Lipids of Subcellular Particles

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Abstract

Methods for isolation and characterization of subcellular particles as well as procedures for analysis of lipid class composition are discussed. The literature on distribution of lipids in subcellular particles is then reviewed. Pertinent new data from our laboratories are presented as well. The isolated particles are related to the organelles to which they correspond in the cell and are discussed with regard to heterogeneity and morphological integrity. Confusion can arise with regard to subcellular particles unless it is appreciated that: 1) preparation of particles of high purity generally requires more than the classical differential centrifugation scheme (both differential and gradient centrifugation may be required); 2) it is hazardous to apply exactly the same procedure for all tissues; 3) all subcellular fractions must be thoroughly characterized.

The more recently devised DEAE cellulose column and thin-layer chromatographic procedures for analysis of lipid class composition are more reliable than the older hydrolytic or silicic acid column or paper chromatographic techniques.

The chief lipid components of mitochondria from all organs and species are lecithin, phosphatidyl ethanolamine, and cardiolipin (diphosphatidyl glycerol). Despite the fact that reports in the literature are in agreement that phosphatidyl inositol is a major component of mitochondria, it is concluded on the basis of new data obtained from highly purified mitochondria and improved analytical methods that phosphatidyl inositol is not a major component of mitochondria. The presence of a relatively large amount of phosphatidyl inositol in mitochondrial preparations is probably related in part to contamination with other particles. Some analytical procedures are demonstrated to give erroneous values for this lipid class. It is also concluded that phosphatidyl serine, phosphatidic acid, sphingomyelin, cerebrosides, and lysophosphatides, reported to occur in mitochondria, are not characteristic mitochondrial components and furthermore that the large amount of uncharacterized mitochondrial phospholipid reported is actually an analytical artifact. Microsomes appear to be similar to mitochondria except that cardiolipin is either low in or absent from microsomes. Available data indicate nuclei to be rather similar to mitochondria and microsomes, at least in some organs.

Studies of the fatty acids of subcellular particles indicate that different particles from one organ have very similar fatty acid compositions. It is clear that there are marked variations in fatty acid composition of particles from different organs and from different species. Differences in dietary fat may be associated with marked changes in fatty acid composition, although brain mitochondrial lipids are largely unchanged. Each lipid class from mitochondria of most organs appears to have a fairly characteristic fatty acid composition. Cardiolipin from some organs contains primarily linoleic acid, phosphatidyl ethanolamine contains large amounts of linoleic and higher polyunsaturates, and lecithin is similar to phosphatidyl ethanolamine except that it does not contain as much arachidonic acid and/or other highly unsaturated fatty acids. New data, the first to be reported, are presented for heart mitochondrial cardiolipin, phosphatidyl ethanolamine, and lecithin.

It is concluded that there are two basically different types of membranous structures. Myelin is the chief representative of the metabolically stable type of membrane structure while mitochondria represent the more labile type. The two types of membranes have very different in vivo properties and very different lipid compositions. Myelin is characterized by a high content of cholesterol and sphingolipids with more long chain saturated or monoenoic fatty acids while mitochondria are characterized by a low cholesterol content, little or no sphingolipid, and highly unsaturated fatty acids. It is clear that formulations of the myelin type membrane structure such as that of Vandenheuvel cannot apply to mitochondria. It is postulated that membrane structures intermediate between the extremes represented by myelin and mitochondria exist.

Introduction

LIPIDS OTHER THAN THOSE in adipose tissue occur primarily as components of biological membranes. Membranes are found at the cell surface (plasma membrane) and in subcellular particles and thus it is essential that individual cellular units be studied in order to define the variations in lipid composition of different membranes and relate lipid composition to membrane structures and functions.

