für die Gesamte Virusforsch., 1961, v11, 209.

8. Moscovici, C., La Placa, M., *Giorn. di Microbiol.*, 1961, v9, 135.

9. Burnet, F. M., Bull, D. R., *Aust. J. Exp. Med., and Biol. Sci.*, 1943, v21, 55.

10. Johnson, K. M., Bloom, H. H., Mufson, M. A., Chanock, R. M., *J.A.M.A.*, 1962, v179, 112.

Received May 24, 1962. P.S.E.B.M., 1962, v110.

Demonstration of Two Kinds of Fat Particles in Alimentary Lipemia with Polyvinylpyrrolidone Gradient Columns.* (27608)

ENOCH GORDIS (Introduced by V. P. Dole)
The Rockefeller Institute, New York City

It has long been known that the fat particles of blood plasma are not all alike. Gage and Fish(1) distinguished large and small particles with the dark-field microscope. More recently, Albrink(2) classified the particles as "light chylomicrons" and "heavy chylomicrons". The "light chylomicrons" floated from plasma into isotonic saline after centrifugation at 20,000 G $\times$ 1 hr (1.2×10^6 G min), while the "heavy chylomicrons" floated up only after additional centrifugation at 105,000 G $\times$ 22 hrs (1.32×10^8 G min). Kuo *et al.*(3) centrifuged the fat particles of alimentary lipemic plasma into saline at 58,000 G $\times$ 30 min (1.7×10^6 G min). Some of the particles were packed into a thick "cream" layer at the top of the centrifuge tube, and others floated as a less turbid "saline" layer just below the "cream" layer. The lipid in these 2 layers differed in fatty acid composition. Centrifugation, however, did not sharply separate one group of particles from another. This paper describes how plasma fat particles may be separated into 2 distinct groups by differential flocculation in a polyvinylpyrrolidone gradient column.

Fat particles must be aggregated in order to float spontaneously in plasma. Burstein and co-workers(4,5) showed that 5% polyvinylpyrrolidone (PVP) in turbid plasma flocculates the fat particles, but not the soluble lipoproteins. Eight per cent PVP flocculates the low-density lipoproteins also, but even at this PVP concentration, flocculation is limited to the fat particles if the plasma contains in addition 100 mg NaCl/ml.

Materials. Stock solutions, stable at room temperature for several weeks, should be sparkling clear. A. 10% NaCl containing 0.01% sodium ethylene diamine tetraacetate (EDTA). The solution should be filtered to remove undissolved solute. B. 5% polyvinylpyrrolidone (average molecular weight 40,000) in solution A. (Polyvinylpyrrolidone powder obtained from General Aniline and Film Co., New York City, N. Y.) C. 25% polyvinylpyrrolidone in solution A. Cellulose nitrate centrifuge tubes (20 ml). These are transparent and can be sliced with a tube slicer.

Procedure. A PVP density gradient is made by displacing fluid of continually increasing PVP concentration from a mixing bottle(6) into the bottom of a test tube. Fifteen ml of solution A is placed in a mixing bottle. From a syringe containing 20 ml of solution B, a plastic tube leads through the mixing bottle stopper to a point above the fluid in the bottle. The tip of a second plastic tube is immersed in this fluid and leads through a second perforation in the stopper to the bottom of a cellulose nitrate tube. The contents of the syringe are infused slowly into the mixing bottle while the fluid in the bottle is stirred. Six gradients can be made at once if a large bottle replaces the syringe, and volumes of the solutions are increased proportionately: 90 ml of solution A is put in the mixing bottle, and the outflow is delivered simultaneously into 6 tubes.

Tubes containing the gradient mixture are warmed in an incubator at 37°C before use.

* This study was supported in part by PHS Training Grant.

Meanwhile 80 mg dry NaCl is dissolved in 0.8 ml lipemic plasma, and this mixture is warmed to 37°C in a water bath for 10 min. Two-tenths ml of solution C is then added dropwise with stirring from a syringe with a fine needle. (The 25% PVP is too viscous to be delivered accurately from a pipette.) The plasma is then carefully layered under the fluid in the gradient tube with a syringe and long needle. After about 16 hours at 37°C, the plasma is clear, and the turbidity is in 2 bands, one at the top of the tube, the other just above the plasma. This separation of the turbidity into 2 bands appears to be due to differential flocculation of particles (Fig. 1, 2). The absence of any bands when clear plasma is put under a gradient indicates that the soluble lipoproteins are not significantly aggregated under the conditions of the analysis.

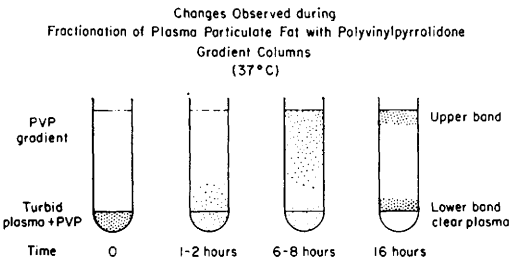

FIG. 1

FIG. 2. PVP gradient fractionation of lipemic plasma.

