ment, as long as the lipid undergoes the inclusion reaction with α-cyclodextrin. The inclusion complexes can be formed on the paper after development of the lipid chromatogram by spraying it with α-cyclodextrin. When reacted with the lipid, α-cyclodextrin is inert to iodine vapors, while in free form it yields a purplish color instantaneously with iodine. Accordingly the lipids are detected as white spots on a purple background. Saturated di- and triglycerides do not react with cyclohexaamylose and cannot be located by this simple device.

	Analysis 1	Analysis 2	Analysis 3
	Acids %	Methyl esters %	Monoacid triglycerides %
Stearic	–	9·3 (10·0)	–
Oleic	30·7 (29·3)	27·5 (26·2)	20·3 (19·2)
Linoleic	23·2 (24·0)	33·4 (31·5)	46·5 (47·0)
Linolenic	46·5 (46·8)	29·8 (32·3)	35·4 (33·9)

These methods are applicable to the quantitative determination of lipids. Mixtures of fatty acids of the C_{18} series, of their methyl esters, or of the unsaturated mono-acid triglycerides have been analyzed by paper chromatography. Some results are given in the following compilation, where the theoretical values are listed in parentheses.

The procedures were essentially the same as previously reported. Siliconized paper (Dow Corning 200 fluid on Whatman No. 1 paper) was used in ascending chromatographic technique. The chromatograms of the fatty acids or esters were developed for 16–20 h at 30° ± 1° C with a mixture of aqueous 88% formic and 85% acetic acids (1:1 by volume) while the triglycerides were separated at 24° ± 1° C with a chloroform-methanol solvent mixture (3:1 by volume). A densitometer was used for measuring the optical densities of brown spots in blue light (filter 445 mμ) and for measuring the transmission of white spots on purple background in green light (filter 525 mμ). Model mixtures of known composition were chromatographed with an unknown on the same sheet, and the areas of the optical absorption curves were compared. After one or two adjustments of the component ratios in the model blends, linear interpolation is permissible for calculating the composition of the unknown.

The direct determination of oleic and stearic acids or their esters, which so far is possible only by paper chromatography, will often be advantageous. Higher unsaturated acids are commonly determined by alkaline isomerization. Notwithstanding merits of this latter method, the smaller amounts required for paper chromatography will make this technique very useful in many cases. To our knowledge, no method for the paper chromatographic separation and assay of triglycerides has been reported previously.

A more detailed report will appear in the near future.

JOANNE L. GELLERMAN and HERMANN SCHLENK

Hormel Institute, University of Minnesota, Austin, Minn., May 24, 1956.

Zusammenfassung

Ein qualitatives Verfahren zur papierchromatographischen Trennung von Fettsäuren und Derivaten liess sich zur quantitativen Bestimmung ausbauen. Die Methode hat eine Fehlergrenze von etwa ± 5% und ist unabhängig von den funktionellen Gruppen der Moleküle. Sie ist für gesättigte Di- und Triglyceride nicht direkt anwendbar.

The Influence of Albumin on the Electrophoretic Mobility of Serum Lipids *

MAURER[1] reported in 1953 that the addition of serum or albumin to the buffer profoundly altered the distribution of phospholipids in paper electropherograms of rat and rabbit sera doubly labelled with S^{35} and P^{32}. While he found almost all of the phospholipids associated with the serum globulins before addition of the "carrier protein", albumin appeared to form a complex with the phospholipids which migrated with the electrophoretic mobility of an albumin.

MAURER's experiments imply that the molecular structure of the lipoprotein complexes, which have been shown to contain most of the serum phospholipids and cholesterol[2] must be based on only very weak associations between the protein and lipid moieties, since albumin appeared to displace the protein component by a simple mass action effect. Such a dependence of lipoprotein structure upon the protein composition of the medium would invalidate much of the work of GOFMAN and PAGE on the determination of lipoprotein subfractions since these ultracentrifugal analyses were carried out in protein-poor solutions. While lipoproteins have long been considered labile structures and have been shown to exchange component lipids even *in vitro*[3] there is some evidence which argues against MAURER's views: It would be very difficult to explain the differences in solubility and electrophoretic mobility between α- and β-lipoproteins within the framework of MAURER's postulate. Moreover, RUHENSTROTH-BAUER[4] has demonstrated recently that the protein moiety of human serum lipoproteins is specific and cannot be replaced by either albumin or γ-globulin. In order to resolve this controversy we have reinvestigated the effect of added albumin on the electrophoretic mobility of serum phospholipid and cholesterol in human, hypercholesterolemic rabbit, and hypercholesterolemic rat sera. The effect upon phospholipid mobility alone was determined in normal rat and rabbit sera, where low cholesterol levels made analysis for this compound impractical.