Significant progress has been made in the isolation and characterization of subcellular particles of various organs and the analysis of the lipid composition of the particles. The major problems are in the isolation of pure particles, the characterization of particle preparations, and the methods for analysis of lipids. A great deal of progress has been made in each of these areas and the purpose of this report is to evaluate present knowledge of subcellular particle lipid composition in the light of recent advances. A general consideration of the problems of isolation and characterization of subcellular particles and methods of lipid analysis is presented followed by consideration of data from the literature. New data obtained from our own laboratories have been included where important points were not covered in the literature or where our findings are in disagreement with the literature.

This review is divided into three separate parts dealing with different subjects and the bibliography has been arranged to reflect this division. References 1–102 pertain to isolation, characterization and functions of cellular organelles. References 103–134 pertain to methods of analysis and 135–182 to lipid composition studies of subcellular fractions. References in each section were arranged by year of publication. This provides a useful guide to the emphasis placed on

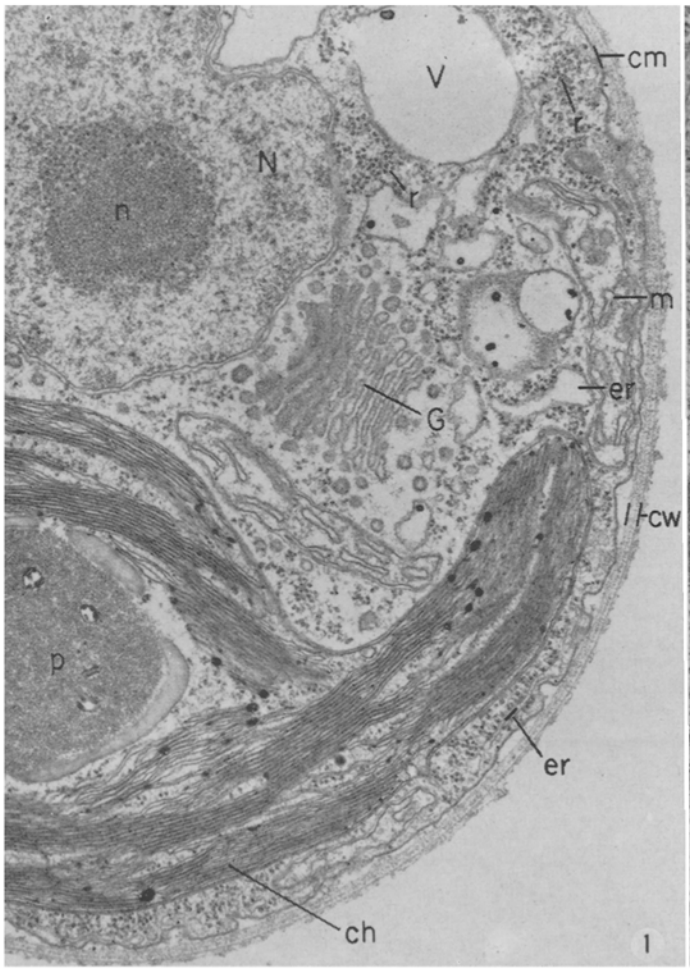


FIG. 1. An electron micrograph of a portion of a plant cell, the alga Chlamydomonas reinhardi (x 25,000). The organelles of the cell are designated: nucleus (N) with a large nucleolus (n), Golgi complex (G), mitochondria (m), endoplasmic reticulum (er), plasma or cell membrane (CM), cell wall (cw), ribosomes (r), vacuole (V), and a large chloroplast (ch) containing a pyranoid. This photograph was provided by Dr. George Palade of the Rockefeller Institute (94).

