Histochemical Demonstration of Triphosphopyridine Nucleotide Diaphorase Activity in the Developing Teeth of the Rat.*

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Since triphosphopyridine nucleotide (TPN) was introduced for the first time by WARBURG and his co-workers (1935) in the liver and other organs, it has been clarified that this enzyme dominates as a co-enzyme of various dehydrogenase. FARBER, STERNBERG and DUNLAP (1955) reported that the histochemical method of succinic dehydrogenase was utilized for a demonstration of TPN diaphorase by employing the tetrazolium salts as an indicator.

In the additional studies, the histochemical demonstration of TPN and DPN diaphorase is made by the use of the modification techniques of FARBER, STERNBERG and DUNLAP (1956). TSOU, CHENG, NACHLAS and SELIGMAN (1956) discovered a new reagent of 2, 2'-di-(p-nitrophenyl)-5, 5'-dimethyl-3, 3'- (3, 3'-dimethyl-diphenylen)ditetrazolium chloride (Nitro BT) showing a desirable result by employing the histochemical indicator of dehydrogenase, and NACHLAS, WALKER and SELIGMAN reported concerning the localization of TPN diaphorase in the stomach, pancreas and kidney of the rats by using this new tetrazolium salt.

The relations between the localization of this enzyme and that of each alkaline phosphatase, acid phosphatase, esterase and β-glucuronidase were reviewed at the present study.

I. Materials and Methods.

The materials were the upper and lower jaws in 15—20 days fetuses and 2—5 days old after birth of healthy Wister strain rats.

The animals were sacrificed by decapitation, followed by prompt excision of the jaw. Fresh frozen sections, 10—20μ thick, were cut in the cryostat using a sliding microtome. Sections were mounted on slides and allowed to dry at room temperature. The Nitro BT method which was reported by NACHLAS et al. was employed. The substrates were as follows: 5 mg TPN (triphosphopyridine nucleotide) + 4 ml sodium DL-isocitrate (0.1 M) + 2 ml manganese chloride (0.005 M) + 10 mg Nitro BT (Nitro blue tetrazolium) + 10 ml veronal buffer pH 7.4 (0.05 M). The incubation time was


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for 30 minutes at 37°C.

Following incubated sections were rinsed briefly in distilled water, fixed in 10% neutral formalin for over two hours, and then mounted in balsam after dehydration.

II. Results.

The activities of triphosphopyridine nucleotide diaphorase (TPN-diaphorase) were as follows: A moderate or slight activity was present in the undifferentiated odontoblasts and ameloblasts, and a most intense activity in the complete differentiated odontoblasts and ameloblasts was observed especially in the part of odontogenesis and amelogenesis. They situated on the labial site of the incisor which belonged to 20 days fetus and 5 days old of the rats.

On the other hand, the enzyme activities were found in the portion of cervical loop and the immaturesd cells in the odontoblasts and ameloblasts. However, in the differentiated ameloblasts and odontoblasts, the enzyme reaction decreased gradually.

The basal portion and the adjacent stratum intermedium of matrix forming ameloblasts showed a high reaction and the intracellular localization of TPN diaphorase was present. In the ameloblasts, a finely granular material was stained in color of intense blue-violet. The basal portion in these ameloblasts was the most stainable region in the developing teeth. No the predentin and matrix forming enamel contained the stainable substances. The dental pulp and outer enamel epithelium were stained lesser than the ameloblasts. Most of those cells contained the very fine granules.

In generally, the distribution and localization of TPN diaphorase activity in the developing teeth and bone of the rat were the same as that of β-glucuronidase as mentioned at previous reports.

