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Quantitative Lipoprotein Studies in Normal and Abnormal Subjects Using Combined Electrophoretic and Chemical Technics.* (22552)

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The general problem of atherosclerosis may be considered to have 3 major facets: 1) Elucidation of pathogenesis of atherosclerosis; 2) establishment of diagnostic laboratory procedures; 3) evaluation of methods to prevent or treat the disease. The relationship of disturbances in serum lipids to pathogenesis of atherosclerosis has been studied extensively. Interest has centered on distribution of cholesterol, phospholipids, and neutral fat between the two major lipoprotein complexes, the so-called alpha and beta lipoproteins. Barr *et al.*(1) demonstrated a definite correlation between percent distribution of cholesterol in alpha and beta lipoprotein complexes and the presence of clinically evident atherosclerosis and diseases predisposing to atherosclerosis. Cohn fractionation, the method used by these investigators for separation of alpha and beta lipoprotein complexes, is technically difficult, expensive and time consuming.

Several reports have dealt with separation of alpha and beta lipoproteins by paper strip electrophoresis and subsequent semiquantitation of total lipid staining material in the alpha and beta fractions, by means of scanning devices(2). Determination of total lipids in lipoprotein complexes by staining, elution, and colorimetric determination has been reported(3). Kunkel and Slater(4a) and Nikkila(4b) have attempted chemical quantitation of serum cholesterol and phospholipid after paper electrophoretic separation, and subsequent elution of alpha and beta lipoprotein entities.

The present report deals with a method for the separation of alpha and beta lipoprotein complexes by paper strip electrophoresis, and subsequent quantitation of total lipid, total cholesterol, and phospholipid in each of these complexes.

Method. Two 6.5 cm wide Whatman No. 3MM filter paper strips are moistened in a barbital buffer solution (pH 8.6, ionic strength 0.05%). They are then placed in each of 3 plastic boxes having the dimensions of 24 x 18 x 6.5 cm. Each end of the paper strips is immersed in a 1.5 liter buffer reservoir placed at the end of the boxes. 0.20 ml

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of serum is applied by pipette evenly across the width of the paper strip, except for a margin of 1.0 cm at each side. Electrophoresis is carried out for approximately 18 hours, maintaining a constant potential of 80 volts. After this process is complete, the strips are removed from the boxes and dried, then fixed and stained in a solution of bromphenol blue, prepared as follows: to 400 ml of 2% acetic acid add 0.2 g bromphenol blue; mix; add 4.0 g of bichloride of mercury and shake; add slowly dropwise strong hydrochloric acid until the solution changes from red to orange. To this stain, add 45 ml of formaldehyde solution (approximately 37%) and mix. This stain is good for several weeks. After staining and washing in 2% acetic acid, the strips are cut into 2 portions between the alpha and beta components. This effectively separates the albumin and alpha globulins from the gamma and beta globulins, respectively, and includes all stainable material. The 2 portions of each strip are dried in room air, and subsequently treated identically. Each is cut into small pieces and placed in 10 cc of boiling water. This is evaporated nearly to dryness, and then eluted with a hot solution containing equal parts of acetone and ethyl alcohol. The eluates from the 3 corresponding paper portions are combined. The solutions derived from the "alpha and beta lipoprotein complexes," respectively, are divided into 3 aliquots for determination of total cholesterol, lipid phosphorus and total lipid (in duplicate). *Cholesterol determination*: The first aliquot is evaporated to dryness, then taken up in a total volume of 5 ml of acetone alcohol. After hydrolysis of the esterified cholesterol with 5 normal potassium hydroxide, total cholesterol is determined. In this laboratory a modification of the Schoenheimer-Sperry technic is used(5). *Lipid phosphorus determination*: The second aliquot is evaporated to dryness and then dissolved in approximately 10 ml of acetone alcohol, and transferred to a pyrex tube. It is again evaporated to dryness and digested with concentrated sulfuric acid. Digestion is completed with 28% hydrogen peroxide (Superoxal). Determination of phosphorus is then carried out by the method of Fiske and Subbarow(6), and reported as lecithin (x 25). *Total lipid determination*: The third aliquot is evaporated to dryness and extracted with petroleum ether. Total lipid is determined, using the method of Bragdon(7). Arbitrarily, we have reported the difference between the sum of phospholipid plus cholesterol, and total lipid, as neutral fat. Total cholesterol, phospholipid, and total lipid are determined macrochemically on whole serum and the recovery of lipid from the paper strips compared with these totals.

