Lead Tetra-Acetate/Schiff Tests in Histochemistry

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SUMMARY

Certain polysaccharides, such as starch and glycogen, do not give consistently positive or negative reactions with all lead tetra-acetate/Schiff techniques. This depends upon the conditions under which the oxidant is used. A simple glacial acetic acid solution of lead tetra-acetate is least active but most specific. Added potassium acetate acts as a catalyst. Dilution with water not only increases the activity of the reagent but also decreases the specificity of the test.

VARIOUS histochemical techniques have been described in which lead tetra-acetate is used as a selective oxidant and followed by Schiff's reagent in a manner similar to the periodic acid/Schiff tests. Not all these lead tetra-acetate methods give the same results with certain polysaccharides. Thus, a positive reaction with glycogen has been reported to be obtained regularly with one technique (Shimizu and Kumamoto, 1952), occasionally with another (Lhotka, 1952, 1954), but not at all at room temperature with yet another (Glegg, Clermont, and Leblond, 1952). Unrecognized, such differences can be misleading in histochemical analyses. They are related to the composition of lead tetra-acetate reagent and the conditions under which it is used.

Comparative studies have been carried out with the methods of Crippa (1951), Shimizu and Kumamoto (1952), Jordan and McManus (1952), Lhotka (1952), Glegg, Clermont, and Leblond (1952), Hashim and Acra (1953), and Graumann (1953), as well as with four unpublished procedures. All of these techniques were tested on 10μ paraffin sections of potatoes and of mouse, rat, and rabbit livers and jejunums fixed in various reagents (formaldehyde-saline, absolute ethanol, Bouin's, Carnoy's, Champy's, Flemming's, Gendre's, Helly's, Rossman's, or Zenker's fluid). Those devised by Hashim and Acra (1953) were studied at 28° C., the temperature of these investigators' laboratory (Hashim, Acra, Afifi, and Shanklin, 1953). The effect of first covering the sections with collodion (Lison, 1953) was determined for all methods. Freshly prepared (Bailar, 1939) lead tetra-acetate was compared with three commercial products (Arapahoe Chemicals Co., Boulder, Colo., U.S.A.; Hopkins and Williams, Chadwell Heath, Essex; Light and Co., Colnbrook, Bucks).

With none of the procedures were there any appreciable differences between the results for the livers or jejunums from mice, rats, or rabbits or

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between those for potatoes purchased in Canada or England. The preservation of the various polysaccharides, especially glycogen, varied to some degree with the fixative but their reactivities were unaffected by this or the collodion film. The latter favoured the retention of Schiff-positive oxidation products, but with some methods it also gave a positive reaction. Although differing in assay (Dimroth and Schweitzer), the four samples of lead tetra-acetate gave similar histochemical results.

The differences between the observations for certain polysaccharides in the tissues could be related to the conditions under which the lead tetra-acetate is used in each method. Where the reaction medium is glacial acetic acid as in the procedures of Crippa (1951), Glegg, Clermont, and Leblond (1952), and Graumann (1953), or glacial acetic acid diluted with toluene or benzene as suggested by Hashim and Acra (1953), starch and glycogen do not react positively under the specified conditions for each procedure. When anhydrous potassium acetate is added (Lhotka, 1952), these polysaccharides occasionally give a weakly positive reaction, especially when the duration of oxidation is prolonged. More strongly positive reactions are usually noted with the methods of Hashim and Acra (1953) in which the stock acetic acid solution of lead tetra-acetate is diluted with water. Intensely positive reactions are always obtained when the diluent is aqueous sodium acetate as used by Shimizu and Kumamoto (1952) and by Jordan and McManus (1952). With all methods, hepatic and intestinal connective tissues, intestinal mucin, and potato cellulose react positively.

These observations demonstrate that while acetate can serve merely as a catalyst for oxidation by lead tetra-acetate, water not only accelerates the reaction but decreases the specificity of the method (compare Fuson, 1950). This has also been noted by Lhotka (1954). The interpretation of histochemical tests is usually more certain when these are carried out under conditions giving results which conform with well established organic or biochemical observations. For example, starch is usually considered to be oxidized only with difficulty by lead tetra-acetate although readily by periodate (Criegee, 1948). The lead tetra-acetate/Schiff methods introduced by Crippa (1951), Lhotka (1952), Glegg, Clermont, and Leblond (1952), and Graumann (1953), and the methods of Hashim and Acra (1953) in which the diluent is benzene or toluene, may be preferable for critical applications. With the techniques of Shimizu and Kumamoto (1952), Jordan and McManus (1952), and those of Hashim and Acra (1953) where water is the diluent, careful consideration should be given to the increased activity of the reagent and the decreased specificity of the methods. In all cases, any report of observations with a lead tetra-acetate/Schiff test should include details of the exact method employed.

Additional studies have shown that not only the reaction medium but also the concentration of the lead tetra-acetate and the duration and temperature at which it is used may affect the results. Careful adjustment of these variables, however, provides a series of tests valuable for the comparative study of

carbohydrates and other tissue constituents containing appropriate reactive groups.

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