ON HISTOCHEMICAL METHODS OF DETERMINING ACID MUCOPOLYSACCHARIDES

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Several methods have been suggested for the histochemical determination of acid mucopolysaccharides. Among them are: Hell's method with colloidal iron- acetate reagent, staining with toluidine blue or thionine, staining with acid fuchsin chloride (Schiff's reagent) after prelimanary acidification with periodic acid or lead triacetate. All these reactions are of a group nature and are not specific for individual acid mucopolysaccharides [1, 2, 3, 4, 5]. However, with parallel enzymatic control (for example, hyaluronidase), the enumerated reactions can be considered sufficiently specific for the determination of certain mucopolysaccharides (for example, hyaluronic acid).

In practice, a number of difficulties arise in using these methods, especially Hell's method. We determined hyaluronic acid in the tissues of the placenta and umbilicus of man and animals and during the work we modified Hell's method somewhat, which permitted the detection of acid mucopolysaccharides with greater completeness and accuracy. Hell's method is based on the detection of acid mucopolysaccharides by a color reaction forming Prussian blue from the colloidal iron adsorbed by the mucopolysaccharide and potassium ferrocyanide. We were convinced that the most important factor in determining the success of the reaction is the quality of the colloidal iron acetate reagent. Descriptions of the method in textbooks [1] do not indicate how the colloidal iron hydroxide is obtained, what proportions of the reagents should be used to obtain a workable solution. Undoubtedly, in each case, the optimum proportions of the solutions must be found experimentally, depending on the fixing and preparation of the material. We recommend iron acetate reagent, obtained in the following manner, as one of the most useful reagents (using the fixing method of Carnoy and some others, using 8% formalin containing 1-4% basic lead acetate or 0.5% acetic acid which was accepted for this method).

The original material is 10% iron chloride solution. Iron chloride solution is added by drops to boiling distilled water in order to obtain colloidal iron hydroxide. The best results were obtained when 8 to 12 ml of iron chloride solution were added to 100 ml of water. Attention should be paid to the careful elimination of hydrochloric acid from the solution by dialysis, which is best carried out in a hot water bath, changing the distilled water often.

Prepared cooled colloidal hydroxide mixed with a two molar solution of acetic acid in equal amounts is recommended for obtaining a workable solution. However, we were convinced that the clearest color reaction was obtained when the hydroxide was mixed with acetic acid in the proportion of 3 : 1 or 2 : 1. The time for treating the preparation with iron acetate reagent and also with the potassium ferrocyanide should be increased from 10 minutes (as Hell recommends) to 20-25 minutes. It is best to wash the iron acetate reagent not in distilled water, but in a two molar solution of acetic acid. This gives a clearer and purer color. The brightness of the color depends not only on the quality of the iron acetate reagent, but also on the purity and freshness of the potassium ferrocyanide solution.

Waterless fixing fluids are recommended for the determination of acid mucopolysaccharides by Hell's method and, in particular, Carnoy's fluid. However, when they are used, the acid mucopolysaccharides are not determined completely: the tender delicate, fine structures remain undetermined as a result of the considerable shrinkage of the tissues. A metachromatic reaction is clearly evident in the umbilical arteries on dyeing with toluidine blue, which is typically localized, while in preparations which were fixed with Carnoy's fluid, acid polysaccharides cannot be found in the wall of the umbilical arteries by Hell's method. Thus, in order to determine acid mucopolysaccharides completely enough histochemically, Carnoy's fixing fluid should be rejected as the fixative. We obtained considerably fuller determination of acid mucopolysaccharides by Hell's method after fixing in a 4% solution of basic lead acetate over 8% formalin. Thus, using this fixative, we obtained complete correlation of the results of staining by both methods.

For determining acid mucopolysaccharides in embryonic tissues which are high in water, a 1% solution of basic lead acetate or 0.5% solution of acetic acid over 8% formalin can be especially recommended. With this fixative, tissue shrinkage is insignificant and the most delicate acid mucopolysaccharide structures are clearly stained.

SUMMARY

For a most complete histochemical detection of acid mucopolysaccharides a following modification of Hell's method is recommended:

1. In order to obtain a colloidal ferric hydroxide, 8-12 mg of 10% solution of ferric chloride should be added in drops to 100 mg of boiling distilled water.

2. Dialyzed colloidal ferric hydroxide is mixed with 2 M acetic acid in proportion 3 : 1 or 2 : 1.

3. The period of action of ferric acetate and solution of ferricyanide upon the preparation is increased to 20-25 minutes.

4. Material in 1-4% solution of basic lead acetate (or in 0.5% solution of acetic acid) is fixed over 8% formalin.

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