Nutrition, 1949, v3, 301.

6. Whitehair, C. K., Peterson, D. R., Van Arsdell, W. J., and Thomas, O. O., *J. Am. Vet. Med. Assn.,* 1952, vCXXI, 285.

7. Gallup, W. D., and Hoefer, J. A., *Anal. Ed. Ind. Eng. Chem.,* 1946, v18, 288.

8. Kimble, M. S., *J. Lab. Clin. Med.,* 1939, v24, 1055.

9. Lesher, M., Brody, J. K., Williams, H. H., and Macy, I. G., *Am. J. Dis. Child.,* 1945, v70, 182.

10. Thomas, J. W., Loosli, J. K., and Williams, J. P., *J. Animal Sci.,* 1947, v6, 141.

11. Long, R. A., Van Arsdell, W. J., MacVicar, Robert, and Ross, O. B., *Okla. Agri. Exp. Sta. Tech. Bull.* No. T-43, 1952.

12. Van Arsdell, W. J., Ross, O. B., and MacVicar, R. W., *J. Animal Sci.,* 1950, v9, 545.

Received May 7, 1953. P.S.E.B.M., 1953, v83.

Chromatographic Separation of the Plasma Lipids.* (20422)

DOROTHY L. FILLERUP AND JAMES F. MEAD.

From the Atomic Energy Project, School of Medicine, University of California, Los Angeles.

Although technics for the analysis of the various components of the plasma lipids are available, methods for their separation are neither simple nor quantitative. Bloor's method(1) of phospholipid separation has been the subject of considerable discussion (2-8) with general agreement that not all the phospholipid is precipitated by acetone-magnesium chloride(3,4,6,9,10) while significant amounts of other water soluble materials such as urea, creatinine, amino acids and Na, K, and Cl salts generally accompany it as impurities(11-13). Resolution in petroleum ether of the alcohol-ether extract may leave as much as 11.8% residue undissolved(13). The separation of the crude mixture into saponifiable and non-saponifiable fractions involves the destruction of both triglycerides and sterol ester fractions and thus precludes their separate analysis. Indeed, the separation and analysis of the sterol and sterol ester fractions has been the subject of considerable investigation, with the resultant disclosure of several procedures(14-21). The more successful of these methods have made use of the technic of chromatography. Trappe(20) investigated the use of alumina and later of silicic acid to separate glycerides from cholesterol esters, whereas Hess(14) used alumina for the separation of sterols and sterol esters. We were unable to repeat the work of Hess, who reported the elution of sterol ester with 10% ether in petroleum ether and of sterols with 10% alcohol in petroleum ether. In our hands both fractions appeared together in the eluate with the alcoholic mixture. Part of the difficulty may lie in the well-known activity of alumina in altering the adsorbed lipid, for example, by hydrolysis of fatty esters(2,20). Silicic acid seemed most suitable for the separation since it is not known to alter the adsorbed materials and shows no reaction with the solvents. Borgstrom(2) used this material to separate phospholipids or cholesterol palmitate from a mixture of stearic acid and glycerides. The last 2 substances could then be separated by extraction of their alkaline alcohol solution with petroleum ether. However, when mono- or diglycerides were present they were only partly extracted and it was necessary to use an ion exchange resin (IRA-400), to effect the separation.

With these considerations in mind, the complete separation of the plasma lipids was attempted with only one column and alterations in the eluting mixture. Regular changes in the eluant were not attempted since it was thought that some advantage could be gained by continuing the elution with a particular solvent mixture until little or no material appeared in the eluate. In this manner, sub-

* This paper is based on work performed under contract between the Atomic Energy Commission and the University of California at Los Angeles.

TABLE I. Chromatographic Separation of an Artificial Lipid Mixture.

Fraction (in order of elution)	Compounds used*	Solvent mixture eluting fraction	Column vol of eluate	% recovery
1. Sterol ester	Cholsterol palmitate, cholesterol oleate	1% ether in petroleum ether	20	98.4–100
2. Triglyceride	Tripalmitin, triolein	4% ether† in petroleum ether	20	96 –100
3. Sterol	Cholesterol	10% ether in petroleum ether	15	99 –100
4. Acid	Stearic, oleic	50% ether in petroleum ether	15	97
5. Phospholipid	Lecithin	25% methanol in ether	15	100

* Sterol esters were purchased from Dougherty Chemical Co., the tripalmitin and oleic acid were prepared and purified by Drs. Nevenzel and Howton of this laboratory. Triolein was purchased from Matheson Co.; cholesterol and stearic acid purchased from Eastman Kodak Co., and lecithin from Bios Laboratories.

† Mallinckrodt analytical grade ether was shaken with aqueous ferrous sulfate, dried over anhydrous magnesium sulfate and calcium chloride and redistilled before use.

fractions of the main lipid classes were obtained which will be examined in the future. Artificial mixtures of lipids were studied before applying the technic to the separation of the plasma lipids. As a preliminary application of the method a spectrophotometrically determinable fatty acid, β-eleostearic(22), was fed as the methyl ester, and its distribution among the plasma lipids was investigated. A trial of the different methods of extraction of plasma lipids revealed that in our hands a modification of the method of Delsal(23) gives the highest and most consistent results. This method was therefore adopted for all plasma lipid extractions.

