A new procedure for staining lipoproteins in ionographic separations

During very recent years there has been an upsurge of interest shown in the study of lipoproteins, largely because of their implication in the genesis of atherosclerosis. One of the most convenient ways of separating the α- and β-lipoproteins from blood is to fractionate serum ionographically[1]. In previous studies of lipids and lipoproteins by this method, in paper-stabilized electrolytes, the chief difficulties encountered were in the inadequate staining of the lipoprotein bands, in the retention of the stain by the paper itself, and in the undue length of time often required for the staining procedure,—sometimes a matter of several hours. By the new procedure described here, involving pre-staining of the lipids before application of the serum sample to the paper, all of these difficulties have been overcome.

Of the several stains which have been proposed for lipoproteins[1], the most favorable experience in this laboratory with the conventional method of staining the ionograms, namely, after a run, was obtained with Sudan Black B[2], or so-called acetylated Sudan Black B[3], which produce ionograms having dark blue bands corresponding to the areas covered by lipoproteins, against a light blue background. With the acetylated dye, the color intensity of the lipoprotein bands was increased and the background lightened, but the result still left much to be desired.

By the new procedure, the lipoproteins are pre-stained directly in the blood serum sample before application to the paper, as follows: To 1 ml of blood serum in a small test tube there is added slowly (to avoid precipitation) 0.1 ml saturated solution of acetylated Sudan Black B in 95% ethanol (or for smaller relative amounts, the same 10 to 1 ratio). The tube containing the serum is now shaken and allowed to stand at room temperature for 30 minutes (although even 3–5 minutes will yield fair results). The excess alcohol is evaporated by applying a suction to the tube and allowing a fine stream of nitrogen (or air) to be pulled over the serum through a thinly-drawn capillary tube.

Five microliters of the serum containing the stained lipoproteins are now applied from a micropipet to the filter paper (Whatman No. 1, 0.5 inch width) as a streak across the paper ribbon. The horizontal open-strip method, employing the Precision Ionograph, was used in making the separations. The other experimental conditions generally used for optimal results were: veronal buffer; pH, 8.6; ionic strength 0.05; potential gradient 6 volts/cm; atmosphere, water-saturated helium at 0–5° C, although runs in a simple water-saturated air atmosphere at room temperature were also quite satisfactory. An adequate separation is obtained in 4–5 hours. Both the α- and the β-lipoproteins migrate in the usual manner and appear as blue zones against a white background.

Since the dye is physically dissolved in the lipoproteins and is insoluble in water, there is no smearing of the colored zones. No dissociation or break-up of the colored materials was evident, as is observed when serum proteins are pre-stained with bromophenol blue[4]. This can be explained as follows: the Sudan Black B dissolves physically in the lipid-protein moiety whereas the protein-bromophenol blue complex involves an ionic bond and an equilibrium which can be upset due to the leaking away of the bromophenol blue ions from the complex, under the influence of the electric field. Using the same technique, except that the Sudan Black B was replaced by Sudan III, Sudan IV and Oil Red O, the results were much less satisfactory.

The new technique eliminates a long and tedious staining procedure, including washing of the ionogram, conserves rather costly reagents and yields crisp, well-demarked zones which enable the relative amounts of α- and β-lipoproteins to be determined more accurately.

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[1] H. J. MCDONALD, R. J. LAPPE, E. P. MARBACH, R. H. SPITZER AND M. C. URBIN, *Ionography; Electrophoresis in Stabilized Media*, Year Book Publishers, 1955.
[2] B. SWAHN, *Scand. J. Clin. Lab. Invest.*, 4 (1952) 98; also 5, Supplement No. 9 (1953) 1–114.
[3] W. G. B. CASSELMAN, *Biochim. Biophys. Acta*, 14 (1954) 450.
[4] R. H. SPITZER AND H. J. MCDONALD, *Federation Proc.*, 14 (1955) 285.

Received April 4th, 1955

Some components of the oxidative phosphorylation system*

Attempts at fractionation of the mitochondrial phosphorylative oxidation system, outlined in this communication, have led to the identification of Mn^{+2} and cytochrome *c* as essential components in the over-all process. ERNSTER AND LINDBERG[1] have recently reported that the inhibition of coupled

* The investigation was supported by a grant-in-aid (No. A-596) from the National Institute of Arthritis and Metabolic Diseases, N.I.H., and another from the American Heart Association, Inc.