ues. The polymeric material from heated-oxidized methyl esters of unsaturated fatty acids has been separated by vacuum distillation and chromatography on silicic acid. It is believed to be principally dimeric in nature.

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# Fat Transport Mechanism

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FATS HAVE LONG BEEN KNOWN to provide the body with rich sources of energy and to act as a protective insulating layer when they are stored in adipose tissue. For many years however the difficulties of fat chemistry largely dissuaded the biochemist from a more detailed study of body lipides. Then this luxury became too costly as the evidence mounted relating certain lipides to heart disease (1, 2).

The body transports its fat from organ to organ *via* the circulating plasma and lymph. An amazing phenomenon, present in this system of fat transport, is the ability of the body to render plasma lipides water-soluble. Although fats are notoriously insoluble in water and tend to coalesce and layer out, the fats which are present in the aqueous medium of plasma remain in solution or in a stable colloidal suspension. Obviously if the lipides in plasma were suddenly to lose this property and coalesce, the resultant fat droplets would soon plug the capillaries of vital organs, resulting in death. This fortunate but seemingly paradoxical behavior of plasma lipides is largely the result of their combination with certain proteins. These impart water-soluble properties to the lipides and by so doing preserve the single-phase aqueous system of plasma. As a result plasma and lymph are able to serve as vehicles for the transport of fat by the body.

A study of fat transport quite naturally evolves into a study of plasma lipides. Already investigation of the circulating lipides has provided a fundamental insight into the mechanism of fat transport for it is now apparent that extremely small amounts of lipide, often regarded previously as insignificant, are in reality of great importance biologically. The lipides in normal plasma total only about 5.0 g. per liter. Comprising this total are several distinct species of lipide, each apparently serving a different and probably important function.

Of the plasma lipides perhaps the most thoroughly understood are the unesterified fatty acids (UFA), which have to be measured by microtitration after being extracted from plasma. Until recently these tiny amounts of "free" acid in plasma were considered to be either a laboratory artifact or of no biological significance. Certainly their normal concentration of 0.1 to 1.0 milliequivalents per liter of plasma is not very impressive. These low amounts however belie their importance.

While precise chromatographic analyses of the plasma UFA have not been published, they appear to consist mostly of 14 to 18 carbon aliphatic fatty acids, a large part of which are normally oleic and palmitic acid (3). All are present in ionized form. In spite of their small concentration in plasma they would still precipitate out as soaps were they not bound to the water-soluble plasma protein, albumin. The ability of albumin to bind UFA increases the solubility of UFA in an aqueous medium by many fold (4). A more detailed understanding of plasma UFA largely stems from experiments in which humans and animals are given an intravenous injection of a radioactive carbon, labelled UFA.

IN A TYPICAL EXPERIMENT palmitate-1-$C^{14}$ bound to albumin is injected intravenously into a human subject (5, 6). The radioactive palmitate mixes rapidly throughout the plasma and thereafter is presumed to behave in exactly the same manner as the nonradioactive palmitate already present. Following the injection, a quick fall of radioactivity in the plasma indicates a rapid disappearance of palmitate. However equally rapid replenishment with UFA from storage tissues, of course, occurs to maintain the constant plasma UFA level observed throughout the experiment. Of the palmitate molecules which disappear from the plasma many are rapidly metabolized by the tissues to carbon dioxide and water, as evidenced by radioactive carbon dioxide which quickly appears in the expired air. Further calculations from these and similar data reveal the startling fact that the small concentrations of plasma UFA are probably the main form in which fat becomes a major source of energy. The explanation for this phenomenon lies in the short period of time an UFA molecule stays in the plasma. The rapid removal of UFA by metabolizing tissue enables the plasma to transport from storage tissues large quantities despite a low UFA concentration.

The remaining plasma lipides are linked to proteins in macromolecules called lipoproteins. They are associated not with albumin but with different types of globulin, the other major class of plasma protein. About 8–12% of the plasma proteins are, in reality, lipoprotein. All of the plasma lipoproteins contain basically the same kinds of lipides, namely, free and esterified cholesterol, phospholipide, and triglyceride. Despite the chemical similarity, distinct species of lipoprotein exist which significantly differ in size, density, type of protein, and relative proportions of lipide. These differences have made possible a variety of methods to separate the lipoprotein types although none as yet yields a truly pure lipoprotein.

Ultracentrifugation, for example, takes advantage of differences in density to separate classes of lipoprotein. This method has been fully developed by Gofman and his colleagues (7). The more lipide a particular lipoprotein molecule contains, the larger its size and the less its density. Those lipoproteins

with density less than the solvent medium float to the top of the centrifuge tube while the heavier molecules sink to the bottom. By appropriately slicing the tube, the lipoproteins can be recovered without their mixing. Through repeatedly changing the solvent density, refined separations are possible. The different fractions of lipoprotein which are separated by this technique are classified according to the range of solvent density within which they were isolated. In addition, by amplifying the technique, rates at which a particular lipoprotein fraction floats in a solution of standard density can be determined and quantitated in Svedberg units of flotation "$S_f$ numbers."

