The Differentiation of Exogenous and Endogenous Hyperlipemia by Paper Electrophoresis *

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In the clinical study of hyperlipemia, it is useful to know whether the excess plasma glycerides are due to inadequate clearance of dietary fat ("fat-induced" hyperlipemia) or to release of glyceride by the liver at a rate exceeding capacity for removal ("endogenous" hyperlipemia). When the endogenous glyceride appears to accumulate in response to increases in dietary carbohydrate, the term "carbohydrate-induced" hyperlipemia is often used (2).

The most direct way to make this distinction is to feed a high-carbohydrate diet containing practically no fat for 1 to 4 weeks, followed by an isocaloric diet in which fat represents at least 40% of calories and to obtain serial plasma glyceride measurements during both dietary periods (2, 3). Sometimes, fat induction can also be inferred from the presence of a normal plasma cholesterol concentration when the glycerides are markedly increased or by the observation that alimentary particles [1] (chylomicrons) rise to the top of the plasma under appropriate conditions, leaving a clear infranatant layer. The preparative ultracentrifuge can sometimes be used to accelerate this separation. If hyperlipemia arises from both exogenous and endogenous sources, however, none of these tests may provide a decisive answer.

Two more specific methods have recently been developed for characterization of the glyceride-rich lipoproteins and particles that occur in hyperlipemia. Starch-block electrophoresis has been adapted to define three different peaks of "particles" in plasma, two being associated with exogenous hyperlipemia and one representing endogenously synthesized glycerides (5, 6). Polyvinylpyrrolidone (PVP) density gradient flocculation has been devised to achieve the same separations (6, 7).

Earlier experience with electrophoresis on paper in buffer containing albumin (1, 8, 9) has suggested that the distinction between the two forms of hyperlipemia might be made by this simple and practical technique. The behavior on paper of the various glyceride-rich lipoproteins and particles in plasma is here compared with the results obtained by other methods, and the possible value of paper electrophoresis in studies of hyperlipemia is explored.

Methods

Subjects

Seventeen volunteers participated in studies of the effects of dietary fat and carbohydrate on plasma lipoproteins. They were defined as normal because their parents and siblings had no history of metabolic disease, their glucose tolerance was normal, and while on a free diet their plasma cholesterol and glyceride concentrations were within 2 SD of "normal" mean values for this laboratory (3). Fifteen of these subjects were 17 to 21 years of age (nine male and six female), and two were males 30 and 40 years of age. Patients with hyperlipoproteinemia were also studied (Table I). Five had the relatively rare familial fat-induced hyperlipemia, the "Type I" syndrome as defined elsewhere (10). Eleven other patients (Table I) represented at least two other types of hyperlipemia.

Metabolic diets

All subjects were fed ordinary foods, in amounts necessary to maintain constant body weight, as one of three major diets: 1) a normal diet in which fat represented 40

[1] The name "particles" has been applied (4) to lipoproteins of size large enough ($> 0.1 \ \mu$) to be seen with the light microscope. These particles all have $D < 1.006$ and $S_f > 400$; they therefore represent a subclass of "very low density lipoproteins" as defined in the ultracentrifuge (Table II).

to 45% of calories, carbohydrate 45 to 55% of calories, and protein the remainder; 2) a high-carbohydrate diet containing 70 to 80% of calories as carbohydrate, less than 5 g fat, and the rest protein; 3) a high-fat diet containing 60 to 85% of calories as fat, 5 to 15% of calories as carbohydrate, and the rest as protein.

Single fat feedings

A single large fat meal was fed to some subjects so that the glyceride particles of alimentary origin might be isolated in quantity. This was usually 250 g of corn oil, emulsified with skim milk, or as a custard with egg white. Occasionally either 100 g of mixed fat as bacon and eggs or 250 g of cocoa butter was fed. Blood was drawn 3 to 12 hours after the meal, and the plasma was stored at room temperature until used. For these and all other blood samples, disodium ethylene diamine tetraacetic acid (EDTA), 1 mg per ml of blood, was used as anticoagulant.

Isolation of lipoproteins and particles

Electrophoresis. Paper electrophoresis was performed by a modification of the Durrum hanging-strip method with barbital buffer containing 1% albumin (8). Starchblock electrophoresis was performed by the method of Kunkel and Slater (11), as modified by Bierman, Gordis, and Hamlin (5). After electrophoresis for 16 to 20 hours at constant current at an initial potential drop of 6 v per cm, the starch block was cut into half-inch segments. These were placed in test tubes to which 3 ml of 0.9% saline in 0.001 M EDTA was added. After shaking and centrifugation at 1,000 × g for 15 minutes, the supernatant was removed for further analysis. Particle recovery, calculated as recovery of the original sample turbidity in the starch-block eluates, was about 75%.

