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Histo-chemical proof of the presence of protein matter in dental enamel.

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There has been much speculation regarding the occurrence of protoplasmic matter in dental enamel but no demonstration of it, although recent histological work, by Bödecker particularly, has suggested the presence of organic structural elements. In a direct histo-chemical study, human teeth, after immersion for several days in 10 percent formalin solution, were sectioned transversely through the crowns. The sections were ground to the thickness of about 1 mm. or less; decalcified by Bödecker's process[1] in a mixture of methyl alcohol, nitric acid, and celloidin; freed from celloidin with a mixture of ethyl alcohol and ether, and kept in ethyl alcohol. Throughout this procedure the residual structure of the enamel retained the original outlines. A portion of the soft yellowish-white enamel residue, about a millimeter wide and several millimeters long was gently teased away, transferred carefully with a pipette to water, in which it was soaked for the removal of soluble extraneous matter; and then pipetted cautiously to a microscope slide, where the identity of the material was confirmed and absence of dentinal admixture established. At this stage the particle was slightly acid in reaction to litmus. The quantities of this material in dental enamel differ widely.

The fragment of soft enamel residue, which remained intact during these manipulations, was then treated on the slide with a few drops of Gies's reagent for Piotrowsky's ("biuret") test. Gradually the particle under microscopic examination acquired a lavender tinge of uneven intensity, the coloration ultimately becoming evident to the naked eye. This result was obtained as well with fully as with incompletely decalcified specimens. The Molisch test for carbohydrate, and the ammoniacal silver test for aldehydes, applied to other pieces, were negative, indicating that

[1] Bödecker: Proceedings of the New York Section of the International Association for Dental Research, and of the New York Academy of Dentistry, *Journal of Dental Research*, 1925 (in press).

the result with Gies's reagent was due neither to a reducing substance derived from the celloidin nor to retained formalin.

Since no insoluble material except a protein mass gives this color reaction, under the severely exclusive conditions of this test (including the prolonged initial treatment of the original tooth with formalin and of the sections with nitric acid, alcohol, ether, and water), the result, repeatedly obtained, presents conclusive histo-chemical evidence of the presence of protein in enamel, and of the occurrence there of true protoplasmic (organic) matter. This general finding is directly corroborated by the fact that pieces of the enamel residue, when gently warmed in Millon's reagent, give the red response that is typical of insoluble protein matter.

The enamel protein is stained deeply by trypan blue. Gies demonstrated some years ago that *while dental tissue is being produced, injected trypan blue* is absorbed into the enamel, from the circulation of dogs, and is permanently retained; but that trypan blue does not pass into the enamel *after* its construction.[2] (A dog nearly eight years old showing such retention was presented.) Since injected trypan blue, after staining the tissues, is removed from all parts of the body except dental enamel (after its passage into enamel under the special conditions just stated), it is probable that the degree of true *nutritional* transformation, as distinguished from simple osmotic change, in enamel *after its production,* is very slight if not wholly negative.

Further histo-chemical study in this relation may be expected to throw new light not only on the distribution and probable structural relationships of the protein matter in enamel, but also on the origin and prevention of dental caries.

[2] Gies: *Journal of the National (American) Dental Association,* 1918, v, 529.