A second method of separating different lipoproteins is the ethanol water method of Cohn (8). Standard concentrations of ethanol in a cold-water solution of plasma precipitate distinct fractions of protein and lipoprotein. The precipitated fraction can then be redissolved. A limitation of this method however lies in its inability to separate the lipoproteins of very low density.

Still another technique employed to characterize lipoproteins is electrophoresis, a method ordinarily used to distinguish between different proteins in solution. A voltage applied across a solution of proteins causes the different types to move in characteristic directions and at rates which depend upon their size, shape, and polar groups. Variations in technique make possible a similar differentiation of some of the lipoproteins, which are classified after the protein type with a corresponding behavior on electrophoresis (9). In this manner lipoproteins with an electrophoretic pattern similar to the $\alpha$ and $\beta$ globulins have been distinguished and are called $\alpha$ and $\beta$ lipoproteins.

These different methods of separation have confused the classification of lipoproteins. At present a classification based upon density is the best available, particularly since most lipoprotein separations are now done by ultracentrifugation. The characteristics of the lipoproteins classified by this method follow. Note that some of the classes correspond to fractions isolated by one of the other methods. Also it should be realized that each class represents a spectrum of molecules, the densities of which vary within the limits of the solvent media used. The lipide values were obtained by analyzing each of the density fractions and therefore represent only average chemical compositions (10).

Chylomicrons are lipoproteins with a density of less than 1.006 and a size sufficiently large to scatter light. They possess a very small amount of protein and about 9% cholesterol, 7% phospholipide, and more than 80% triglyceride. They will be discussed further. Those lipoproteins, exclusive of the chylomicrons, with a density of less than 1.019 contain an average of 7% protein, 52% triglyceride, 22% cholesterol, and 18% phospholipide. Between densities 1.019 and 1.063 are the so-called low-density lipoproteins. They correspond to the $\beta$-lipoproteins as well as the lipoproteins found in Cohn fraction III-O and consist roughly of 21% protein, only 9% triglyceride, but 47% cholesterol and 23% phospholipide. Finally between densities 1.063 and 1.21 are found the high density lipoproteins, identical with the $\alpha$-lipoproteins and those found in Cohn fraction IV-I. They contain approximately 46% protein, 8% triglyceride, 19% cholesterol, and 26% phospholipide.

The fact that such distinctly different species of plasma lipoproteins exist suggests, of course, different reasons for their transport by the circulation. Present knowledge however is limited to the chylomicron class of lipoprotein.

Chylomicrons are macromolecules up to 1.5 $\mu$ in diameter, and because of their large size they scatter light and impart to plasma a creamy appearance. They exhibit Brownian movement upon dark-field microscopy and exist in plasma and lymph as true colloidal particles. The chylomicrons are made by the walls of the intestines. The major portion of the chylomicron consists of the long-chain fatty acid triglycerides derived from ingested fat. The ingested shorter-chain fatty acids are not incorporated in the chylomicron and are taken directly to the liver by the portal vein (11). For some unknown reason the chylomicrons, with their incorporated long-chain fatty acids, are shunted away from the liver by being collected in the lymph channels of the small intestines. These channels join to form the thoracic duct, which conveys the chylomicrons from the abdomen, through the thorax, into the left jugular vein, where they enter the blood stream to be transported and distributed by the circulation.

Experiments have been devised to study the fate of chylomicrons. If a dog is fed cream and palmitate-1-$C^{14}$, the intestines will manufacture chylomicrons, incorporating into the triglyceride some of the radioactive fatty acids. This material is harvested by placing a polyethylene catheter in the dog's thoracic duct and collecting the flowing lymph. When the lymph is centrifuged, the chylomicrons float to the top of the centrifuge tube, forming a firm "butter" layer which can be easily recovered. These packed chylomicrons may be resuspended readily in saline, providing a preparation which, when injected into another animal, will reproduce under controlled conditions the arrival of chylomicrons into the blood stream from the thoracic duct.

Serial samples of plasma taken after the intravenous injection of such a chylomicron suspension demonstrate that the chylomicrons rapidly disappear from circulation (12, 13). The manner in which these large molecules leave the circulation is not known. Two possibilities present themselves: either they somehow escape through capillary pores or they are removed by phagocytic cells, in much the same fashion as foreign particles are screened from the blood. In any event a rising level of radioactivity in the plasma UFA indicates that once the chylomicrons are removed from the circulation, many are hydrolyzed to fatty acids and glycerol. As would be expected, those fatty acids which reappear in the plasma as UFA are metabolized almost immediately, and their radioactivity appears in the expired carbon dioxide.

Chylomicrons therefore represent the means whereby certain of the ingested lipides are transported from the intestines to the circulation, then *via* the circulation to various tissues. These tissues rapidly remove the chylomicrons and hydrolyze the triglyceride. Many of the resultant fatty acids re-enter the plasma and are metabolized. The fate of those fatty acids which do not re-enter the circulation remains to be discovered. Undoubtedly some are placed in adipose tissue for storage while others may be shunted back into the circulation as part of a lipoprotein other than chylomicrons.