In all experiments the sera were labelled biologically by administering radiophosphorus** to the donor 48 h before bleeding. Hypercholesterolemia was induced in rabbits by feeding a diet enriched with 1% cholesterol and 5% corn oil for 14 weeks, and in rats by the intra-

* Supported in part by a grant (H-2013) from the National Heart Institute, U.S. Public Health Service.

[1] W. MAURER, Klin. Wschr. *30*, 323 (1952). – W. MAURER and E. R. MULLER, Biochem. Z. *324*, 255 (1953). – W. MAURER, Arch. exper. Path. Pharmakol. *218*, 26 (1953).

[2] *Cf.* J. L. ONCLEY and F. R. N. GURD, in: J. L. TULLIS, *Blood Cells and Plasma Proteins* (Academic Press, New York, 1953), p. 337.

[3] H. G. KUNKEL and H. G. BEARN, Proc. Soc. exper. Biol. Med. *86*, 887 (1954). – W. H. FLORSHEIM and M. E. MORTON, unpublished results.

[4] G. RUHENSTROTH-BAUER, Z. ges. exper. Med. *121*, 475 (1953).

** The Radioisotopes used in this investigation were supplied by the Oak Ridge National Laboratory on authorization from the Isotopes Division, U. S. Atomic Energy Commission.

venous injection of 100 mg "Triton WR-1339"***. Electrophoresis was carried out in barbital buffer of pH 8·8 and ionic strength 0·1 by the "Starch Block" or "Paper Sandwich" techniques of KUNKEL and SLATER[5]. The separation of the α-lipoproteins was better by the "Starch Block" technique since lipoproteins appear to be adsorbed less on starch than on paper. With rat sera, especially, good separations could not be obtained by paper electrophoresis. 1 or 2 cm³ of serum were used for each experiment. Human serum albumin, when employed, was added to the barbital buffer to a concentration of 2·50 g%. When albumin was present in the buffer

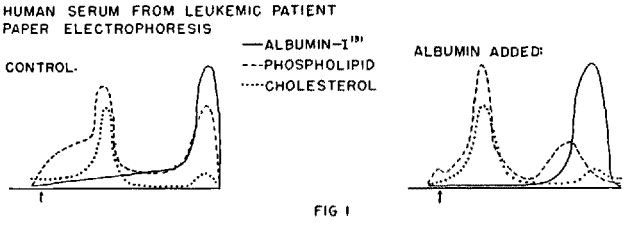

FIG 1

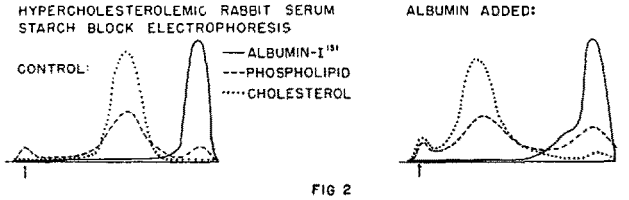

FIG 2

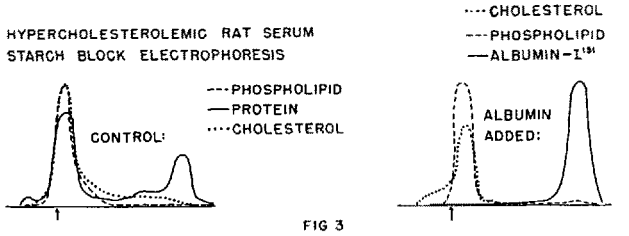

FIG 3

the position of the albumins in the experimental serum was marked by adding tracer amounts of I^{131}-labelled human serum albumin[6], which was shown to be a satisfactory marker for the animal albumins also. Electrophoretic separation was carried out for 24–38 h at room temperature with a potential of 300 V and a current of 10 mA. The electropherograms were cut into 1 cm segments and protein was measured in saline eluates by the Biuret reaction. Cholesterol was determined by the methods of ZLATKIS[7] on aliquots of a Bloor extract. Albumin-I^{131} was assayed with a scintillation counter using a thallium-activated sodium iodide crystal, and radiophosphorus was determined by Geiger counting of a protein-free lipid extract.

