LABORATORY NOTE

IDENTIFICATION OF TYPE III HYPERLIPOPROTEINEMIA BY ELECTROPHORESIS ON CELLULOSE ACETATE AND INDIRECT (SCHIFF'S) STAINING*

LOUIS A. ROSATI, M.D., EDWARD R. NAPIER, JR., Ph.D., AND JOHN G. BATSAKIS, M.D.

Departments of Pathology and Biochemistry, The University of Michigan, Ann Arbor, Michigan, U.S.A.

(Received August 29, 1969)

CELLULOSE ACETATE AS THE ELECTROPHORETIC SUPPORT MEDIUM for the separation of serum lipoproteins, combined with either direct lipid (1) or indirect

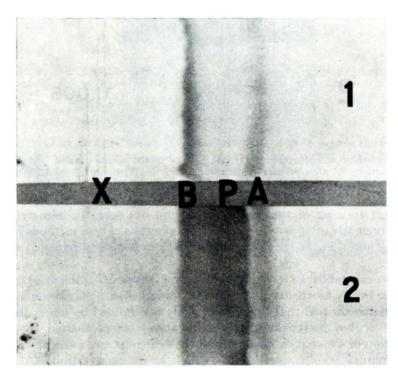


FIG. 1. Comparison of normal lipoprotein pattern (1) with that from patient with type III hyperlipoproteinemia. Note "broad beta" migration. Application site (x); betalipoproteins (B); pre-betalipoproteins (P); alpha-lipoproteins (A).

^{*}Supported in part by the USPHS (AM-07354) and in part by a grant from the Michigan Heart Association.

TYPE III HYPERLIPOPROTEINEMIA (cellulose acetate)

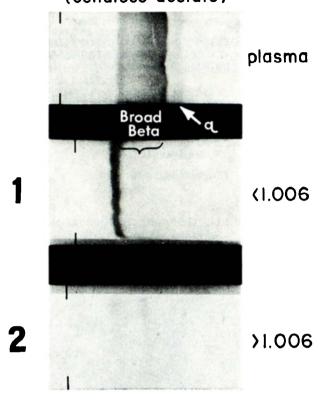


FIG. 2. Comparison of lipoprotein electropherograms of the natine plasma; and top (1) and subnatant fractions (2) after preparative ultracentrifugation. Virtually all stainable lipoproteins manifesting beta-mobility appear in the top layer fraction.

(Schiff's) (2) staining of the electropherogram, has recently been reported to possess distinct advantages over paper electrophoresis. These methods have been particularly useful in the study of patients with hyperlipoproteinemia of type II and IV (3).

Of the types established in the NIH system, (4) type III is the most difficult to characterize from a biochemical standpoint. Nonetheless, Fredrickson and associates have demonstrated that lipoprotein analysis obtained by either paper electrophoresis or by analytical ultracentrifugation, complemented by chemical determination of the concentration of the chylomicrons, are interconvertible (5).

This communication emphasizes that such an interconvertibility pertains to type III hyperlipoproteinemias as identified by cellulose acetate electrophoresis and indirect staining of the lipids by Schiff's reagent.

The sera were obtained from a 58-year-old man manifesting" adult onset"—diabetes mellitus. The initial lipoprotein electrophoresis demonstrated a "broad

beta" fraction (Fig. 1). The serum cholesterol in this sample was 336 mg/100 ml and serum triglycerides were 408 mg/100 ml. The serum was opalescent.

Following the demonstration of the abnormal beta lipoprotein fraction, a second serum sample was spun in a preparative ultracentrifuge† at 30,000 rpm for 16 hours (6) and the top layer (d < 1.006, S_t 20–400) and the subnatant fractions separately subjected to electrophoresis on cellulose acetate (Fig. 2).

Virtually all of the lipoproteins having beta mobility appeared in the top layer (d < 1.006), in contrast to the expected "sedimentation" of the higher density beta (d > 1.006, S_t 0-20) and alpha lipoproteins.

Thus, the findings reported here meet the criteria of the NIH system for the definition of the type III pattern. This is of practical importance because rapid, simple and inexpensive procedures as provided by cellulose acetate and Schiff's stain are needed for routine and more importantly, for large scale population screening. Type III disease has proven to be somewhat of an obstacle in this regard due to the insistence of ultracentrifugal data for precise definition. While we do not disagree that the most reliable definition of this disease should include such data, we feel that the gross abnormality of this pattern can be adequately demonstrated by electrophoresis on cellulose acetate, thereby avoiding the limitations of ultracentrifugation in the routine clinical laboratory.

REFERENCES

- CHIN, H. P. & BLANKENHORN, D. H. Separation and quantitative analysis of serum lipoproteins by means of electrophoresis on cellulose acetate. Clin. Chim. Acta 20, 305-314 (1968).
- FARBER, E. R., BATSAKIS, J. G., GIESEN, P. C. & THIESSEN, M. Lipoprotein electrophoresis: a comparison of cellulose acetate and paper technics. Am. J. Clin. Path. 51, 523-538 (1969).
- 3. CHIN, H. P. & BLANKENHORN, D. H. On the precision of lipoprotein electrophoresis on cellulose acetate and its use in the diagnosis of hyperlipoproteinemia. Clin. Chim. Acta 23, 239-240 (1969).
- 4. FREDRICKSON, D. S., LEVY, R. I. & LEES, R. S. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. New Eng. J. Med. 276, 148-156 (1967).
- FREDRICKSON, D. S., LEVY, R. I. & LINDGREN, F. T. A comparison of heritable abnormal lipoprotein patterns as defined by two different techniques. J. Clin. Invest. 47, 2446-2457 (1968).
- DELALLA, O. F. & GOFMAN, J. W. Ultracentrifugal analysis of serum lipoproteins. In Methods of Biochemical Analysis, Glick, D., ed. Interscience Publications, New York, 1954, Vol. I, pp. 459-478.