TABLE I. Recovery of $\alpha + \beta$ Lipid Entities (Expressed as Percent of the Same Lipids in Whole Serum).

	Mean % recovery	Stand. dev.
Cholesterol	98.9	4.4
Phospholipid	101.5	6.6
Total lipid	98.2	5.2

Results. Recovery. Data obtained from twenty sera from 14 individuals are included in this report. (Table I).

Normal and abnormal sera. Fig. 1 shows the percent concentration of total lipid, neutral fat, total cholesterol and phospholipid present in the "alpha lipoprotein" (in relation to serum levels of the same lipids) from 6 normal young adult females, 3 normal young adult males, and 5 patients with atherosclerosis or diseases predisposing to atherosclerosis. These patients include a male with diabetes of 18 years' duration associated with hypercholesterolemia; a young female with diabetes of 4 years' duration, with no clinical evidence of vascular disease; a 54-year-old male with angina pectoris and gout; a middle aged male with familial hypercholesterolemia with no clinical evidence of vascular disease; and lastly, a young female with familial hypercholesterolemia with no clinical evidence of vascular disease.

Findings to date show: 1) No obvious separation of normals from abnormals in terms of total lipid or neutral fat distribution; 2) overlapping of normals and abnormals in terms of total cholesterol distribution; 3) sharp separation of normals from abnormals, in regard to phospholipid distribution.

Under way at the present time are com-

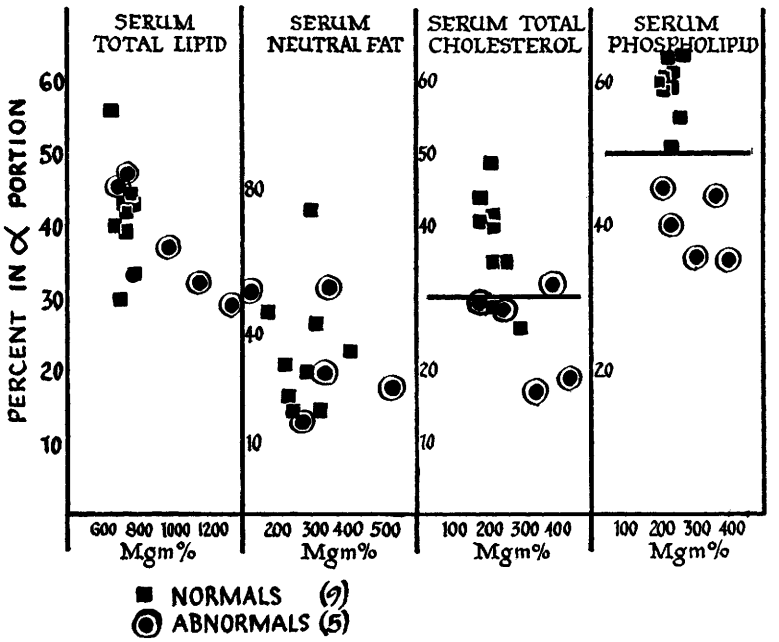

FIG. 1. Serum "α lipid" constituents in normal and abnormal subjects.

parative studies using paper electrophoretic separation and Cohn fractionation in identical sera.

Summary. 1) A method for paper electrophoretic separation and quantitative determination of serum alpha and beta lipoprotein neutral fat, cholesterol and phospholipid, respectively, is described. Studies in 9 normal and 5 abnormal subjects are included. 2) The sums of total lipid, cholesterol and phospholipid recovered after electrophoretic separation of alpha and beta lipoprotein are compared with those values determined macrochemically in untreated sera. Separation of abnormal from normal individuals appears to have been achieved in this small series in terms of distribution of phospholipid, and possibly of cholesterol, in the alpha and beta lipoprotein complexes.

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