Brief Notes

A Method for the Histochemical Differentiation of Cholesterol and Its Esters.*

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The Schultz (1) method for cholesterol, a modification of the Liebermann-Burchardt reaction, is probably the most specific and the most widely used histochemical method for cholesterol and its esters. Both are visualized by the production of a transient green or blue-green color, this developing somewhat more rapidly with respect to the esters than the free cholesterol, and lasting a few minutes or hours. The free cholesterol forms an insoluble complex with digitonin (2), generally of a needle-like, birefringent, crystalline character, and the microscopic identification of such crystals has been suggested as a means of identifying free cholesterol. This cholesterol-digitonin complex is insoluble in a variety of fat solvents, such as an alcohol-ether mixture, which mixture will extract the cholesterol esters. The complex remains reactive to the Schultz procedure, probably being disrupted by the reagents used, and this would be anticipated from data with methods which utilize the reactivity of the complex to the Liebermann-Burchardt procedure in biochemical determinations.

These properties may be utilized for the histochemical differentiation of cholesterol and its esters.

1. Immerse formalin-fixed frozen sections in a 0.5 per cent solution of digitonin in 40 per cent ethyl alcohol for 3 hours at room temperature.

2. Drain and immerse in a mixture of equal parts of absolute ethyl alcohol and ether for 3 hours at room temperature.

3. Drain and immerse in a 2.5 per cent aqueous iron-alum solution at 37°C. for 2 to 4 days. Simultaneously, place a duplicate section of the same material not treated as in 1 and 2 above, into the same solution.

4. Drain fairly dry. (Blotting dry has not been found necessary.) Cover both sections with a mixture composed of glacial acetic acid to which an equal volume of concentrated sulfuric acid has been added while the mixture has been kept in an ice bath. The appearance of a transient blue-green color is the characteristic positive reaction.

The digitonin solution complexes with the free cholesterol but does not affect the cholesterol esters. The alcohol-ether mixture extracts the cholesterol esters, but not the free cholesterol-digitonin complex. The acetic–sulfuric acid mixture reacts with the remaining free cholesterol-digitonin complex, to produce the characteristic blue-green color. It acts on the control slide to disclose both the free and esterified cholesterol, so that the differ-

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ence between the sections indicates the esters. It is probable that this method will not disclose or differentiate small quantities of these materials, but it has served to identify and differentiate the character of the lipides in neural xanthomatosis (Figs. 1 and 2).

Other methods which have been suggested include the use of digitonin followed by bismuth trichloride (3), and identification by polariscopic recognition of birefringent crystals. The latter method is now generally recognized as being non-specific, even the characteristic Maltese cross birefringence being exhibited by other lipides, and often being absent when cholesterol or its esters are present (4).

SUMMARY

Free cholesterol is demonstrated in formalin-fixed frozen sections when treated successively by digitonin, alcohol-ether, and the Schultz technique, in which circumstances cholesterol esters are not visualized. Cholesterol esters and free cholesterol are both demonstrated in comparable sections treated by the Schultz method alone, so that the difference between such sections indicates the sites at which cholesterol esters may be considered present.

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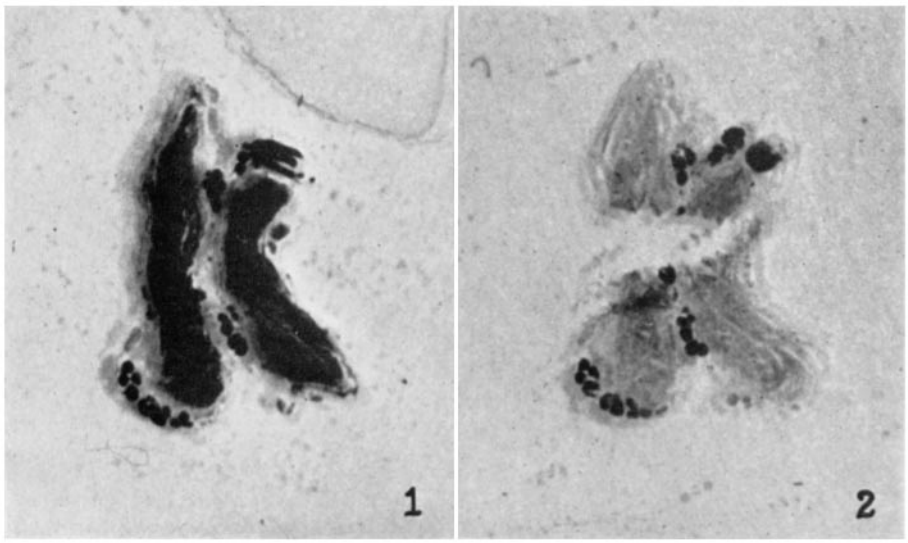
EXPLANATION OF PLATE 35

FIG. 1. Frozen section of spinal cord showing free cholesterol in the normal myelin of the nerve roots, and cholesterol esters in xanthomatous lesions within the cord substance, both stained blue-green by the Schultz method. × 5.

FIG. 2. Frozen section of same segment of spinal cord treated with digitonin and alcohol-ether and stained by the Schultz method. The cholesterol esters within the cord are not stained, but the free cholesterol in the nerve roots is stained blue-green. × 5.



(Feigin: Histochemical differentiation of cholesterol)