Analytical methods. Nephelometry. Turbidities of the bands from gradient tubes were measured in arbitrary units with a locally made nephelometer. Tubes were sliced just below the upper bands and the upper bands removed. The tubes were then sliced again just above the lower bands, and from each tube the lower band and clear plasma were removed together. Suspensions of upper band and lower band particles were then sufficiently diluted with solution A so that measurements could be made within the linear range of the nephelometer.

Lipid analysis. Particles for lipid analysis were obtained from gradient tubes by slicing the tubes beneath the upper bands and removing them, then aspirating the lower bands with a fine-tipped pipette. The particles from both bands were then centrifuged upward through a sucrose density gradient into isotonic saline at 100,000 G $\times$ 30 min (3×10^6 G min) in the S-40 head of the Spinco Model L ultracentrifuge. The turbid saline layers were removed for lipid extraction with the aid of a tube slicer. Lipids were extracted with chloroform-methanol (2:1 v/v). Triglyceride was obtained from this lipid by scraping off the appropriate areas of thin-layer silicic-acid chromatographic plates developed in petroleum ether-diethyl ether (90:10 v/v)(7). Methyl esters of the triglyceride fatty acids were prepared by refluxing the silicic acid scrapings in dry HCl-methanol 2 hours at 120°C(8). Gas liquid chromatography of the fatty acid methyl esters was performed on a 6-foot packed column with ethylene glycol succinate polyester as the stationary phase, using a strontium-90 ionization detector(9) calibrated with NIH "Metabolism Study Section Standard Mixtures A, B, and C."

Results. 1. *Chyle.* Chyle from a chylous pleural effusion was suspended in clear plasma, and the plasma was fractionated with the PVP gradient. More than 95% of the turbidity was in the upper band.

2. *Alimentary lipemia.* Normal fasting

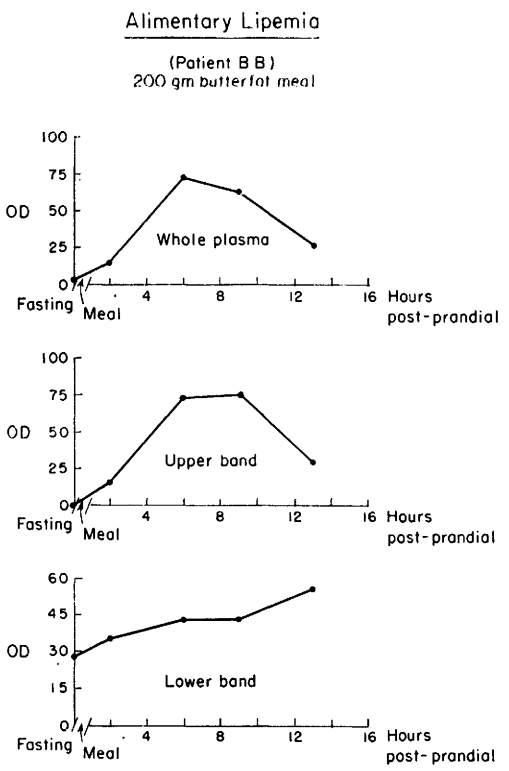

FIG. 3

medical students were fed 200-250 g of butter or corn oil. Bloods were drawn before the meal and at intervals after it, using Na EDTA as the anticoagulant. Buttock subcutaneous fat was aspirated according to the method of Hirsch *et al.*(10). Fig. 3 shows the turbidities of whole plasma and upper and lower bands during alimentary lipemia. Whole plasma turbidity reaches a maximum at 6-9 hours. Two groups of particles contribute to the turbidity of whole plasma. Early in lipemia, plasma turbidity results mainly from the presence of upper band particles. Later, as the whole plasma and upper band turbidities are falling, the lower band turbidity becomes predominant, and at the end of lipemia it is the only detectable band.

Not all subjects showed an upper band in alimentary lipemia. Fig. 4 shows the gradients made during a 24-hour study of alimentary lipemia in a 35-year-old diabetic man fed 250 g of corn oil. An upper band was seen only at 9 hours. The changes in the lower band progressed as usual.

