A Modification of the Method for Prestaining Alpha and Beta Lipoproteins Separated by Paper Electrophoresis

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When one employs the classic method for staining lipoproteins after separation by paper electrophoresis (3, 4) one encounters difficulties in attempting to evaluate the alpha and beta bands. These problems are manifested in: 1. detection of alpha lipoprotein present, 2. problems of elution, and 3. the inability to scan strips on a photoelectric scanner. The disadvantage in using postelectrophoretic staining is that it not only stains the lipid bands, but also results in a high color background to all of the paper strip. Because of these inadequacies we decided to develop a prestaining technique that would clearly stain only the lipid material but would not affect the background color of the filter paper.

EQUIPMENT AND REAGENTS

1. Petroleum ether–alcohol solvent. Mix 1 part of reagent grade petroleum ether with 4 parts of absolute ethyl alcohol.

2. Saturated solution of Sudan Black “B” (certified stain) prepared in the above solvent.

3. Barbiturate buffer, pH 8.6, ionic strength 0.05. 1.85 Gm. barbital (diethylbarbituric acid) and 10.30 Gm. of sodium barbital are dissolved in water and diluted to 1 liter in a volumetric flask. The buffer may be purchased preweighed as Spinco No. 300-811 buffer B-1.

4. Electrophoresis apparatus consisting of:
   (a) Whatman No. 3 mm. filter paper, width 3 cm.
(b) Micropipette 25 μ, graduated in 5 μ
(c) Serum stripper
(d) Power supply, Spinco Model R Duostat or equal
(e) Hanging-strip paper electrophoresis cell of the Durrum type (1).

PROCEDURE

Into a 15 × 100 mm. test tube 1.0 ml. of plasma or serum is pipetted. To this is slowly added 0.1 ml. of saturated Sudan Black “B” solution, and 0.05 ml. of Absolute Ethyl Alcohol is added. The tube and its contents are then incubated for 20 minutes at 37°C.

During the incubation of the dye and plasma the electrophoresis cell is prepared and the strips are allowed to equilibrate for 15 minutes in the atmosphere of the buffer.

At the end of the incubation period 40 μl of the prestained plasma is applied to the paper strips with a stripper using two applications of 20 μl each.

The system is then sealed and power supply set at a constant current of 15 ma for three-and-one-half hours. At the end of this period the power is turned off, the strips are removed, dried at 110 to 120°C and scanned with a densitometer.

DISCUSSION

Prestaining of lipoproteins has been described by McDonald (2), but we experienced many difficulties with it: when the strips were wet the alpha lipoprotein band could be observed, but after drying this band would disappear.

In an attempt to solve this problem various lipid specific dyes were used in the system and evaluated. As a result of trial and error the final procedure was established.

Sudan Black “B” saturated in petroleum ether–alcohol mixture proved to be the ideal dye for prestaining lipoproteins because:

It provided ideal separation of the serum lipid fractions.

It provided clear-cut distinctions between the Alpha and Beta lipoproteins, and more accurate quantitation.

It made possible the determination of neutral fats.

With its clear-cut lines of demarcation between the alpha and beta fractions of lipoproteins it was possible to establish easily the ratio of the fraction to each with a scanner equipped with automatic integration.* It was found that the proposed technique gave values for the lipid constituents as other more complicated techniques.

*Spinco Model R. Analytrol, with B-3 cam and a Corning 5031 blue filter.
For the elution techniques it provided ideal "scissor separation" areas.

SUMMARY

A procedure is described for prestaining serum lipids for paper electrophoretic evaluation.

REFERENCES