Flocculation techniques. Flocculation in PVP density gradients was carried out according to the method of Gordis (7).

Preparative ultracentrifugation. Ultracentrifugation was performed at 15° C in the Spinco model L ultracentrifuge with the 40 and 40.3 rotors. Supernates were removed by tube slicing. Particle concentrates were prepared by centrifugation of plasma or starch-block eluates at D 1.006 for 3×10^6 g minutes (4); the packed particles were resuspended by repeated passage through a no. 25 needle. These preparations inevitably contained some very low density lipoproteins, since ultracentrifugal separations in this density range are not perfect. Particle-free infranates were used for isolation of very low density lipoproteins (see Table II) by further centrifugation at D 1.006 for 16 hours at 100,000 × g (4). The preparations were washed once by ultracentrifugation into overlying 0.9% saline solution in 0.001 M EDTA.

Nomenclature. Various names have been applied to the lipid-protein complexes in human plasma, depending on the analytical system used. These operational definitions are correlated in Table II with alpha, beta, and pre-beta lipoprotein and chylomicron bands defined by the paper electrophoretic method.

TABLE I

Characteristics of the hyperlipemic patients participating in the studies*

Subject	Sex	Age	Lipoprotein pattern	Glyceride
		years		*mg/100 ml*
J.P.	M	32	Type I	2,014
P.P.	M	18	Type I	4,361
L.W.	M	21	Type I	4,345
G.W.J.	F	18	Type I	3,374
G.S.	F	2	Type I	5,272
I.R.	M	60	Type III	811
E.A.	M	44	Type III	480
J.G.	M	45	Type III	1,310
F.S.	F	54	Type III	600
J.I.	M	36	Type III	1,424
E.G.	F	·64	Type IV	7,760
S.L.	F	62	Type IV	1,721
S.P.	M	58	Type IV	285
E.M.	F	51	Type IV	250
G.G.	M	23	Type IV	>1,000
J.Gr.	M	1	Type IV	497

* The lipoprotein pattern, expressed in terms of a classification developed for familial hyperlipoproteinemia (10), and glycerides were determined while the patient was on an *ad libitum* diet. More detailed description of most of these patients appears elsewhere (3).

Determination of glyceride fatty acid composition. Lipoprotein bands from paper electrophoretic strips were eluted for analysis of glyceride fatty acids as follows: Strips were run in replicate. One was stained with oil red O, and areas were cut from one or two unstained strips corresponding to the lipoprotein bands revealed by staining. These were further subdivided into pieces of approximately 1 × 0.1 cm and shaken for 5 minutes in 12.5 ml of chloroform-methanol (2:1) in a glass-stoppered centrifuge tube. The phases were split by addition of 2.5 ml of aqueous H_2SO_4, 1:2,000. The chloroform phase was evaporated to dryness, taken up in 50 μl of chloroform, and applied to thin-layer plates coated with silica gel G.[2] The chromatograms were developed with petroleum ether: ethyl ether: acetic acid (85:15:1) for approximately 45 minutes and air dried. The plates were sprayed with a solution of 2,7-dichlorofluorescein in ethanol and the triglyceride spot scraped into a glass-stoppered tube and transesterified with sodium methoxide in absolute methanol for 30 minutes at 65° C (13).

Gas-liquid chromatography. Fatty acid methyl esters were quantified by gas-liquid chromatography on 12-foot ethylene glycol succinate columns at 190° C with argon as carrier gas and a radium ionization detector. The composition of a National Heart Institute Standard D as determined by this system agreed within 10% for the major and within 20% for the minor components of this mixture (14).

The corn oil and cocoa butter that were fed to induce alimentary particles to appear in plasma, and lipoprotein

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[2] Merck, Darmstadt, Germany.

TABLE II

Definitions used in various procedures for lipoprotein analysis

Paper electrophoresis (8)* (See Figure 1)	Chylomicrons	Mobility: zero, all remains at the origin.
	Beta lipoproteins	Mobility: that of β_1-globulin.
	Pre-beta lipoproteins	Mobility: slightly greater than beta lipoprotein.
	Alpha lipoproteins	Mobility: between α_1-globulin and albumin.
Ultracentrifugation (12)	Very low density lipoproteins	$S_f > 20$ and $D < 1.006$.
	Low density lipoproteins	S_f 0–20 and D 1.006–1.063; identical to beta lipoprotein.
	High density lipoproteins	$D > 1.063$, identical to alpha lipoproteins.
Starch block electrophoresis (5, 6)	Primary particles	Mobility: alpha$_2$; found only after fat ingestion.
	Secondary particles	Mobility: beta; found only after fat ingestion.
	Endogenous particles	Mobility: alpha$_2$; found postprandially in plasma, unrelated to fat ingestion.
PVP† density gradient flocculation (6, 7)	Primary particles	Particles that flocculate at the top of the density gradient tube.
	Secondary particles	Those that flocculate at the bottom of the tube.
	Endogenous particles	Those that are distributed as a hazy flocculate throughout much or all of the tube.

