Estimations of serum cholesterol from protoporphyrin-stained electrophoretic strips*

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» Lipophylic dye measurements of serum lipoproteins separated by filter paper electrophoresis cannot be converted into chemical units (1). Therefore several investigators (2 to 5) have proposed techniques whereby lipoproteins fractionated electrophoretically are estimated in terms of cholesterol. These methods, however, require preparation of duplicate electrophoretic strips, one of which is stained for lipids. One strip can then be used as a marker for identification of alpha- and beta-lipoprotein areas on the other strip. The staining of reference strips is not only time-consuming but limits by one-half the number of determinations that can be carried out simultaneously in the electrophoretic cell. There is some degree of uncertainty in locating lipoprotein zones in this manner, since adjacent strips rarely demonstrate identical electrophoretic migrations. However, it is not feasible to elute cholesterol directly from strips stained with Oil Red O or Sudan Black B. These dyes, when dissolved in 60% ethanol, are capable of extracting considerable amounts of lipid material from strips during the staining process (1). In addition, the solubility of the lipophylic dyes in the usual cholesterol extractants might be expected to interfere appreciably with subsequent colorimetric measurements. To circumvent these difficulties a fluorescent technique has been employed in our laboratories, whereby electrophoretic strips are stained for lipid with protoporphyrin in a pure aqueous system (6). The use of protoporphyrin as a lipid stain was first described by Kôsaki *et al.* (7, 8, 9) and was utilized by Sulya and Smith (10) to locate purified lipids applied to filter paper. More recently it has been observed that serum cholesterol may be eluted directly from stained strips, since protoporphyrin does not interfere with colorimetric analysis of this lipid.

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Filter paper strips are dried in an oven at 110° for 15 minutes following the electrophoretic separation of 50 μl of serum. The strips are then immersed for 5 minutes in 0.05 N HCl containing 5.0 mg/100 ml of commercially available purified protoporphyrin.[1] Rinsing is achieved by placing the filter papers in distilled water for another 5 minutes. For maximum sensitivity Sulya and Smith (10) suggest that protoporphyrin-stained lipid chromatograms be viewed under ultraviolet light while still damp. However, in our hands, exposure of serum electrophoretic strips to 110° for approximately 15 minutes enhances ultraviolet fluorescence. These strips retain fluorescent qualities for several days, while those allowed to dry at room temperature fade appreciably in a matter of hours.

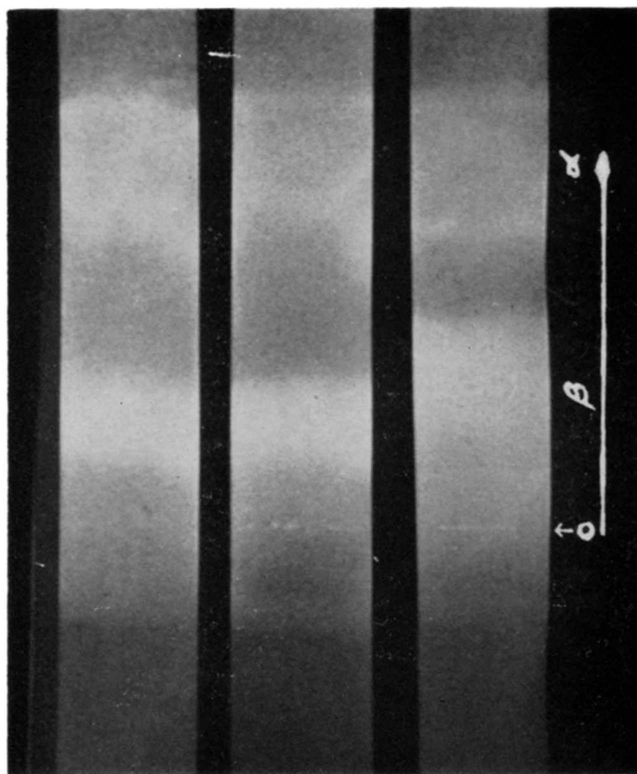

Fig. 1. Fluorescent electrophoretic strips stained with an acid solution of protoporphyrin showing location of α- and β-lipoproteins.

In ultraviolet light the protophorphyrin-treated electrophoretic strips prepared from 50 μl of serum show the major lipid-bearing proteins as brilliant red fluorescent zones against a blue background (Fig. 1). In

[1] California Corporation for Biochemical Research, Los Angeles, Calif.