Fig. 5 shows the fatty acid composition of the particle triglyceride from upper and lower bands in a normal subject fed 250 g of corn oil. Plotted on the ordinate is the ratio of linoleic acid (18:2) to oleic acid (18:1) in the triglyceride fatty acids. This ratio sharply distinguishes the composition of corn oil from that of adipose tissue. The composition of the upper band resembled closely, but

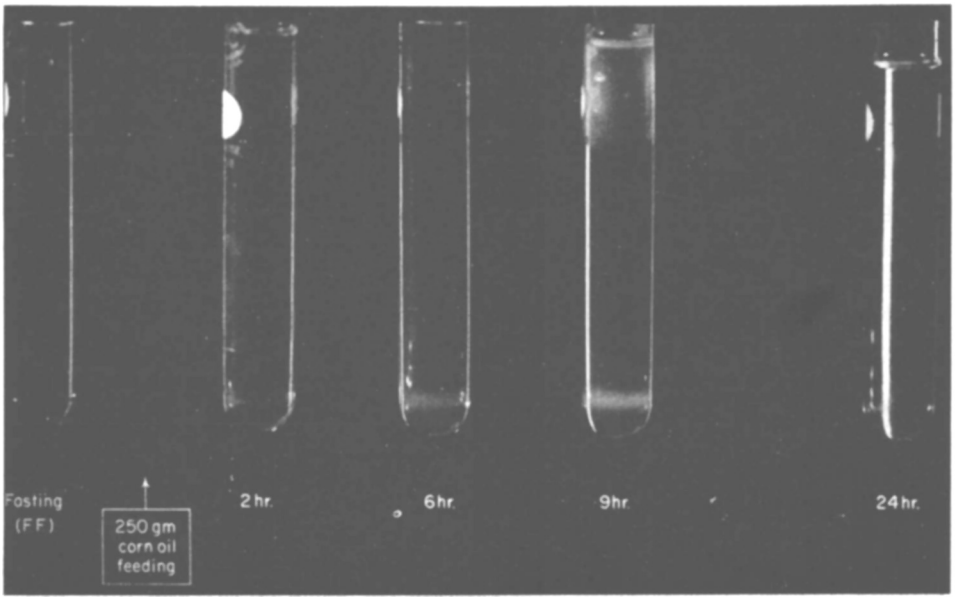

FIG. 4

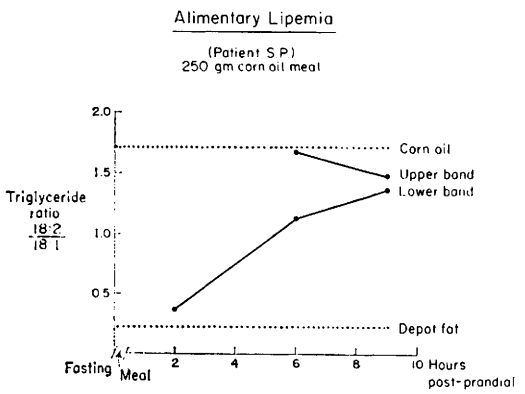

FIG. 5

not identically, that of the fed fat. The lower band triglyceride was initially similar to body fat, but as lipemia proceeded, its composition approached that of fed fat. The data from this subject and from 2 others are summarized in Table I.

dietary fat may occur also *in vitro* if some lower band particles flocculate with the upper band.

The marked discrepancy between dietary fat and lower band particulate fat, especially at the onset of lipemia, suggests that the triglyceride of the lower band particles is derived from both body fat stores and dietary fat. There is much to suggest the liver as the source of the lower band particles. Lymph chylomicron triglyceride which enters the liver is initially unhydrolyzed; hydrolysis and loss of the glycerol moiety of the triglyceride takes place subsequently(14,15). The liver not only re-esterifies fatty acid(16,17), but, when perfused *in vitro,* can manufacture fat particles large enough to be seen in the dark-field microscope(18). The predominant role of the liver in producing lower band particles is not proved, however, and other possible sources have not been ruled out.

TABLE I. Linoleic Acid/Oleic Acid Ratio in Triglyceride of Upper and Lower Band Particles during Alimentary Lipemia.

Subject	Fat meal		Time after meal: 2 hr	6 hr	9 hr	Depot fat	Fed fat
S.P., ♂, age 25	250 g corn oil	Upper band	No band	1.67	1.46	.21	1.7
		Lower "	.37	1.13	1.37		
J.H., ♂, age 25	"	Upper band	No band	1.80	1.51	.21	"
		Lower "	.50	1.48	1.89		
A.E., ♀, age 23	"	Upper band	No band	1.32	.89	.29	"
		Lower "	.61	1.07	1.32		

Discussion. The results suggest that upper band particles are those which enter the plasma from thoracic duct chyle, since the composition of the upper band is close to that of fed fat, and chyle particles suspended in plasma flocculate almost entirely in the upper band. The discrepancy between the fatty acid composition of upper band particles and fed fat is least marked at the peak of lipemia, and more marked as lipemia subsides. Evidence that plasma fatty acids can be incorporated into lymph chylomicron triglyceride (11) suggests that ingested fat mixes with plasma fatty acid in the intestinal mucosa, a site of re-esterification(12,13). The contribution of plasma fatty acids would be proportionally least at the peak of lipemia. Dietary fat may possibly be diluted with bile lipids and desquamated epithelium. Dilution of

Summary. 1. A gradient column of polyvinylpyrrolidone in 10% saline sharply divides the fat particles of alimentary lipemic plasma into 2 groups. The particles of both groups float in isotonic saline, density 1.006. 2. During alimentary lipemia the 2 groups differ in rates of appearance and disappearance, and in fatty acid composition. Evidence is presented that one group consists of particles from thoracic duct chyle, and that the other group consists of particles originating elsewhere, possibly the liver.