* Numbers in parentheses refer to pertinent references.
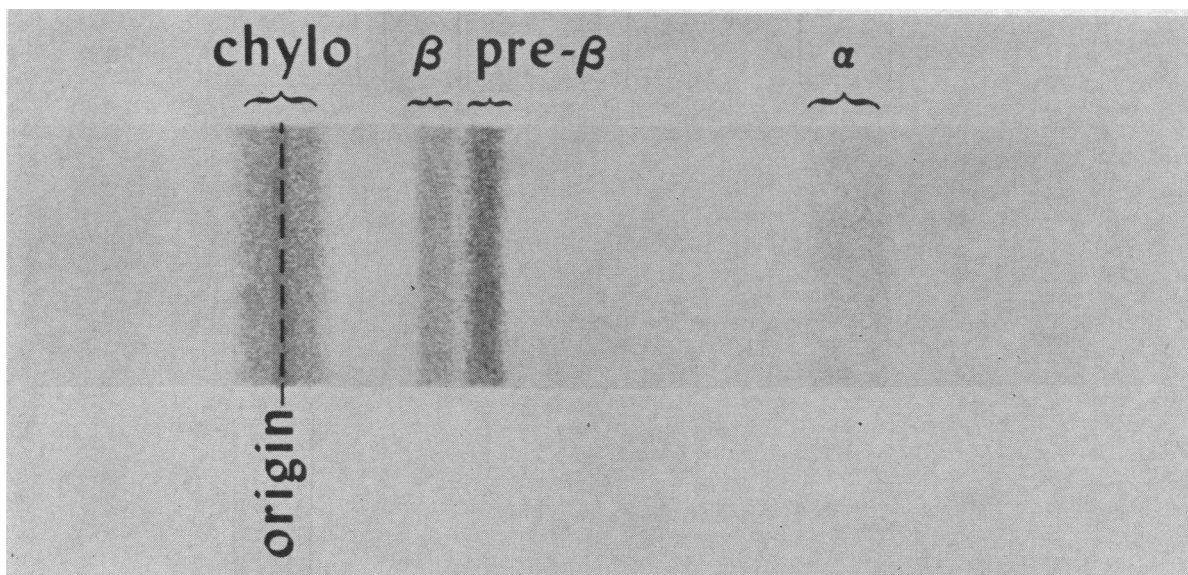† PVP = polyvinylpyrrolidone.

FIG. 1. A DIAGRAM OF THE FOUR BANDS THAT MAY BE SEEN IN HUMAN PLASMA ON PAPER ELECTROPHORESIS IN BARBI-TAL-ALBUMIN BUFFER (6). The anode is to the right.
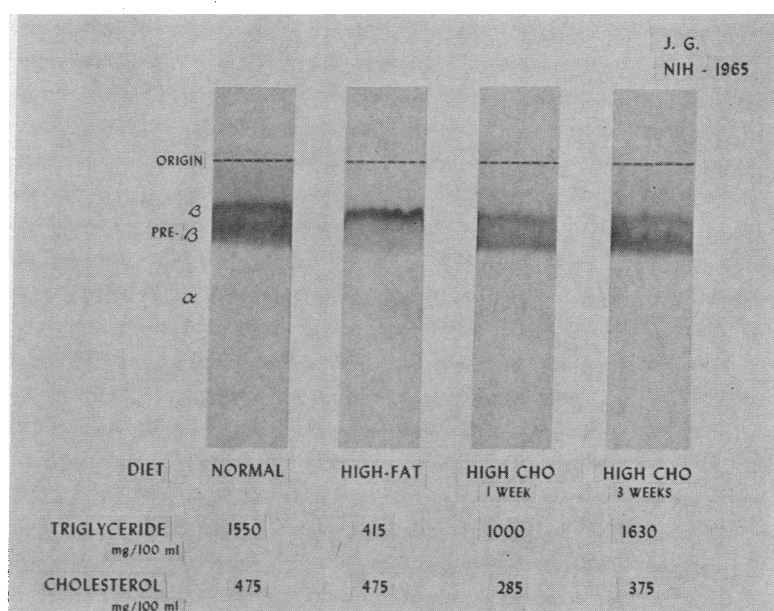
FIG. 2. LIPOPROTEIN AND LIPID ANALYSES DURING METABOLIC STUDY OF PATIENT J.G. The plasma lipoprotein patterns (reading from left to right) are those obtained after 2 weeks of normal diet, 3 weeks of high-fat diet, and 1 and 3 weeks of high-carbohydrate (CHO) diet.

fractions isolated by other methods, were extracted by the method of Folch, Lees, and Sloane Stanley (15) ; the chloroform extracts were treated as described for the extracts from paper electrophoretic strips.

