to occur (enamel) or is proceeding (dentin) would seem to emphasize the rôle of phosphatase in calcification.

Summary. 1. Phosphatase activity and localization in developing tooth germs of rats (1 and 4 days old) is correlated with the degree of cellular differentiation and the status of the calcification process. 2. The enzyme is shown to be localized (a) in the stratum intermedium, (b) in the outer enamel epithelium of the incisors, (c) to a lesser extent in the stellate reticulum about the first molar in the more advanced stages (4 days old), and (d) in the pulp contiguous to those odontoblasts in the regions of dentin calcification. 3. No phosphatase activity was demonstrated in the ameloblasts or odontoblasts. 4. The connective tissue surrounding the crypt bone showed extensive phosphatase activity.

[13] Hampp, E. G., *Anat. Rec.*, 1940, **77**, 273.