The β-lipoproteins possibly represent an example of this latter phenomenon. Many individuals placed on a diet containing large amounts of saturated fatty acids have an increase in low-density or β-lipoproteins. The high cholesterol content of this type of lipoprotein accounts in large measure for the elevation of serum cholesterol observed in these people. However, the explanation of this phenomenon lies outside our present-day knowledge and within the province of future research.

From the foregoing it is evident that understanding of fat transport is far from complete. Little is known about the structure of the lipoprotein molecule, how the protein is affixed to the lipide, why different proportions of the various lipides occur with each type; nor do we completely understand the functions of the different kinds of lipoproteins, how they are released or removed from the circulation, or the factors which regulate their concentrations in plasma. Research is hampered not only by a methodology still inadequate to cope with the complexities of fat chemistry but also by the variations in fat metabolism present between different species of experimental animals. Enlightenment in these difficult areas is essential to a more complete understanding of the body's fat transport mechanism.


## Summary

The body uses several different mechanisms for the transport of fat in the aqueous medium of the blood. Fat is absorbed from the intestine in the form of chylomicrons, which are discrete particles, visible under the microscope. They consist primarily of triglycerides of the longer fatty acids. The chylomicrons are absorbed *via* the lymphatic system, which ultimately empties into the blood stream. They are rapidly removed from the blood by several tissues, which either oxidize the fatty acids, store them, or return them to the circulation incorporated in liproproteins or bound to serum albumin. This latter mechanism of transporting fatty acids by binding them to albumin has recently been shown to be of great physiologic significance. Although the quantity of lipide so bound represents only a small percentage of the total serum lipides, it has a rapid turnover. The bulk of the serum lipides are carried as large lipoprotein molecules, of which there is a wide spectrum. Although several methods are now available for characterizing and quantitating these lipoproteins, little is known of their physiologic significance.



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# ABSTRACTS . . . R. A. REINERS, Editor

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## • Oils and Fats

**Autoxidation (of oils), volatile products and the role of antioxidants.** N. A. Khan(Pakistan Council Sci. Ind. Research, Tejan, Dacca). *Oléagineux* **13**, 331–5(1958). Moisture was the sole volatile product during the initial 125 hours of autoxidation of methyl oleate at 75°. After 150 hours volatile organic material was formed. The hydroperoxides of methyl oleate and methyl linoleate were decomposed under vacuum at 150° for one hour and the volatile products collected in a capillary at low temperature. Infrared spectra showed the disappearance of *trans* absorption in the residue of methyl oleate and the appearance of *trans* absorption in the products from methyl linoleate. (*C.A.* **52**, 12424)

**New method for extracting oils with acetone and neutralizing them within the mixture.** C. Vaccarino. *Oléagineux* **13**, 233–6 (1958). The process involves extraction of oil with acetone and neutralization with sodium hydroxide while still in the micella stage. (*C.A.* **52**, 12424)

**Fractionation with superheated steam in the laboratory, with particular emphasis on the conditions for the separation of fatty acids. I.** H. Stage, R. Bünger, and A. Jonas(Fa. Distillationstech. Dr. Hermann Stage, Cologne-Niehl, Ger.). *Fette u. Seifen* **55**, 513–16(1953). The theory and apparatus used are described. (*C.A.* **52**, 12423)

**Ethanol number as a new constant for oils and fats.** L. Rozental(Akad. Med., Warsaw). *Acta Polon. Pharm.* **14**, 95–9(1957). Differential solubility of various oils and fats in mixtures of ethyl ether and ethyl alcohol was applied to their evaluation. A sample (5 g.) of the oil or fat is dissolved in 25 ml. of anhydrous ethyl ether, and titrated at 20° with 95% ethyl alcohol to a stable turbidity. The amount of ethyl alcohol in ml. used is proposed as a new characteristic, ethanol number. The method is not applicable to crude fats containing many alcohol insoluble contaminations. (*C.A.* **52**, 12423)

**Constants of the fatty acids from animal or vegetable oils—their use for identifying some esterified oils.** L. Martarese. *Oléagineux* **13**, 157–63(1958). (*C.A.* **52**, 12423)

**Fractional separation of fatty acids.** A. Fichoux. *Oléagineux* **13**, 127–9(1958). The batch, continuous, and semicontinuous systems for rectifying fatty acids are critically compared. (*C.A.* **52**, 12423)

**Molecular association in fat mixtures.** D. G. Dervichian. *Oléagineux* **13**, 113–17(1958). A study was made of the variation of X-ray diagrams as a function of the composition of lauric acid-myristic acid, lauric acid-stearic acid, and lauric acid-palmitic acid mixtures. A definite correlation exists between the variation in the position and intensity of the bands observed and the variation of the melting point curves of the fatty-acid binary mixtures. The existence of molecular associations between fats, phosphatides, and cholesterol was evident. These associations are in simple molecular proportions and mainly in the presence of water. Molecular spacings were also detected which either correspond to each of the pure constituents or to their association. (*C.A.* **52**, 12423)

**Comparison of the influence of continuous and discontinuous heating on the stability of animal fats.** A. Uzzan and R. Lobry (ITERG, Paris). *Rev. frcnç. corps gras* **5**, 233–6(1958). The