Materials and methods. All solvents were analytical grade when purchased and were redistilled before use. Data on the purification or preparation of other materials are given in the body of the following section.

Total plasma lipid extraction. Blood is removed from the anticubital vein of an adult human and is immediately placed in a 15 ml centrifuge tube containing a few grains of heparin-sodium. This anticoagulant is used in preference to oxalate or citrate since the last two are reported to lead to erroneous values for lipids(9,24-27). The tube is shaken gently to mix the contents and is centrifuged for 15 minutes at 1000 g. To 2 ml of plasma is added, with vigorous stirring, 30 ml of a 4:1 mixture of methylal:methanol (23). After 30 minutes, the precipitate is centrifuged down and the supernatant solution is evaporated to dryness under nitrogen at reduced pressure and room temperature(4,5, 9,28). The residue is extracted with petroleum ether and this extract is evaporated to dryness in the same manner; the residue is taken as the total lipid. The method appears superior because of its reproducibility and high yields. An average of 752.7 mg % total lipid can be compared with that of 545 mg % obtained by the method of Bloor(29) with samples of the same plasma.

Chromatography. For about 50 mg of lipid, 13 of silicic acid was packed into a tube of 2 cm diameter to a height of 7 cm. The column was washed under reduced pressure with 3 column volumes (one volume = about 14 ml) of absolute methanol, followed by equal volumes of acetone; anhydrous, peroxide-free ether; and petroleum ether (b.r. 60-70°). Pure lipids were added to the column in petroleum ether singly, in pairs, or, finally, as a complete mixture, and were eluted from the column with various ether-petroleum ether mixtures. A particular solvent mixture was continued until evaporation of the eluate showed it to contain little or no lipid and it became apparent that a more polar mixture would be necessary to elute the next fraction.

Results. Separation of artificial mixtures. Table I gives the composition of the artificial mixture and the solvent system required to elute each fraction. The average percentage

TABLE II. Chromatographic Separation of Plasma Lipids; 7 Determinations. (Values given as mg % of plasma.)

Total lipid	Sterol ester	Neutral fat	Sterol	Phospholipid	Total recovery, %
1047.5	30.0	477.5	70.0	455.0	98.6
953.3	86.7	260.0	110.0	446.7	94.8
786.7	73.3	250.0	113.3	316.6	95.7
750.0	44.0	288.0	96.0	312.0	98.7
737.0	24.3	370.0	77.0	233.0	95.6
620.0	35.0	293.0	62.5	242.0	102.0
572.5	41.3	278.8	167.5	75.9	98.3

recovery, as judged by the weight of the fraction and qualitative† as to its nature are also given.

Separation of plasma lipids. When plasma lipids from various subjects, extracted as described above, were subjected to the same treatment, the data shown in Table II were obtained.

Transportation of β-eleostearic acid in blood. Approximately 5 g of methyl β-eleostearate was fed to 2 normal adults under conditions previously described(22). After 4 hours a sample of blood was drawn and analyzed as described above. Each fraction was analyzed spectrophotometrically for β-eleostearate as previously described(22). In one case a total of 1.90 mg of β-eleostearate was present in the plasma sample used. Of this amount 79% was found in the first 10 volumes of the 2% ether eluate. No eleostearate was found in any but the neutral fat fraction. In previous determinations in which methyl β-eleostearate was added to the serum before extraction, the eleostearate was recovered totally in the first 10 volumes of 4% ether in petroleum ether.

Discussion. Trappe(20), Hess(14), and Borgstrom(2) showed that a partial separation of the plasma lipids could be achieved with a chromatographic technic. It remained only to simplify and generalize the method so that with one absorbent and a simple series of solvents all the major classes of lipids could be separated.

It should be emphasized that the technic is not intended as a quantitative determination of the plasma lipids; however, the partition may be even more sensitive than is claimed since some separation of subfractions within the major classes was observed. Nevertheless, the procedure has already found several applications. The easily traceable β-eleostearic acid appeared only in the triglyceride fraction after its ingestion as the methyl ester. Since Borgstrom(30) has reported that ethyl oleate appeared in the blood of rats not as the ethyl ester but as the triglyceride, we have assumed, in this preliminary experiment, that eleostearic acid also is transported as the triglyceride, and not as the methyl ester, which might also appear in this fraction. Continuation of this research with the aid of these technics may reveal the mode of transport of other types of fatty acids under a variety of conditions.

Summary. By chromatography on a silicic acid column, artificial lipid mixtures and plasma lipids have been separated into fractions composed mainly of the following groups: sterol esters, triglycerides, sterols, fatty acids and phospholipids. By this technic eleostearic acid is found exclusively in the triglyceride fraction after having been fed as the methyl ester.

† The Liebermann-Burchard test for sterols was positive only in fractions 1 and 3.

