METHOD FOR DETECTION OF CHROMAFFIN CELLS

I. N. Molchanova

From the Department of General Biology (Head — Doctor of Biological Sciences Professor V. V. Izosimov) of the Kazan Medical Institute

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Animal chromaffin cells may be detected by methods based on chroming [1], and on silvering.

However these methods failed when used on invertebrate cells.

We tried a modification of the method proposed by Ogata for the detection of chromaffin cells in invertebrates.

According to A. M. Utevskii and N. S. Levantseva [3, 4], adrenalin may combine with formaldehyde and still preserve unchanged the diphenol groups on which its reducing power depends.

It might be supposed therefore, that cells containing adrenalin should be fixed in formalin before silvering, because the formalin, instead of destroying the adrenalin, actually preserves its reducing properties.

After fixation in 12 % neutral formalin and silvering, we succeeded in demonstrating cells containing adrenalin which differed markedly from other structures. Here, just as in vertebrate cells, we found brown granules, while the other cells which did not contain adrenalin remained colorless.

A still greater difference between chromaffin and non-chromaffin cells was obtained by using a 12 % alkaline solution of formalin for fixation; this was obtained by adding ammonia or 3-5 drops per 20 ml of 0.1 N. sodium hydroxide. After fixing in this way the chromaffin cells stained dark brown or black.

We also introduced certain modifications into the silvering method of T. Ogata and A. Ogata.

We recommend the following method of silvering for the detection of chromaffin cells: 1) fixing the living tissues in 12 % neutral or slightly alkaline formalin for not less than 1 hour; fixation may be prolonged further without harm; 2) washing in distilled water; 3) preparing the sections for the freezing microtome; 4) treatment for 30 minutes with 1 % ammonia; if alkaline formalin was used for fixing, this step may be omitted; 5) treatment with 5 % ammoniacal silver nitrate for not less than 3 hours; 6) treatment with 1 % ammonia solution for 30 minutes; 7) washing for 1-2 minutes or more with a 3 % solution of hyposulfite ; 8) washing with tap water for 10-20 minutes; 9) washing with distilled water; 10) dehydration through increasing strengths of alcohol, clearing in oil, and mounting in Canada balsam.

This method of silvering is equally effective for the chromaffin cells of both vertebrates and invertebrates. The surrounding tissues, which remain colorless, may be counterstained.

Good results were obtained in invertebrate animals when prior to the fixation and the silver treatment the animals were anesthetized with chloral hydrate or when vital staining with methylene blue was used. In the first case a solution of chloral hydrate of approximately 0.1 % was used, and was added to the water in which the leeches were kept; in the second case the methylene blue was prepared by the method of A. L. Shabadash [5] (distilled water — 1000 ml, sodium chloride — 7.5 g, glucose — 1.5 g, methylene blue — 0.25 g), but 6.5 g instead of 7.5 g of sodium chloride was taken, i.e. the methylene blue solution was made up in the saline normally used for invertebrates.

After being vitally stained blue, the chromaffin cells took up the silver very strongly and stained dark brown; they stood out clearly from the other non-chromaffin cells whose nuclei and protoplasm were also stained, but much more weakly. There was therefore no need to counter-stain the sections.

Thus the chromaffin cells are more strongly argyrophilic than those of other tissues, and this is strongly borne out by use of this method. We can therefore recommend it in cases where these cells are to be deomonstrated in animals.

Also this method allowed us to show that in vertebrates the sympathetic nerve fibers are more strongly argyrophilic than the parasympathetic, and this no doubt results from the presence in them of the adrenalin-like substance sympathin.

Ogata's method, as modified by us, is not definitely specific for adrenalin, but in comparison with chroming, which is also non-specific for adrenalin [2], it is more sensitive, and by its use adrenalin can be demonstrated in the outgrowths as well as in the bodies of the cells.

SUMMARY

A modification of Ogata's method is proposed, based on treating the tissues by 12 % formalin previous to the application of silver.

Chromaffin elements are stained brown. This method is not strictly specific for detection of adrenalin, but is is more sensitive than others and reveals chromaffin elements in the cells of invertebrate animals.

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* In Russian.