Chemical analyses. Cholesterol was estimated with the autoanalyzer (16). Triglyceride was determined by the method of Jagannathan (17).

Results

Origin of the glycerides in the pre-beta band

Plasma was obtained from 12 normal subjects and from 7 patients with hyperlipemia after they had fasted overnight. All had been fed the high-carbohydrate diet for periods of 3 to 21 days. A pre-beta band was now present in the plasma of every normal subject and was enhanced if present earlier in the hyperlipemic patients. In none of the plasma samples was there a chylomicron band. This effect of the high-carbohydrate diet on the lipoprotein pattern of J.G. (Table I), a patient with carbohydrate-induced hyperlipemia, is shown in Figure 2. After he had eaten a normal diet for 2 weeks, his plasma had a milky appearance in the

TABLE III

*The fatty acid composition of the glycerides of pre-beta lipoprotein**

Subject	Diet	14:0	16:0	16:1	18:0	18:1	18:2
		%	%	%	%	%	%
Normal B.W.	High CHO	2.8	41.3	7.8	2.7	40.0	5.4
Normal L.K.	High CHO	2.2	34.7	9.5	1.5	42.6	9.5
Normal S.M.	High CHO	4.2	35.7	8.3	2.9	40.2	8.7
Normal N.R.	High CHO	3.4	46.5	7.6	4.3	34.3	3.9
Carbohydrate-induced hyper-lipemic F.S.	High CHO	3.4	43.7	7.9	4.3	33.0	7.7
Carbohydrate-induced hyper-lipemic J.I.	High CHO	2.0	38.8	4.7	3.9	44.8	5.8

* The acids between 14:0 and 18:2 were quantified, and each was expressed as a per cent of the total.   The fatty acids are represented by chain length and number of double bonds as suggested by Dole and co-workers (18).   CHO = carbohydrate.
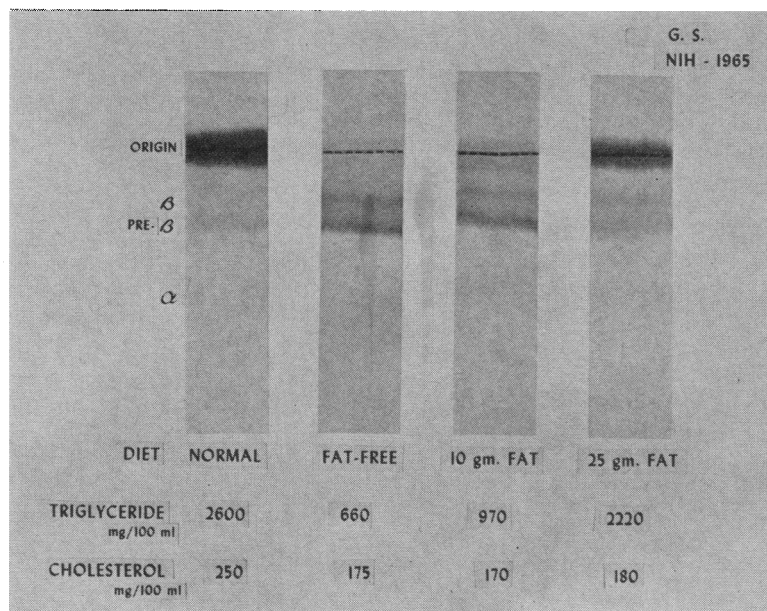
FIG. 3. LIPOPROTEIN AND LIPID ANALYSES DURING METABOLIC STUDY OF PATIENT G.S. The plasma lipoprotein patterns (reading from left to right) are those obtained after 1 week of normal diet, 10 days of fat-free diet, 4 days at 10 g of fat per day, and 10 days at 25 g per day.

postabsorptive state. There were dense beta and pre-beta bands but no chylomicron band (Figure 2). After he had been fed the high-fat diet for 3 weeks, his plasma became clear and the pre-beta band practically disappeared. Within 1 week after he was changed to a high-carbohydrate diet, hyperlipemia reappeared with accumulation of pre-beta lipoproteins, but again there were no detectable chylomicrons (Figure 2).