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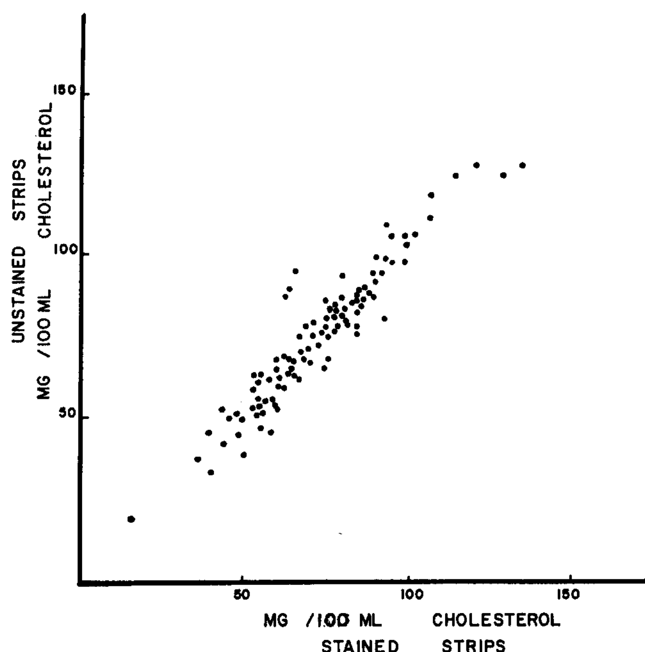

FIG. 2. Comparison of α-lipoprotein cholesterol values obtained from protoporphyrin-stained and unstained electrophoretic strips.

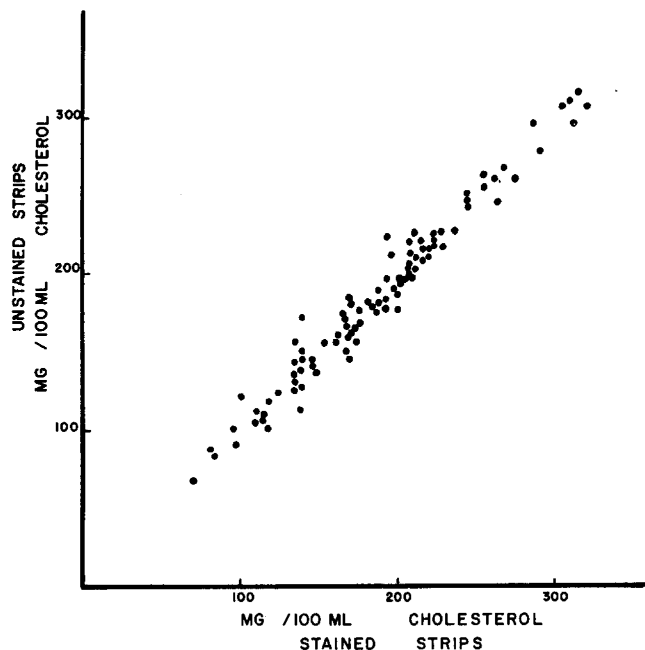

FIG. 3. Comparison of β-lipoprotein cholesterol values obtained from protoporphyrin-stained and unstained electrophoretic strips.

this manner, areas occupied by the alpha- and beta-lipoproteins can be easily identified, marked, and cut from each strip. Cholesterol is extracted in 1 hour by placing each filter paper segment in a test tube containing a measured amount of glacial acetic acid saturated with ferrous sulfate (5). Color is developed from cholesterol by addition of concentrated sulfuric acid to an aliquot of the eluate and estimated on a spectrophotometer at 490 mμ (11).

A series of 100 sera has been subjected to filter paper electrophoresis in order to compare cholesterol recoveries from stained and unstained strips. Lipoprotein cholesterol was isolated directly from one group of strips stained with acid protoporphyrin. Using a duplicate set of electrophoretic separations, the alpha- and beta-lipoprotein areas were identified by a dye marker technique (12), and cholesterol was isolated from unstained strips. No significant differences were observed in the amounts of cholesterol isolated from alpha- or beta-lipoproteins from protoporphyrin-stained or unstained strips (Figs. 2 and 3). These data indicate that lipid-bound protoporphyrin does not significantly interfere with extraction or quantitation of cholesterol by the methods employed. Furthermore, no loss of lipoprotein cholesterol was apparent when strips were treated in the aqueous protoporphyrin system.

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