DETECTION OF DOUBLE- AND TRIPLE-CHARGED CATIONS IN TISSUE CELLS OF CHICK EMBRYOS

F. N. Kucherova

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A method of simultaneous detection of copper, manganese, calcium, strontium, iron, and mercury in the tissue cells of chick embryos is described. Not only can the presence of these cations be detected in the cell body, but their content in cells of different tissues can be compared.

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The possibility of using techniques of microchemical analysis based on crystal optics on biological objects was demonstrated in a previous paper [4] which gave the results of qualitative determination of 30 cations detected in the liquid part of the albumen of a hen's egg before incubation by the use of spot tests. The presence of these ions in the liquid albumen was also confirmed by spectral analysis.

In another paper [5], methods of detecting a double-charged cation, especially Co^{++}, in tissue cells of chick embryos are described. To detect some double- and triple-charged cations, the substance cupferon $C_6H_4\begin{matrix}NO\\OH\end{matrix}$ [2,3] is used as reagent in microcrystalloscopy [2, 3]. When this reagent acts on the material a crystalline precipitate is thrown down, in which most of the crystals detectable in it, containing a particular cation, especially Cu^{++}, Mn^{++}, Ca^{++}, Sr^{++}, Hg_2^{++}, and Fe^{+++}, possess a different and characteristic color [3]. For this reason cupferon can be used to detect these cations in tissue cells.

EXPERIMENTAL METHOD

The test material consisted of chick embryos at 106, 130, and 154 h of incubation. The embryos were fixed in 10% formalin. The procedure was then carried out on paraffin sections (7 μ) before and after microincineration, and also on frozen sections (20 μ).

In the first case dewaxed (xylol) sections were taken through to absolute alcohol, and after evaporation of the alcohol from this slide a drop of 5% aqueous solution of cupferon was applied to the sections, the slides were covered with a Petri dish, allowed to stand for 50-60 min, after which the reagent was absorbed with ash-free filter paper, mounted in glycerol, and covered by a cover slip.

In the second case, the sections were dewaxed and carried through to absolute alcohol, after evaporation of which microincineration was carried out to the phase of "carbonization." The slides were then cooled, a drop of 5% cupferon solution was applied to the sections, and subsequent treatment was given as in the first case.

Frozen sections were placed on a glass slide, dried for 24 h, and then treated as in the first two cases.

Specimens were examined under the "Mikrofot" Mark D microscope giving a magnification of 10×65.

EXPERIMENTAL RESULTS

With many repetitions of the experiment, in all three cases a distinct coloration was observed in certain parts of the body of the tissue cells. These colors were identical with the color of the crystalline precipitate thrown down by cupferon and containing one of the cations listed above. To compare the color of individual cations detected in the embryonic tissue cells, a color determination scale [1] was used. Because

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of the different sizes of crystals of different cations and because of differences in their shape, an accurate picture can be obtained by differential focussing for each of them. Ca^{++} crystals are seen as long, thin, curved, black threads, Sr^{++} crystals as thin, short, dark needles, with bifurcating ends; Mn^{++} gives a dark brown precipitate; Hg_2^{++} gives thin, light brown threads; Fe^{+++} gives spherical crystals of a brick red color, and Cu^{++} gives greenish rosettes.

Although this method cannot be used to determine the distribution of cations in different parts of the cell, it nevertheless reveals them very clearly if they are present in the cell body. In addition, it can be used to compare the content of these cations in cells of different tissues. Erythrocytes, for instance, contain more Ca^{++} and Sr^{++} than leukocytes, while the Fe^{+++} and Cu^{++} precipitates are about equal. The Ca^{++} precipitate is very slight in heart muscle and the walls of the renal tubules, while the content of Fe^{+++}, Cu^{++}, Sr^{++}, and Mn^{++} is about the same as in the leukocytes.

Precipitates containing any of these ions retain their color for a long time.

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