The triglycerides in the pre-beta lipoproteins from four of the normal subjects and two of the patients were isolated by preparative paper electrophoresis. As shown in Table III, these glycerides contained a preponderance of fatty acids consistent with their having originated mainly from endogenous synthesis (2, 18).

Origin of the triglycerides in the chylomicron band.

One hundred g of mixed fat was fed to five normal subjects in whose plasma neither chylomicron nor pre-beta bands were seen in the fasting state. Blood was drawn 8 to 12 hours later and the plasma subjected to paper electrophoresis. In each case, a faint chylomicron band, but no pre-beta band, appeared. The effect of feeding fat was much more dramatic in a patient with fat-

induced hyperlipemia (Figure 3). While this child (G.S., Table I) was on a normal diet and in the postabsorptive state, her plasma contained on electrophoresis an extremely dense chylomicron band. The beta, pre-beta, and alpha lipoprotein bands were barely perceptible. The chylomicron band disappeared after she had been switched to a high-carbohydrate, virtually fat-free diet. At this time there now appeared a dense pre-beta band similar to that seen in other subjects on the high-carbohydrate diet. The beta and alpha lipoprotein bands were also more apparent. Four days after she was placed on a diet including only 10 g of fat per day, chylomicrons were again present in her plasma in the postabsorptive state. After she had been on 25 g of fat per day for 10 days, her plasma contained an excess of chylomicrons similar to that present when she ate a normal diet (Figure 3).

The divergence of the glyceride fatty acid patterns in the chylomicron and pre-beta bands was demonstrated simultaneously in E.M. (Table I), whose plasma, when she was in the fasting state, contained a distinct pre-beta band. Three hours after she was fed 50 g of corn oil, her plasma glycerides had risen from 265 to 435 mg per 100 ml,
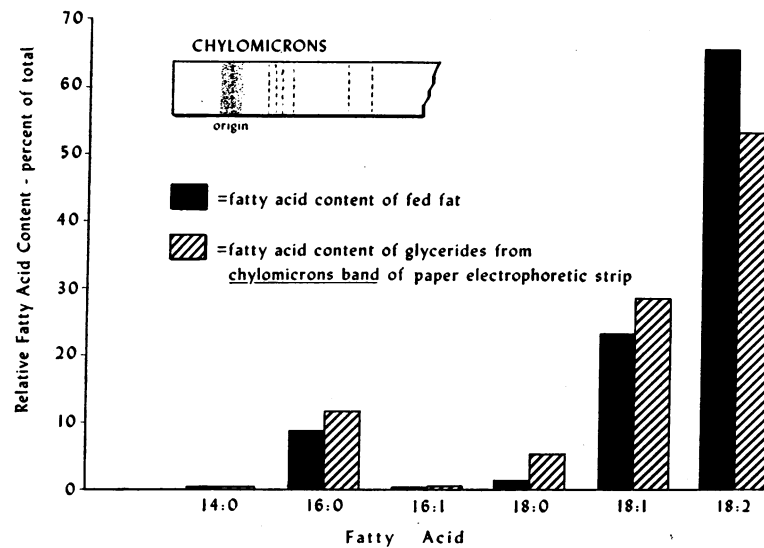
FIG. 4. THE FATTY ACID PATTERN OF CHYLOMICRON GLYCERIDES IN 3-HOUR POSTPRANDIAL PLASMA OF PATIENT E.M. Each fatty acid is represented by chain length and number of double bonds as suggested by Dole and co-workers (18).

and there were both a chylomicron and a pre-beta band. These were eluted and the glyceride fatty acid compositions compared (Figures 4 and 5). The fatty acids in the chylomicrons (Figure 4) closely resembled those of the corn oil, whereas those in the pre-beta band (Figure 5) had a higher content of palmitic and oleic acids than did the chylomicron glycerides and a much lower content of linoleic acid. This fatty acid pattern of pre-beta glycerides was comparable to that seen in the
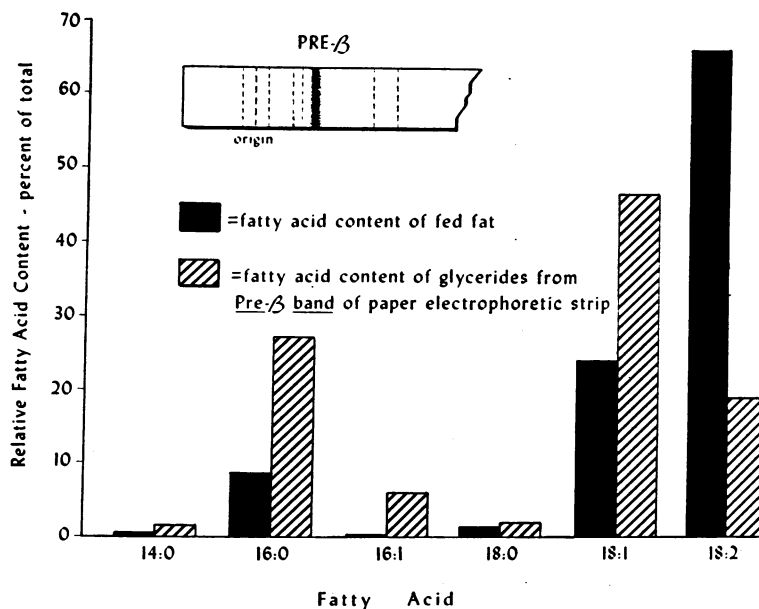

