Fig. 3. The pigment epithelium of dark-adapted frog retina. When ethanol was used as substrate, formazan deposition are visible on the mitochondria and endoplasmic reticulum (arrows).

Fine Structural Localization of Phosphatase Activities in Chorio-Allantoic Placenta of Rat

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Many studies have been carried out on the function and morphology of the placenta, where active materno-fetal exchange of various substance is taking on¹⁾. The present investigation was undertaken to establish the fine structural localization of phosphatase activity in the chorio-allantoic placenta of rat in alkaline, neutral and also in acid ranges. The function of the most phasphatases is implicated in transport of material in number of tissues. The placentas of three rats were removed on each 19th and 21 st gestation day, fixed with 3-4% cold glutaraldehyde solution buffered by cacodylate (pH 7.3-7.4)²⁾ for about one hour, washed with 0.05M. cacodylate buffer containing 10% sucrose and kept in the refrigerator until use less than a week. These frozen sections (40 μ) were used for the electron microscopic studies and thin sections (10 μ) for light microscopic studies.

Histochemical reactions

1) Phosphatase reactions in neutral range: Modified Wachstein and Meisel's solutions³⁾ containing 5mg substrate, 4ml of 0.1M Tris-maleate buffer, (pH 7.2), 1ml of 0.1M MgCl₂, 0.4ml of 2% lead nitrate and 4.6ml of distilled water, were used, and ATP, AMP and TPP were used as substrate.

2) Alkaline phosphatase reactions: Cadmium method by the present authors²⁾ was applied for the demonstration of phosphatase activities in high alkaline pH.

The substrate solution contained 2ml of 5% sodium barbital, 1ml of 0.1M magnesium chloride, 0.5 ml of cadmium acetate (final concentration 5 mM), distilled water to make a final volume of 10ml, and 20mg of sod. glycerophosphate or 5mg of ATP or AMP. The pH of each solution was adjusted to pH 9.2-9.4 with NaOH or HCl.

3) Acid phosphatase reaction: Gomori's method⁴⁾ was used. The incubation was carried out at room temperature for 5-15 minutes.

In neutral and alkaline ranges, most of the phosphatase activities, survived with glutaraldehyde fixation, were observed on the luminal surface of trophoblasts, and on the plasma membranes of both two trophoblastic layers in the labyrinthine barrier. However, ATP-reaction was almost negative there at pH 9.4. The marked enzymatic activity, capable of hydrolyzing ATP, also occurred at the giant cell membranes, where the reactions with AMP and glycerophosphate as substrate were almost negative.

Thiamine pyrophosphatase activity was also revealed in Golgi apparatus, particularly in saccles. Acid phosphatase activity using glycerophosphate as substrate mainly occurred in dense bodies (lysosomes) and Golgi vesicles.

These results were discussed in reference to transport of materials through the barrier.

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Golgi Apparatus and Thiamine Pyrophosphatase (TPPase) Activity in the Rat Intestinal Epithelium

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Using Novikoff-Goldfischer method (1961), the localization of TPPase activity was studied in the epithelia of the rat jejunum, ileum and colon.

After fixation in cold 2% glutaraldehyde in 0.1M cacodylate buffer, pH7.2, for 2 hours, specimens were washed overnight in cacodylate buffer and incubated in Novikoff-Goldfischer medium for 15-30 minutes at 37°C. Following incubation, they were post-fixed in cold Caulfield solution for 1 hour, dehydrated in graded ethanols and embedded in epon.

The incubated specimens were also reacted with 1% yellow ammonium sulfide solution to be observed under the light microscope.

In order to test the specificity of the reaction, in particular to rule out the possibility of involvement of non-specific alkaline as well as acid phosphatase, inhibitors (10 mM cysteine for alkaline phosphatase and 10-25 mM sodium fluoride for acid phosphatase) were added in the medium. In some cases the substrate TPP, was replaced by thiamine monophosphate (TMP) or β -glycero-