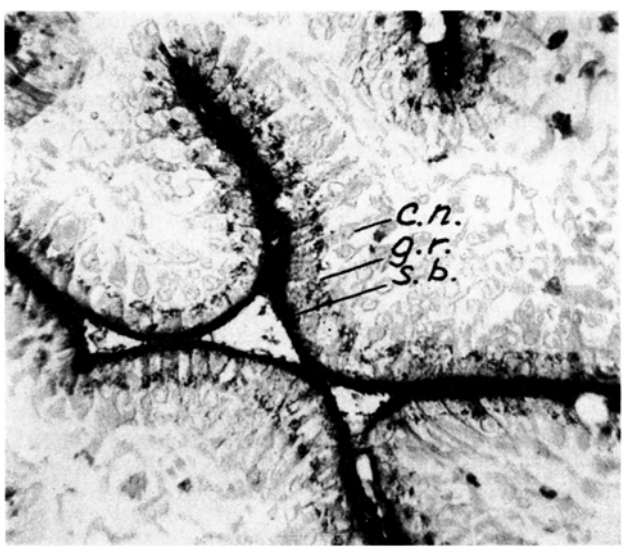
with the probable formula TiY^- and TiY, which are reduced reversibly and independantly of pH at the dropping Hg-electrode. Temporary values are given for the dissociation constants. At higher pH values, the electrode processes are irreversible and dependant on pH. From this fact, and from the decrease in wave height, the presence of complexes containing oxygen and numerous nuclei is concluded.

A Modification of the Histochemical Method for Demonstration of Alkaline Phosphatase in which the Diffusion Phenomenon is Reduced

The method for histochemical demonstration of alkaline phosphatase presented by GOMORI[1] and TAKAMATSU[2] has been the object of a number of critical studies upon which modifications have been based.

The diffusion phenomena, first demonstrated by MARTIN and JACOBY[3], are now generally considered to represent a serious objection to the method, inasmuch as the diffusion is thought to effect principally the cell nuclei which could thereby simulate phosphatase activity. Any possible original enzyme activity is thereby masked. For this reason, the question as to whether alkaline phosphatase exists at all in cell nuclei must remain unanswered. Cytochemical phosphatase determinations indicate that there is only minor activity in cell nuclei in liver, kidney, and intestine[4].



Rat intestine (duodenum). Fixation: acetone. Stained for alkaline phosphatase. Personal modification. Incubated for 1 hour. Cobalt nitrate. Note that cell nuclei (*c.n.*) are not stained. *g.r.* = Golgi region. *s.b.* = striated border. 280:1.

A number of methods for the extraction of phosphatase make use of acetone in various concentrations. The major part of the enzyme is contained in the protein fraction which precipitates in the presence of 38 to 50% acetone[5]. Therefore, the effect of acetone in the incubation mixture has been studied with regard to diffusion, following MARTIN and JACOBY[1], using superimposed sections.

The diffusion observed by MARTIN and JACOBY[1] and others has been verified. This diffusion decreases with rising concentration of acetone in the incubation mixture, ceasing entirely at a concentration of 50%. However, at that concentration, the enzyme activity has diminished considerably, in part, because of lowered solvent effect upon the substrate. The optimal results, from this point of view, were obtained with an incubation mixture containing 40% acetone concentration. Quantitative chemical controls show that this lowers the phosphatase activity by approximately 20%. Histochemical controls show that no new nonspecific reactions arise.

Preparations made in this manner differ from those described earlier, in that phosphatase activity could not be shown in cell nuclei in liver, kidney, or intestine. No other deviations from the current conception concerning the localization of alkaline phosphatase in these tissues could be demonstrated.

On the basis of the aforementioned observations, a modification of the histochemical method for determining alkaline phosphatase is recommended. This involves incubation in the presence of 40% acetone after deparaffination. Continuous controls must always be made.

The introduction of this modification lowers not only the solubility of the enzyme, but also that of calcium phosphate. In addition, the milieu in which the diffusion takes place is changed in that the protein of the tissue is further coagulated. The lack of the phosphatase reaction in the cell nuclei in the organs studied must not be interpreted as meaning that the enzyme is entirely absent there. It is possible that the nuclei contain enzyme in such low concentrations that the phosphate concentration there never becomes high enough for precipitation to occur unless phosphate or possibly enzyme diffuse from other places with higher enzyme concentration[2]. A precipitation is then obtained through additive effect, but when the diffusion has been reduced, the necessary phosphate concentration is never attained.

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Zusammenfassung

Die Diffusionsphänomene bei der histochemischen Phosphatasereaktion im alkalischen Gebiet können reduziert werden, wenn die Inkubationsmischung 40% Azeton enthält. Dabei treten keine neuen unspezifischen Reaktionen oder eine grössere Inaktivierung des Enzyms auf.

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Sur la présence de pigments jaunes non caroténoïdes chez *Mucor hiemalis*

Mucor hiemalis (Phycomycète) est un très fort producteur de caroténoïdes[1]. Comme *Phycomyces*[2], *Mucor hie-*

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