III. Discussion.

It is described that triphosphopyridine nucleotide (TPN) affects as a co-enzyme, when glucose-6-phosphate is dehydrated and it becomes to gluconic acid 6-phosphate. This co-enzyme was purified from cows and pigs by PAGE et al. (1949). Histochemical demonstration of TPN diaphorase was first introduced by FARBER, DUNLAP and STERNBERG (1955). They used 2, 2' 5, 5'-tetraphenyl (3, 3'-dimethoxy-4, 4'-biphenylen)ditetrazolium chloride (BT) as an indicator, and chiefly in the kidney tissue of the rats. FARBER, DUNLAP and STERNBERG (1956) continued the experiment on the stainability of the kidney tissue in the rat by using three different substrates such as isocitrate, glucose-6-phosphate and malate, and their results were the same.

Further more, 2, 2'-di-(p-nitrophenyl)-5, 5'-dimethoxy-3, 3'-dimethoxy-4, 4'-diphenylene) ditetrazolium chloride (Nitro BT) was synthesized by TSOU, CHENG, NACHLAS and SELIGMAN (1956). Nitro BT was more sensitive and showed better localization than that of blue tetrazolium and neotetrazolium, until hitherto used as an indicator in the histochemical demonstration of succinic dehydrogenase.

NACHLAS (1958), for a histochemical study concerning TPN diaphorase in the
stomach, pancreas and kidney of the rat by employing the Nitro BT, noted more excellent results in the localization of TPN diaphorase than conventional histochemical results. To our regret, however, it has not been clarified as yet what part TPN diaphorase plays in the metabolism of teeth and bone.

Various investigators have reported that polysaccharide, nucleic acid and alkaline phosphatase related to their part in the maturation of the undifferentiated cells in the developing teeth. At previous experiment, the correlation was found between each activity of acid phosphatase, esterase and β-glucuronidase in the odontoblasts and ameloblasts which took place in dentinogenesis and amelogenesis. This would suggest that TPN diaphorase is related to the matrix formation and calcification in the developing teeth. According to the report published by WATSON and AVERY, concerning their electron-microscopic study on the developing teeth, a number of filament substances were found in the ameloblasts and those substances

All figures show the distribution and localization of TPN diaphorase in the developing tooth and bone.

Fig. 1. A strong staining is seen in the basal portion of the differentiated odontoblasts and ameloblasts, while the pulp is slightly reacted. ×36.
A ameloblasts, DD developing dentine, EM enamel matrix, O odontoblasts, P pulp, SI stratum intermedium.

Fig. 2. Higher magnification of Fig. 1. ×90.

Fig. 1 and 2 show the distribution and localization of TPN diaphorase in the developing tooth and bone.
gradually were changed from the status of the ameloblast cytoplasm in the developing enamel. Also it was reported that they could not observed any clear cell mem-

Fig. 3. Higher magnification of Fig. 1. This figure shows the enamel matrix formation ×360

Fig. 4. Higher magnification of Fig. 1. A strong activity is observed in the basal layer of the ameloblasts and the stratum intermedium. No enzyme reaction is exhibited in the calcifying dental hard tissues. ×360,
brane in the ameloblast adjacent enamel forming areas, and similarly in the cytoplasm of ameloblasts which were separated from enamel.

Observation was made by them in detail about the relation between the odontoblast and the predentin, and it was summarized as follows;

Odontoblast was a slender cell of 70 μ except for its process and the cell was contained mitochondria, endoplasmic reticulum and terminal bar.

There was neither mitochondria nor endoplasmic reticulum at the protuberant site of the odontoblasts.

We have discussed the result in our histochemical experiments, and also the report at the electron-microscopic findings by WATSON and AVERY. The enzyme activity was most intense in the odontoblasts and ameloblasts, and it was the same as that of the area where existed of many mitochondria. It may be suggested that TPN diaphorase activity is related to the enamel and dentin formation.

IV. Summary.

We have made a histochemical study concerning the TPN diaphorase in the developing teeth and alveolar bone of the rats (15-20 days fetuses and 5 days old after birth) by adapting Nitro BT method.

TPN diaphorase in the developing teeth showed a strong activity in the differentiated odontoblasts and ameloblasts. On the other hands, a weak activity was observed in the undifferentiated odontoblasts and ameloblasts.

References.