HISTOCHEMICAL DEMONSTRATION OF THE ACTIVITY OF PHOSPHATASES BY MEANS OF LEADRHODIZONATE

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Summary. A method for the histochemical demonstration of the activity of phosphatases by means of a pinkish-red staining obtained with leadrhodizonate is described. This method was specially developed for demonstrating sites of phosphatase activity in structures and cells containing melanin. The pinkish-red reaction product contrasts well with the brown melanin pigments. After drying and mounting of the sections in malinol, the staining is colour fast.

Introduction

The presence of melanin in tissues complicates determination of the localization of enzymes by methods using the metal-ion principle in the form of cobalt- or leadsulfide because of inadequate contrast between the colour of the melanin pigments and that of the two sulfides. For the enzymes alkaline and acid phosphatase this difficulty can be solved by the use of an azo-technique to reveal the localization of the enzymes. The resulting red or blue reaction products give good contrast with the brown melanin pigments. These azo-techniques, however, are not applicable for other phosphatases and another method is required to enable their demonstration of localization when melanin is present.

Melanin can be bleached by exposure to 3% hydrogen peroxide for 24 hours, but because of inactivation this pretreatment of sections is not suitable for the enzymes adenosinetriphosphatase, 5-nucleotidase, thiaminpyrophosphatase and glucose-6-phosphatase. The same treatment applied after staining is also unsatisfactory because the hydrogen peroxide oxidizes the sulfide present in the sections, producing sulfate. No other means of eliminating the brown colour of the melanin without interfering with the activity of the enzymes is known.

The present communication describes a simple technique for obtaining pinkish-red deposits of leadrhodizonate at the sites of activity of various phosphatases, making it possible to demonstrate their presence and localization. The pinkish-red colour of the leadrhodizonate contrasts very well with the brown colour of the melanin pigments.

Material and Methods

Small blocks of tissue containing melanin were rapidly frozen in isopentane cooled to \(-150^\circ\)C with liquid nitrogen. Frozen sections were cut at 7 \(\mu\) and incubated in a substrate solution as used for adenosinetriphosphatase, 5-nucleotidase, thiaminpyrophosphatase and glucose-6-phosphatase. The same treatment applied after staining is also unsatisfactory because the hydrogen peroxide oxidizes the sulfide present in the sections, producing sulfate. No other means of eliminating the brown colour of the melanin without interfering with the activity of the enzymes is known.

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After incubation the sections were washed for 3 min with distilled water. Experimental sections were then placed for 1 min in a rhodizonate solution containing 30 mg disodiumrhodizonate \(Na_2C_3O_6\) in 40 ml tartrate buffer (pH 3.0). The tartrate buffer contained 1.5 g sodiumhydrogentartrate and 1.2 g tartaric acid per 100 ml. This buffer can be held indefinitely. The rhodizonate solution must be made up just before use. The sections were then washed...
again for 3 min in distilled water. Lastly, the sections were dehydrated in alcohol, cleared in xylene and mounted in malinol. Sections stained with leadrhodizonate should be stored in the dark.

Results and Discussion

Rhodizonic acid in combination with certain uni- and divalent metal-ions gives intensely-coloured inner-complex salts which are only very slightly soluble. It is for this reason applied in analytical chemistry (Feigl, 1949). An aqueous solution of sodiumrhodizonate gives coloured precipitates of basic leadrhodizonates. The violet substance Pb(C₆O₆). Pb(OH)₂·H₂O is produced from neutral solutions and the scarlet-red substance 2 Pb(C₆O₆). Pb(OH)₂·H₂O is produced from slightly acid solutions. These rhodizonate reactions are so sensitive that positive results are given by even such poorly soluble substances as leadphosphate and leadsulfate.

Lillie (1954) and Pearse (1961) have both described a method for demonstrating lead salts in sections with a rhodizonate solution. Neither method gave satisfactory results for our purposes. Lillie’s method gives a dark-brown leadrhodizonate differing little in colour from melanin. As a result of diffusion, Pearse’s method gave too little sharpness of localization and also produced nuclear staining.

In an attempt to obtain improvement of these factors, the natriumrhodizonate was dissolved in various buffer solutions at various pH values and then tested. The best results were obtained with a tartrate buffer having a pH value of 3.0. At this pH value localization is sharply defined and nuclei are not stained (Fig. 1).

The results of the leadrhodizonate method were compared with control sections stained with ammoniumsulfide. No differences were found with respect to either sites of enzymic activity or sharpness of localization. In addition, the sites of acid phosphatase activity in structures and cells containing melanin found with the rhodizonate method were the same as those revealed by the azo method according to Burstone (1961). Sections containing melanin deposits showed no colour after incubation in media lacking substrate and then treated.