FIG. 5. THE FATTY ACID PATTERN OF PRE-β GLYCERIDES IN 3-HOUR POST-PRANDIAL PLASMA OF PATIENT E.M. The fatty acids are represented as in Figure 4.

six subjects shown in Table III, with the exception of a moderate increase in linoleic acid content derived from the fed fat. Similar appearance of some fed fat in endogenous lipoproteins has previously been reported (18).

Correlation of bands with particles separated by other methods

The lipoproteins and particles separated by paper electrophoresis, starch block electrophoresis, and PVP flocculation were compared in several ways, particularly to avoid artifacts that might arise during attempts to isolate particles by one technique and sequentially run them on another system.

Sequential electrophoresis on starch and paper. In plasma samples from a normal subject fed fat and from a patient with fat-induced hyperlipemia (P.P., Table I), primary and secondary particles were separately isolated on starch blocks. These were resuspended in isotonic saline and applied to paper for electrophoresis. Both the primary and secondary particles remained at the origin on paper. Plasma was obtained from another patient abnormally subject to fat induction while he was on the normal diet (J. P., Table I). The particles
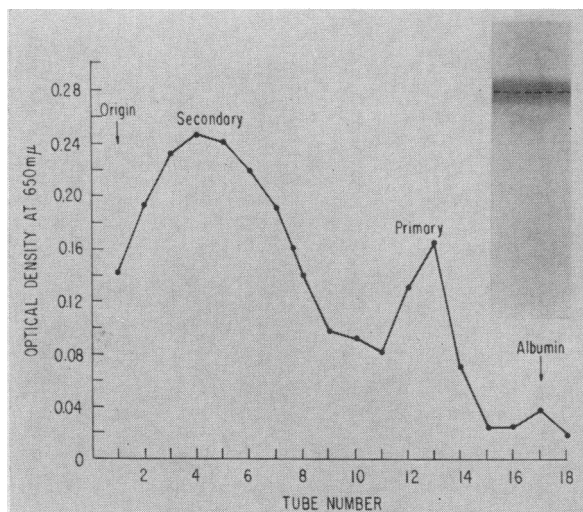

FIG. 6. A COMPARISON OF STARCH-BLOCK AND PAPER ELECTROPHORESIS OF ULTRACENTRIFUGALLY ISOLATED NORMAL ALIMENTARY PARTICLES. The line drawing represents the turbidity of particles eluted from half-inch segments from a starch block. The inset shows the identical sample run on paper electrophoresis and stained for lipid with oil red O.

in this plasma were distributed on starch in a single broad peak that extended from the beta to alpha$_2$ regions. In the PVP gradient tube, the turbid particles formed distinct top and bottom layers, but some opalescence was also present throughout the tube.

The starch block was cut in narrow regions in the beta and alpha$_2$ zones. The eluate from the beta zone, corresponding to secondary particles as defined by Bierman and his associates (Table II), all remained at the origin in the position of chylomicrons. The eluate from the alpha$_2$ zone of the starch block was separated into two bands on paper. One remained at the origin, and the second ran as a pre-beta band. These results are compatible with the presence of both "primary" and "endogenous" particles in the alpha$_2$ region on starch (6). The primary particles behaved as chylomicrons on paper; the endogenous material migrated to the pre-beta region.

Simultaneous electrophoresis and flocculation. Plasma samples obtained from four normal subjects not less than 9 to 12 hours after they had eaten 100 g of mixed fat were also simultaneously separated by the three methods. In all samples, only secondary particles were present as defined by the starch-block (5) or PVP technique (7). On paper electrophoresis, each plasma sample contained a chylomicron band but no pre-beta band. Plasma samples from patients E.G. and I.R. (Table I), which contained opalescence only in the intermediate zone in the PVP gradient tubes as described for endogenous particles (6), contained by paper electrophoresis a pre-beta band but no chylomicrons.

