NEW METHODS

METHOD FOR THE HISTOMETRIC DETERMINATION OF POTASSIUM

M. G. Shubich

From the Chair of Histology (Head – Assistant Prof. G. F. Berezentseva)
of the Kuban Medical Institute, Krasnodar (Director – Prof. V. K. Suprunov)

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Despite the importance of histological determination of potassium, the possible methods for this are very limited. The Macallum method [6] (precipitation of potassium by sodium hexanitrocobalt in the form of $K_2Na[Co(NO_2)_6]$) and conversion of this compound into cobalt sulfide, which can be detected microscopically, has been doubted by many authors [1, 3, 4]. According to Lison [5] the Macallum technique is of no value in histochemical studies of potassium location.

The Carere-Comes method [4], based on the formation of an orange-red precipitate by sodium dipicrylamine with potassium, has a number of advantages: the colored product is formed by direct reaction between sodium paradipicrylamine with potassium, because of the rapid penetration of the reagent into the tissue the colored product is formed at the site of location of the potassium, the preparation technique is relatively simple, and the preparations themselves are stable and do not fade. However, the specificity of this reagent in histological applications gives rise to doubt. Sodium paradipicrylamine has an orange-red color, and the color of the final product, potassium paradipicrylamine, which serves to show the location of potassium in the tissues, is the same. It is therefore possible that, in addition to the formation of potassium paradipicrylamine, simple staining of one microstructure or another by sodium paradipicrylamine may occur.

In such cases control experiments are of fundamental importance.

It must be pointed out that Carere-Comes did not give either the method for preparing the reagent or the concentrations of the solutions in his paper. Aqueous sodium paradipicrylamine solution was patented by him under the name of "Carere-Comes Sienna Orange," and is distributed by one foreign firm. This makes the use of the Carere-Comes method very difficult, and is probably the reason why our national literature contains no reports of its use.

The usual method for preparing paradipicrylamine by nitration of diphenylamine requires a well-equipped chemical laboratory; we obtained a negligible yield because of resinification of the reaction mass. A more convenient method used by us, is preparation of sodium paradipicrylamine from an available product – ammonium dipicrylamine (aurantia dye). A saturated solution of aurantia is made in ethyl alcohol, decanted, and 10% sulfuric acid is added to it until precipitation ceases. The precipitate is filtered off under suction, transferred to a flask, and excess sodium carbonate is added to it. The mass is boiled for an hour on the water bath and filtered. The solution can be used for the experiments. $1\frac{1}{2}$ to 2-fold dilution is permissible.

To ensure the specific nature of the histochemical reaction for potassium with sodium paradipicrylamine, a control technique was developed. The reagent for treatment of the control preparations is phospho-12-tungstic acid which, as was shown by Nikitina [2] precipitates potassium according to the equation:

$$5K^+ + 2H_3(PO_4 \cdot 12WO_3) \rightarrow K_5H(PO_4 \cdot 12WO_3)_2 \cdot 6H_2O + 5H^+.$$

When a preparation is treated with phospho-12-tungstic acid all the potassium present in the section is converted into potassium phospho-12-tungstate, and does not react with sodium paradipicrylamine. Simple staining of microstructures by sodium paradipicrylamine, if it occurs, is not specifically influenced by treatment with phospho-12-tungstic acid.

The control experiments showed that the orange-red, orange-brown, orange, orange-yellow, and clear yellow colors of microstructures treated by the Carere-Comes method are the result of the histochemical potassium reaction. The scarcely noticeable diffuse pale yellow color is not the result of the histochemical reaction, but arises by simple staining.

For the control tests in the histochemical reaction for potassium, the sections are immersed for 1 hour into 10% solution of phospho-12-tungstic acid, or for longer periods into solutions of lower concentrations, thoroughly washed in distilled water, and subjected to the Carere-Comes reaction. The resultant preparations are compared with sections treated by the Carere-Comes procedure without treatment with phospho-12-tungstic acid.

The sections for histochemical tests for potassium must be prepared from freshly frozen pieces. Embedding in paraffin wax is permissible after preliminary freezing and vacuum drying. Fixation in 10% formalin followed by embedding in paraffin wax, as recommended by Carere-Comes, apparently reveals potassium which is bound with the cell proteins and which therefore does not dissolve while the material is in the fixing agent and alcohols.

With this method it is possible to carry out histochemical studies of the location of potassium in cells, and selective staining of elements rich in potassium, such as erythrocytes and other cells of the erythroblastic series. Great interest attaches to histochemical investigation of potassium in tumors, in which it is present in considerably larger amounts than in normal tissues.

In conclusion, it is my duty to express my deep gratitude to Prof. L. I. Falin, who drew my attention to the need for control experiments in histological tests for potassium.

SUMMARY

A simple method of preparing sodium paradipicrylamine patented by Carere-Comes under the name of "Sienna Orange" as a histochemical test for potassium is described. The following method of controlling the specificity of the histochemical reaction for potassium is offered: the preparation is treated phospho-12-tungstic acid which reacts on potassium contained in the section; therefore, potassium cannot be combined with sodium paradipicrylamine.

LITERATURE CITED

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* In Russian.