1. Bloor, W. R., *J. Biol. Chem.*, 1929, v82, 273.
2. Borgstrom, B., a) *Acta Physiol. Scand.*, 1952, v25, 101; b) *ibid.*, 111.
3. Boyd, E. M., *J. Biol. Chem.*, 1931, v91, 1.
4. Ellis, G., and Maynard, L., *J. Biol. Chem.*, 1937, v118, 701.
5. Erickson, B., Avrin, I., Teague, D., and Williams, H., *J. Biol. Chem.*, 1940, v135, 671.
6. Favarger, P., *Helv. Physiol. Pharmacol. Acta*, 1949, v7, 371.
7. Marenzi, A. D., and Cardini, C. E., *J. Biol. Chem.*, 1943, v147, 371.
8. Sinclair, R, and Dolan, M., *J. Biol. Chem*, 1942, v142, 659.
9. Kirk, E., Page, I., and Van Slyke, D., *J. Biol.*

Chem., 1934, v106, 203.

10. Page, I., Kirk, E., Lewis, W., Jr., Thompson, W., Van Slyke, D., *J. Biol. Chem.*, 1935, v111, 613.

11. Christensen, H. N., *J. Biol. Chem.*, 1939, v129, 531.

12. Folch, J., and Van Slyke, D., *J. Biol. Chem.*, 1939, v129, 539.

13. Pernokis, E., Freeland, M., and Kraus, I., *J. Lab. Clin. Med.*, 1941, v26, 1978.

14. Hess, W. C., *J. Lab. Clin. Med.*, 1947, v32, 1163.

15. Kingsley, G. R., and Schaffert, R. R., *J. Biol. Chem.*, 1949, v180, 315.

16. Koehler, A., and Hill, E., *J. Biol. Chem.*, 1949, v179, 1.

17. Schoenheimer, R., and Sperry, W., *J. Biol. Chem.*, 1934, v106, 745.

18. Selbach, H., and Trappe, W., *Arch. Phychiat. Nervenheilk*, 1944, v117, 541.

19. Smith, R., and Marble, A., *J. Biol. Chem.*, 1937, v117, 673.

20. Trappe, W., a) *Z. Physiol. Chem.*, 1942, v273, 177; b) *Biochem. Z.*, 1940, v306, 316; c) *Biochem. Z.*, 1941, v307, 97.

21. Yasuda, M., *J. Biochem.* (Japan), 1936, v24, 443.

22. a) Mead, J. F., Fillerup, D. L., Decker, A. B., and Bennett, L. R., *J. Nutrition*, 1952, v46, 499; b) Fillerup, D. L., and Mead, J. F., UCLA-148.

23. Delsal, J. L., *Bull. soc. chim. biol.*, 1944, v26. 99.

24. Gardner, J., Gainsborough, H., and Murray, R., *Biochem. J.*, 1938, v32, 1457.

25. Man, E., and Gildea, E., *J. Biol. Chem.*, 1932, v99, 43.

26. Schmidt, L. H., *J. Biol. Chem.*, 1935, v109, 449.

27. Shope, R. E., *J. Biol. Chem.*, 1928, v80, 125.

28. Man, E. B., *J. Biol. Chem.*, 1937, v117, 183.

29. Bloor, W. R., *J. Biol. Chem.*, 1928, v77, 53.

30. Borgstrom, B., *Acta Physiol. Scand.*, 1952, v25, 322.

Received May 18, 1953. P.S.E.B.M., 1953, v83.

Method for Establishing Human Cells in Culture: Susceptibility to Poliomyelitis after Prolonged Cultivation.* (20423)

H. E. SWIM AND R. F. PARKER.

From the Department of Microbiology, School of Medicine, Western Reserve University, Cleveland, O.

The application of tissue culture technics to the study of mammalian viruses has received renewed impetus since Enders and his associates showed that the viruses of poliomyelitis would multiply in cultures of human tissues (1). Quantitative studies have been greatly restricted by the existing tissue culture technics for virus propagation, which usually depend on the explantation of fresh tissue for each virus passage. It would obviously be of great value, therefore, to have established cell strains susceptible to viral infection. Of the established cell cultures, the "L" strain (Earle) has been shown to be susceptible to infection with the virus of Rous sarcoma(2), and to pseudo-rabies and herpes simplex(3). Recently, Scherer, Syverton, and Gey(4) have described a strain of cells susceptible to poliomyelitis. The technics described by Earle for the initiation of cell strains on glass or cellophane surfaces have not been widely used. The purpose of this communication is to describe a simple technic for establishing human cells in mass culture and to show how such cell strains may be maintained. These cultured cells appear to retain their initial susceptibility to viral infection.

Methods. The standard technic for cultivation of tissue in roller tubes has been employed. The lower two-thirds of an 18 x 150 mm culture tube is coated with 0.05 ml chicken plasma. The desired number of tissue fragments are placed on the side of the tube and 0.05 ml chick embryo extract (EE 50) is added and mixed with the plasma. The plasma-extract mixture is then rolled over the surface of the tube, allowed to drain for a few seconds, and the excess is removed, leaving the tissue embedded in a very thin clot. The tube is allowed to stand for 5-30 minutes and

* Aided by a grant from The National Foundation for Infantile Paralysis.