Figures 1 to 3 show the results of typical experiments. Our results differ from MAURER's on two crucial points: We were able to demonstrate both α- and β-lipoprotein peaks, but could not detect any albumin-induced shift of either lipid phosphorus or cholesterol from the β-globulin to the albumin region of the electropherograms.

Several possible explanations of these differences can be postulated. In our work much larger volumes of serum were employed, and this tends to minimize adsorption effects[8]. Lipoprotein denaturation appears likely in MAURER's experiments, judging from the large amounts of lipid phosphorus remaining near the origin and from diffuse distribution of the phospholipids migrating in the absence of added albumin. We established that albumin is capable of binding lipids by adding the lipids from deproteinized human lipoproteins to rat serum. Adding albumin to the buffer doubled the amount of extraneous lipids which migrated in electrophoresis with the rat proteins. ARMSTRONG[9] has noted that partial denaturation may increase the electrophoretic mobility of human lipoproteins considerably, which may explain the abnormally high electrophoretic mobility of "lipoproteins" observed by MAURER[10] after the addition of human α- and β-globulin. Denaturation also appears indicated by the fact that MAURER was unable to demonstrate two lipoprotein peaks in rabbit sera. The data of LEWIS[11] show that at least a third of the total lipids is carried as a β-lipoprotein in this species. We believe that under the conditions employed by MAURER an almost complete denaturation of lipoproteins occurred, and that the conclusions drawn by MAURER regarding the nature of protein-lipid binding in serum lipoproteins are therefore not sufficiently substantiated.

W. FLORSHEIM, B. DIMICK, and M. E. MORTON

Radioisotope Service, Veterans Administration Hospital, Long Beach, California, and Department of Physiological Chemistry, University of California Medical School, Los Angeles 24, California, June 4, 1956.

Zusammenfassung

Im Gegensatz zu MAURERS Angaben konnte keine Wirkung des dem Puffer zugegebenen Serum-Albumins auf die elektrophoretische Wanderung von Serumphospholipiden und Cholesterin im menschlichen und tierischen Serum festgestellt werden. MAURERS Befunde scheinen auf einer durch Adsorptionseffekte bewirkten Veränderung der Lipoproteine zu beruhen.

*** Rohm & Haas Co., Philadelphia, Pennsylvania, U.S.A.

[5] H. G. KUNKEL and R. J. SLATER, Proc. Soc. exper. Biol. Med. *80*, 42 (1952).

[6] R. C. GILMORE, M. C. ROBBINS, and A. F. REID, Nucleonics *12*, 65 (1954).

[7] A. ZLATKIS, B. ZAK, and A. J. BOYLE, J. Lab. clin. Med. *41*, 486 (1953).

[8] A. MARIANI, R. C. Ist. sup. Sanità *15*, 337 (1952).

[9] S. H. ARMSTRONG Jr., M. J. E. BUDKA, and K. C. MORRISON, J. Amer. chem. Soc. *69*, 416 (1947).

[10] W. MAURER and E. R. MULLER, Biochem. Z. *324*, 255 (1953).

[11] L. A. LEWIS, A. A. GREEN, and I. H. PAGE, Amer. J. Physiol. *171*, 391 (1952).

Hypertension expérimentale et protéines sériques

Différents groupes d'auteurs (BOUCHNUT, FROMENT et GRASSET[1], PAGE et CORCORAN[2], RAASCHOU[3], LOYKE[4])

[1] L. BOUCHNUT, R. FROMENT et E. GRASSET, Lyon méd. *160*, 3 (1937).

[2] I. PAGE and A. CORCORAN, Ann. Rev. Med. *2*, 51 (1951).

[3] F. RAASCHOU, Circulation *10*, 511 (1954).

[4] H. LOYKE, Amer. J. med. Sci. *230*, 627 (1955).