6. Since this paper was submitted for publication, nine additional sublines of cell cultures were checked for contamination with pleuropneumonialike organisms. One of the sublines yielded growth of pleuropneumonialike organisms.

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Comparative Studies of Lipoproteins by Starch and Paper Electrophoresis

Previous investigations have been concerned with the cholesterol and phospholipid content of serum lipoproteins separated either by paper electrophoresis (*1*) or by starch electrophoresis (*2–4*). This study is concerned with comparative analyses of cholesterol and phospholipid in the various serum lipoproteins separated simultaneously by starch and paper electrophoresis (*5*). Normal serum and serums from patients with idiopathic hypercholesteremia and with idiopathic hyperlipemia were analyzed.

Starch electrophoresis was performed in two parallel blocks 8 by 36 by 1.5 cm using 2.0 ml of serum for each block and applying 450 volts and a current of 18 to 35 ma for 16 to 18 hours in a cold room at a steady temperature of 5°C. Barbiturate buffer of *p*H 8.6 and of ionic strength 0.05 was used. Extraction of the

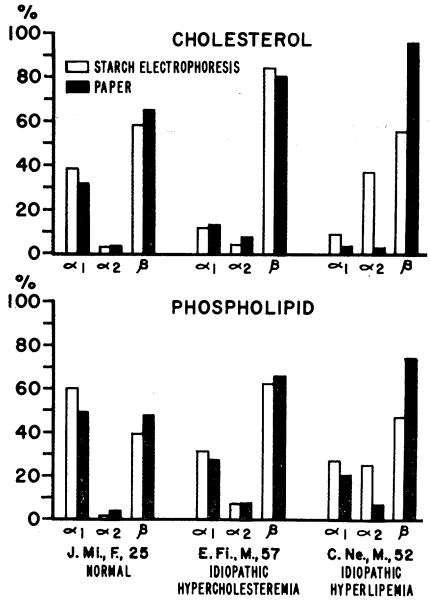

Fig. 1. Cholesterol and phospholipid contents in alpha-1, alpha-2, and beta lipoprotein fractions separated by starch and paper electrophoresis. No significant differences were observed between the two methods of electrophoresis in a normal person or, in persons with idiopathic hypercholesteremia. In idiopathic hyperlipemia, marked increase of cholesterol and phospholipid was seen in the alpha-2 lipoprotein by starch electrophoresis, whereas, by paper electrophoresis, these lipids were mainly increased in the beta lipoprotein.

starch segments (1 cm wide) was performed with a mixture of chloroform and methanol (2/1). The extracts were analyzed for total and esterified cholesterol (*6*) and phospholipid (*7*). Analogous segments of the second block were used for protein determinations by the biuret method (*8*).

Paper electrophoresis was performed in two parallel strips of Whatman 3-MM filter paper (15 by 30 cm) using 0.4 ml of serum and applying 250 volts and a current of 15 to 20 ma for 7 hours. A 2-cm-wide part of one strip was stained with Amidoblack 10B dye for localization of the protein fractions. The buffer and the lipid solvent were identical with those used in starch electrophoresis. Each paper segment (1 cm wide) was analyzed for total and esterified cholesterol and phospholipid (*6*, *7*).

The control serum showed normal lipid partition: cholesterol, 228; phospholipid, 304; triglycerides, 28; and total lipids, 560 mg percent. The lipoprotein fractions alpha-1, alpha-2, and beta that were separated either by starch or by paper electrophoresis showed no significant differences in the distribution of cholesterol and phospholipid (Fig. 1).

In idiopathic hypercholesteremia, the serum was characterized by decided elevation of both cholesterol and phospholipid with a slight elevation of triglycerides and total lipids: cholesterol, 334; phospholipid, 328; triglycerides, 118; and total lipids, 880 mg percent. Regardless of the method of electrophoresis used, the cholesterol and phospholipid contents were markedly increased in the beta lipoprotein fraction and decreased in the alpha-1 lipoprotein fraction (Fig. 1).

In idiopathic hyperlipemia, the serum was characterized by lactescence and by marked elevation of all lipid fractions: total cholesterol, 776; phospholipid, 764; triglycerides, 1460; and total lipids 3000 mg percent. Marked differences in the distribution of cholesterol and phospholipid were observed with the two methods of electrophoresis. When starch was used as the supporting medium, elevation of cholesterol and phospholipid in the alpha-2 lipoprotein fraction was the prominent feature, whereas when paper was used the elevation of these lipids was seen in the beta lipoprotein (Fig. 1). By both methods, decrease of these lipids was observed in alpha-1 lipoprotein.

Considerable adsorption of serum triglycerides (chylomicrons) to the paper was noted at the point of application (*2*). A comparison of the distribution curves of cholesterol and phospholipid by the two methods of electrophoresis showed that large amounts of cholesterol and phospholipid were present in both alpha-2 and beta lipoprotein when starch electrophoresis was used, while the major pattern of these lipids was found in the beta lipoprotein by paper electrophoresis.

The adsorption of serum lipids at the point of application in paper electrophoresis interfered with the migration of some of the cholesterol and phospholipid molecules. When starch was used as supporting medium, no accumulation and adsorption of triglycerides was observed at the point of origin. The triglycerides migrated freely (*4*) and the cholesterol and phospholipid molecules migrated with them. This observation is probably related to the easier extractability of cholesterol "enmeshed in lipids" (*9*).

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Missing Step in Guinea Pigs Required for the Biosynthesis of L-Ascorbic Acid

Man, other primates, and guinea pigs are the only mammals known to be unable to synthesize L-ascorbic acid; thus they require vitamin C in their diet to prevent scurvy. It has not been known

Table 1. Conversion of L-gulonolactone to L-ascorbic acid by rats and guinea pigs.

Species	Conversion* (%)
Rat	9.1
Rat	7.2
Guinea pig	< 0.2
Guinea pig	< 0.2

* Estimated from the amount of C^{14}-labeled L-ascorbic acid present in the animal 24 hours after intraperitoneal administration of 12-mg doses of L-gulonolactone-1-C^{14} (*3*).