1. Gage, S. H., Fish, P. A., *Am. J. Anat.,* 1924, v34, 1.

2. Albrink, M. J., *J. Clin. Invest.,* 1961, v40, 536.

3. Kuo, P. T., Whereat, A. F., Bassett, D. R., Staple, E., *Circulation,* 1961, v24, 213.

4. Burstein, M., Prawerman, A., *Pathol. Biol.,* 1959, v7, 1035.

5. Cornwell, D. G., Kruger, F. A., *J. Lipid Res.*, 1961, v2, 110.

6. Kunkel, H. G., in *The Plasma Proteins*, V. 1, p294, 1960, Ed., F. W. Putnam, Academic Press, New York.

7. Mangold, H. K., *J. Am. Oil Chem. Soc.*, 1961, v38, 708.

8. Stoffel, W., Chu, F., Ahrens, E. H., Jr., *Anal. Chem.*, 1959, v31, 307.

9. Farquhar, J. W., Insull, W., Jr., Rosen, P., Stoffel, W., Ahrens, E. H., Jr., *Nutrition Rev.* (Suppl.) 1959, v17.

10. Hirsch, J., Farquhar, J. W., Ahrens, E. H., Jr., Peterson, M. L., Stoffel, W., *Am. J. Clin. Nutrition*, 1960, v8, 499.

11. Havel, R. J., Goldfien, A., *J. Lipid Res.*, 1961, v2, 389.

12. Dawson, A. M., Isselbacher, K. J., *J. Clin. Invest.*, 1960, v39, 150.

13. Dawson, A. M., Isselbacher, K. J., *ibid.*, 1960, v39, 730.

14. Borgström, B., Jordan, P., *Acta Soc. Med. Upsaliensis*, 1959, v64, 185.

15. Dustin, J.-P., Fredrickson, D. S., Laudet, P., Ono, K., *Fed. Proc.*, 1961, v20, 270.

16. Laurell, S., *Acta Physiol. Scand.*, 1959, v47, 218.

17. Stein, Y., Shapiro, B., *Am. J. Physiol.*, 1959, v196, 1238.

18. Kay, R. E., Entenman, C., *J. Biol. Chem.*, 1961, v236, 1006.

Received May 25, 1962. P.S.E.B.M., 1962, v110.

Extractable Lipid, Water and Residue of Rat Epididymal Fat Bodies.* (27609)

C. R. BOSHART, L. WILL, A. PIRRÉ (Introduced by E. H. Dearborn)
Experimental Therapeutics Research, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

The epididymal fat bodies of male rats or mice constitute a convenient source of adipose tissue for the study of fat metabolism either *in vivo* or *in vitro*. Smith *et al.*(1) suggested that changes in lipid content of the epididymal fat bodies might provide a reasonable means for estimating the influence of hormones and other compounds on adipose tissue in general. Tracht *et al.*(2), Levy and Ramey(3,4) and White and Engel(5) have used the change in lipid content of the fat bodies before and after a period of fasting, as an index of lipid mobilization. In addition, concentration of lipid has been used to show changes in the character of epididymal fat as the result of various treatments(1). Consequently, the relationships between the weight of the fat bodies, their lipid content and lipid concentration are important, especially as these are altered by treatment effects. The present study shows the excellent correlation between lipid content and wet weight of epididymal adipose tisue. The relation of lipid, water and residue concentration of this fat depot are also reported.

Method. Male albino rats of the Sherman strain bred at the Lederle Laboratories Wyckoff Colony were housed individually and given free access to Purina Laboratory Chow Pellets or ground pellets and tap water. Rats weighing between 50 and 330 g were sacrificed and either one or both epididymal fat bodies were taken for analysis. Lipid extractions were performed with chloroform-methanol (2:1) according to a procedure similar to that of Folch *et al.*(6). The weight of extractable lipid obtained after evaporation of solvent and drying was not changed significantly by reextraction of the lipid with petroleum ether (6). The residue remaining after extraction was dried to constant weight. Water content of the tissues was calculated by difference. All percentages were calculated on the basis of fresh weight of the adipose tissue. The regression relations were calculated by the method of least squares.

For convenience the quantities are represented symbolically as follows:

*This work was conducted in the Metabolic Chemotherapy Dept.