HISTOCHEMICAL DETECTION OF ZINC BY MEANS OF SULFARSAZENE

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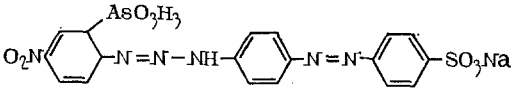
Methods of histochemical detection of zinc by staining frozen and paraffin tissue sections with 0.02% solutions of sulfarsazene, made up in distilled water, alcohol, and a masking mixture, have been developed. The location of zinc in the sections was shown by orange granules. The chief advantages of sulfarsazene over dithizone and other reagents are solubility in water and high selectivity with respect to zinc.

Existing methods of histochemical detection of zinc are based mainly on the use of dithizone [1, 4, 5, 7, 8]. Although highly sensitive with respect to zinc, dithizone has very low selectivity: it can form complexes with 16 other heavy metals [6]. Another disadvantage of dithizone, which considerably restricts its scope is its insolubility in water.

For these reasons, attempts have been made to find other reagents for the histochemical detection of zinc which would be free from these defects. The writers' attention was drawn to sulfarsazene, a compound synthesized by the All-Union Research Institute of Chemical Reagents and Specially Pure Chemical Substances and recommended by them for the photometric and complexometric determination of zinc [2, 3].

Sulfarsazene (the monosodium salt of 4-nitrobenzene-1", 4-diazoamino-1,1'-azo-benzene-2" arseno-4"-sulfonic acid) is a reddish-brown powder, soluble in water, slightly soluble in alcohols, and insoluble in nonpolar organic solvents. In 1 N NaOH solution it gives a bluish-purple color.



$C_{18}H_{14}O_8N_6SAsNa$ Mol. wt. = 572.32

The color reaction of sulfarsazene with zinc is most selective in the presence of a mixture of tartaric and citric acids in ammonia buffer at pH 9.3-9.6. The sensitivity of the reaction is 0.2 μg Zn in 5 ml solution. The determination of Zn is not interfered with by equal quantities of Cd, of 10 times the quantity of Co, and of larger quantities of Cu and other elements [2, 3].

EXPERIMENTAL METHOD

When developing the methods, tissues with a known high zinc content were used: the pancreas and parotid gland of rabbits, the small intestine and prostrate gland of rats, the eye membranes of cats.

The animals were sacrificed by decapitation, one part of each organ was taken for cutting from sections, and the other part was fixed in solutions.

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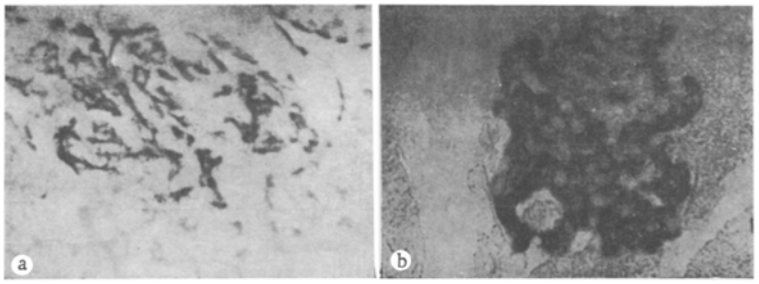

Fig. 1. Sulfarsazene histochemical reaction in an islet of Langerhans: a) paraffin section stained with sulfarsazene solution in masking mixture; b) the same, incinerated preparation. Fixation: 70% alcohol saturated with hydrogen sulfide, 280×.

Frozen sections 10-20 μ in thickness were cut in a cryostat, and paraffin sections were cut to a thickness of 5-10 μ. Some of the frozen sections were treated with formalin vapor for 5 min or with absolute acetone at 4°C for 30 min. Sections of the fixed tissue were also incinerated at 450°C for 3-5 h. Pieces of the organs were fixed in absolute alcohol, acetone, and 70° alcohol saturated with hydrogen sulfide. The duration of fixation was 3-6 h at 4°C. The pieces were then either used for cutting frozen sections or taken through to paraffin.

The sections were stained with 0.02% solutions of sulfarsazene made up in distilled water or absolute alcohol, and also in a mixture masking the other cations (pH 9.5). The mixture contained: 5.4 ml water, 1.6 ml 10% tartaric acid solution, 0.4 ml 10% citric acid solution, and 1.6 ml 5% ammonia solution. The sections were treated for 5 min with one of the above-mentioned solutions of the reagent, and then washed with distilled water, mounted in glycerol, and examined under the microscope.

EXPERIMENTAL RESULTS

Orange granules of the chelate were found in places where zinc was located in the stained sections. The size of the granules varied, they were irregular in shape, and were located in the cytoplasm of the cells, none being found in the nuclei. The granules were seen best under high power of the microscope (more than 400×).

The greatest intensity of staining was obtained by the use of an alcoholic solution of sulfarsazene, and the least intensity by the use of the masking mixture. The reason for this was evidently the better penetration of the alcohol, in which the reagent was dissolved, into the cells.

The tissue structures were much better preserved in specimens fixed with formalin vapor and solutions, and this procedure did not significantly affect the histochemical reaction. Taking the fixed material through to paraffin led to slight diffusion of the zinc. If 70° alcohol, saturated with hydrogen sulfide, was used as the fixative, this diffusion was virtually nonexistent.

In the rabbit pancreas the islets of Langerhans stained strongly, and were easily distinguished against the background of the exocrine tissue. Granules of the chelate were concentrated in the B cells, mainly in their apical portions, especially on the side facing the sinusoidal capillary, while in the A cells they filled the cytoplasm regularly (Fig. 1a). A positive sulfarsazene reaction was found in the islets after incineration (Fig. 1b). Cells which gave a positive staining reaction in the other tissues were the cells of the demilunes of Heidenhain in the submandibular gland of the rabbit, cells located at the base of the crypts (Paneth's cells) in the small intestine of the rats, the retina of the cat's eye, and the epithelium of the terminal divisions and the secretory contents of the efferent ducts in the rat pancreas.

The results of these tests showed that sulfarsazene can be used successfully as a reagent for the histochemical detection of zinc. Unlike the dithizone, which is usually used for this purpose, it does not require the use of potassium cyanide as chelating agent to mask other metals. Other valuable qualities of sulfarsazene are its solubility in water, and the convenience and simplicity of work with it.

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