Analysis of particles isolated by ultracentrifugation. Plasma was obtained from two normal subjects 12 hours after they had eaten 250 g of cocoa butter. The particle fraction was isolated by ultracentrifugation. Human albumin was added to a final concentration of 4% to provide a marker for measurement of mobility and the suspension subjected to simultaneous starch-block and paper electrophoresis. Figure 6 illustrates that whereas both primary and secondary particles were present in the starch block, only a dense chylomicron band appeared on paper.

Trailing of pre-beta lipoprotein. On paper electrophoresis of hyperlipemic plasma, a "trail" of lipid-staining material is frequently seen extend-

ing from the pre-beta region to the origin. This effect is due to the presence of lipoproteins and particles of very low density (8, 9, 19). In the present studies, trailing was seen in some degree whenever pre-beta lipoprotein was present, even in pure preparations of this material from patients on fat-free diets (Figures 2 and 3). To examine the relationship of the size of pre-beta lipoproteins to their tendency to trail, plasma was obtained from J.G. after he had been on the fat-free diet for 3 weeks (Figure 2). Lipoprotein fractions were separated from this lactescent plasma in the preparative ultracentrifuge, under conditions defined in the nomogram of Dole and Hamlin (4), to obtain classes of lipoproteins corresponding approximately to $S_f > 10^5$, S_f 400 to 10^5, and S_f 20 to 400. The preparations were not further washed after the initial separation. On paper (Figure 7), the $S_f > 10^5$ fraction extended from the pre-beta region to the origin as a light and uniformly stained area. The major fraction of endogenous particles (S_f 400 to 10^5) and the S_f 20 to 400 lipoprotein fraction each formed a distinct pre-beta band again with a faint trail extending uniformly to the origin. The staining of the trail produced by particles of S_f 400 to 10^5 was proportionately heavier. The tendency of the pre-beta lipoproteins and particles to trail appears to be directly related to their size and inversely related to their density.

Pure chylomicron preparations do not show any tendency to migrate away from the origin, but instead diffuse as a discrete band around it (Figure 6). When there are sufficient chylomicrons to form a discrete peak or band in the starch or PVP separation, they can be seen as a discrete band around the origin even in the presence of the trail that commonly follows pre-beta lipoproteins. An example of this can be seen in Figure 3 (strip identified as on 10-g fat diet). When the plasma is extremely lipemic, a one- or twofold dilution with saline sharpens this distinction.

Discussion

These studies demonstrate that paper electrophoresis in buffer containing albumin is capable of separating chylomicrons from the particles and very low density lipoproteins in which glycerides of mainly endogenous origin are transported.
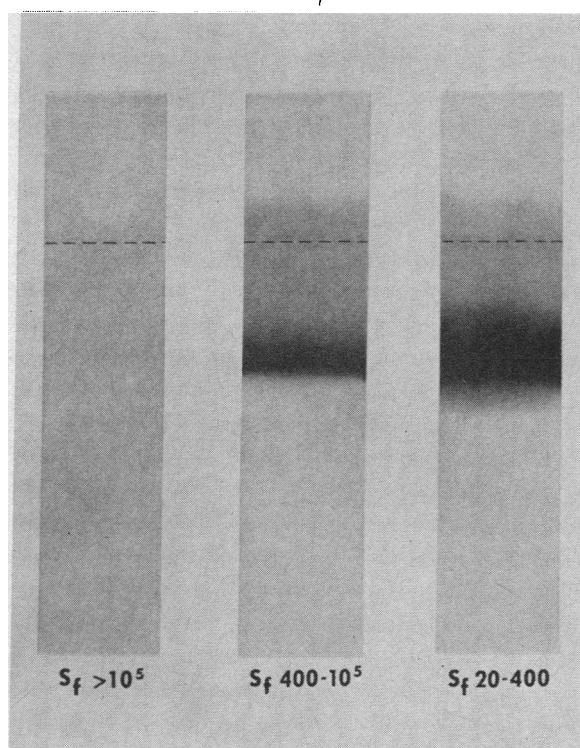

FIG. 7. THE TRAILING OF PRE-BETA LIPOPROTEINS. The electrophoretic patterns are those of ultracentrifugal subfractions of pre-beta lipoproteins obtained from patient J.G. The $S_f > 10^5$ fraction, of which there is very little, trails from the pre-beta region to the origin as a uniformly stained area. There is proportionately greater trailing of the S_f 400 to 10^5 than of the S_f 20 to 400 subfraction.

