METHODS OF DETECTING COBALT IN CHICK EMBRYONIC TISSUES

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UDC 612.646.015.2:546.73-088.1

It is contended [9] that the localization of cobalt in the tissues can be accurately determined by the sulfide method. However, when cobalt salts are revealed by conversion into sulfides, which are brown in color, a similar picture may be given by the compounds of copper, iron, and other metals present in the tissues, and this is a disadvantage of the method. No other histochemical methods of determining cobalt in the tissues are described in the known textbooks [5, 7, 8].

To detect cobalt in chick (Russian White breed of hens) embryonic tissues the techniques used for this purpose in microcrystalloscopy have been adopted. The techniques selected were those in which the reagent testing for cobalt gives a color reaction. Allowance was made for the specificity of the reagent, whether it revealed cobalt only or cobalt with minimal amounts of other ions. In one such method dibasic sodium ammonium phosphate NH_4NaHPO_4 is used [4, 6]. This reagent is used in crystalloscopy for detecting several different doubly charged cations, but if a drop of 0.1 N $KMnO_4$ solution is added to the precipitate and it is then heated with a drop of glycerol, only cobalt compounds give a blue color. A minimum of 0.4 μg Co^{++} can be detected.

The embryos are fixed with 10% formalin, washed, and taken through alcohols, xylene, and paraffin wax. Sections are cut to a thickness of 7 μ, taken through to water, and dried with filter paper. A drop of distilled water containing 3-5 small crystals of NH_4NaHPO_4 and a drop of 0.1 N $KMnO_4$ solution are then applied to the sections, and allowed to stand for 20 min. The solutions are then poured off the slide, the slide is dried with ash-free filter paper, a drop of glycerol is applied to the sections, and the slide is heated and a cover slip applied. Blue granules are deposited in the embryonic tissue cells, indicating the presence of cobalt. The granules stand out in contrast against the general yellowish-brown background of the cell body (see figure). By the use of this method not only can the localization of cobalt in the cell body be determined accurately, but its content in the cells of different tissues can be compared. The blue color of the granules is stable.

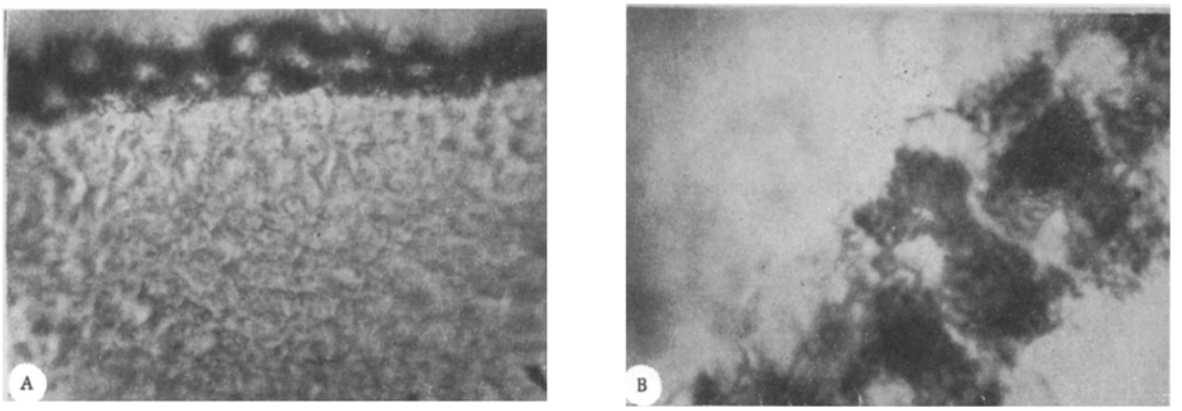

Fig. 1. Cobalt granules in ependymal cells in longrtudinal section through wall of cerebral vesicle. A) Objective 40, ocular 7; B) objective 90, ocular 7.

Laboratory of Embryology and Histology, Department of Human and Animal Physiology, Rostov-on-Don University (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 9, pp. 123-124, September, 1967. Original article submitted November 20, 1965.

Another method used to detect cobalt in embryonic tissues is by means of a reagent ($NaNO_2$, urotropin, and CH_3COOH) giving a precipitate of urotropin cobaltinitrate in the form of yellow aggregates, stars, octahedra, and cubes [3]. This reagent, according to Korenman [3, 4], can detect cobalt in a minimal content of 0.008 μg Co^{++}. The presence of other mineral components in the tested substance does not interfere with the reaction.

To detect cobalt in embryonic tissues by this method the material again is fixed in 10% formalin, washed, and passed through alcohols and xylene and embedded in paraffin wax. Sections are cut to a thickness of 7 μ, deparaffinized, and taken up to 70% alcohol. A drop of 6% acetic acid into which 3-5 small crystals of $NaNO_2$ have first been introduced, followed by 3-5 small crystals of urotropin when the first crystals have dissolved, is then added to the sections and allowed to stand for 20-30 min. The solution is then poured off the slide and the sections are dried with ash-free filter paper and mounted in glycerol. Cells of different tissues stain different intensities of yellow, indicating variations in their cobalt content. For instance, chicken's blood containing 5.6 mg vitamin B_{12}/liter (vitamin B_{12} contains 4.5% cobalt [1,2]), stained with this reagent to an intensity in sharp contrast with that of other tissues.

This method cannot be used to show the local distribution of cobalt in the cell body, but from changes in the intensity of staining of the tissues and, in particular, in the blood it is possible to trace the changes in their cobalt content during development of the embryo. The yellow color of the stained tissue cells is stable.

A similar effect is obtained also by the use of saturated solutions of $NaNO_2$ and urotropin. In these cases the sections are deparaffinized and taken up to 70% alcohol. A drop of 6% acetic acid, a drop of $NaHO_2$ solution, and a drop of urotropin are added in succession, and the sections are allowed to stand in the fluid for 30 min. The solution is then poured off, and the sections are dried with ash-free filter paper and mounted in glycerol.

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