Chylomicrons do not migrate on paper electrophoresis but remain at the origin as a discrete band. The glycerides in this band have a fatty acid pattern closely resembling the ingested fat. The chylomicron band includes both the primary and secondary particles as defined by starch-block electrophoresis (5) or PVP gradient flocculation (7).

In contrast, endogenous particles as defined by using starch or PVP (6) and very low density lipoproteins as defined in the ultracentrifuge migrate to the pre-beta position on paper. The pre-beta band contains glycerides with a fatty acid pattern consistent with their predominantly endogenous origin. A correlation of the results obtained by paper electrophoresis with those of other techniques is presented in Table IV.

The chemical or physical bases for the convenient separation obtainable on paper, which occurs only in the presence of albumin in the buffer

TABLE IV

The relationship of the chylomicron and pre-beta bands obtained by paper electrophoresis to other lipoprotein analytical methods

Band on paper electrophoresis	Migration in starch-block electrophoresis (5, 6)	Location in PVP density gradient (6, 7)	Approximate S_f by ultracentrifugation (8)
Chylomicron	Alpha₂ (primary particles)	Top	> 400
Chylomicron	Beta (secondary particles)	Bottom	> 400
Pre-beta	Alpha₂ { Endogenous particles / Very low density lipoproteins }	Throughout the tube	> 400
			20–400

(8), are not entirely known. The pre-beta lipoproteins have been shown to contain both alpha and beta lipoproteins, or at least their characteristic proteins (20), and this probably accounts for the intermediate mobility of these lipoproteins on paper and starch. Any relationship between the protein content of chylomicrons and their immobility on paper is difficult to assess. There is no agreement as to the nature of possible "chylomicron proteins" (4, 21, 22), but their total protein content is very small (21), giving a low ratio of charge to mass. It seems most probable that the chylomicrons remain at the origin on paper because of this and because of their large size.

Paper electrophoresis can be used to distinguish only two groups of glyceride-bearing particles in contrast to either starch or PVP, which segregate three types. The consolidation of what on starch are separately isolated as primary and secondary particles, however, can be considered a practical value of the paper technique, providing the simplest distinction between exogenous and endogenous glycerides. Although the earlier work suggested otherwise (5), Bierman and Strandness have recently found (23) that primary and secondary particles represent different particulate forms of alimentary glycerides.

The first two patients whose responses to diet were described in the Results offered a clear-cut distinction between fat and carbohydrate induction of hyperlipemia. In many patients, however, hyperlipemia is exacerbated by both high-fat and high-carbohydrate diets. Both chylomicrons and endogenous particles may simultaneously collect in plasma even while such patients are on a normal diet. The distinction between the two types of particles is preserved even though there may be

trailing of endogenous glycerides in the region where the chylomicrons are concentrated.

We have used the paper electrophoretic technique for the diagnosis and follow-up of several hundred patients with abnormal blood lipid concentrations. These include more than 50 patients in whom the results were correlated with direct demonstration of fat or carbohydrate induction of hyperlipemia under carefully controlled metabolic diets. Dietary testing and plasma glyceride analyses are still necessary to establish whether endogenous hyperlipemia is due to abnormal carbohydrate induction, which is only quantitatively different from the normal response to increased dietary carbohydrate (24). However, the paper technique does permit the recognition of fat-induced and "mixed" hyperlipemia without dietary testing and further enables one to estimate, at any point in time, the proportion of plasma glycerides that has come from endogenous and exogenous sources. It provides a simple and rapid way to follow and record studies of lipoprotein metabolism. The lipoprotein patterns provided by this paper electrophoretic system have been proposed as the basis of a new classification of familial hyperlipoproteinemia (10).

Summary

The plasma lipoproteins and "particles" separated by paper electrophoresis in barbital buffer containing 1% albumin have been compared with those separated by starch electrophoresis, polyvinylpyrrolidone gradient flocculation, and the ultracentrifuge. The lipid-protein complexes utilized were obtained from both normal subjects and patients with hyperlipoproteinemia who were fed diets high in fat or in carbohydrate to produce an

increase in plasma glycerides of exogenous or endogenous origin, respectively. The fatty acid content of these glycerides was analyzed after elution from the paper.

Chylomicrons, which contain exogenous or alimentary glycerides, remain at the origin on paper and correspond to both primary and secondary particles as defined by starch and polyvinylpyrrolidone. Particles and very low density lipoproteins (D < 1.006) containing endogenously synthesized glycerides migrate to the pre-beta position on paper.

This simple system makes possible the immediate recognition of fat-induced and "mixed" hyperlipemia; it is a useful adjunct to dietary testing in the establishment of carbohydrate induction of hyperlipemia. It also provides, at minimal expenditure, a useful means to follow and record studies of lipoprotein metabolism.

Acknowledgment

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