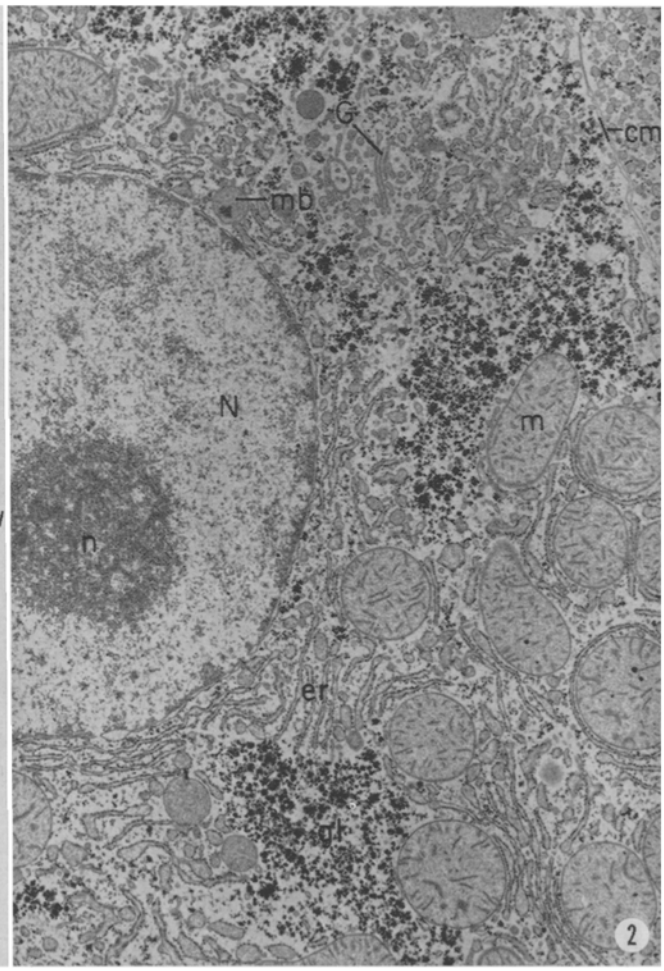


FIG. 2. An electron micrograph of a portion of an animal cell from rat liver (x 9500). The organelles of the cell are designated: nucleus (N) with a large nucleolus (n), Golgi complex (G), mitochondria (m), microbody (mb), endoplasmic reticulum (er), and the cell membrane (cm). This photograph was provided by Dr. George Palade of the Rockefeller Institute.

recent work. No attempt was made to be comprehensive in citation of the literature. Citations were made in Parts I and II to give the reader additional sources of information, and emphasis in Part III was placed on recent work as being most significant.

I. Isolation and Characterization of Subcellular Particles (References 1–102)

The constituents of cells are compartmentalized into morphologically and functionally distinct substructures (organelles). The extent and nature of the compartmentalization varies with cell type. Bacterial cells have fewer organelles than plant and animal cells (94). Knowledge of the composition and function of the organelles is based largely on analysis of subcellular particles which can be isolated with retention of some or most of their original structure.

The detailed fine structure of the cell is revealed only with the use of the electron microscope. A resolution of 10 A is readily obtained. The range of resolution obtained by electron microscopy and light microscopy, i.e. 10 A to 2000 A (0.2 μ) includes macromolecules, membranes, and organelles. The composition of such morphologically distinct components can be determined and provide the basis for construction of models from the component macromolecules (57, 70,90).

Figures 1 and 2 illustrate typical electron microscopic findings with plant and animal cells and a diagrammatic representation of a highly differentiated cell illustrating the more commonly observed organelles is given in Figure 3. The cell is subdivided into the nucleus and the cytoplasm. The nucleus, containing one or more nucleoli, is separated from the cytoplasm by the nuclear envelope (membrane). The cell has as its outer border the cell membrane. The endoplasmic reticulum (ER) and the Golgi complex (GC) form a continuous and interconnected tubular system (the cytoplasmic membrane system) which seems to be connected with the nuclear envelope on one end and with the cell membrane on the other. This system separates the cytoplasm into two main compartments, the intracisternal space which it encloses and the remainder of the cytoplasm or the cytoplasmic space proper (94). Within the latter are found a number of subcompartments: mitochondria, lysosomes, microtubules, centrioles, free ribosomes, and cytoplasmic inclusions. Cytoplasmic inclusions include lipid droplets and glycogen which serve as food storage depots.

Disruption of the cell membrane is the first step in

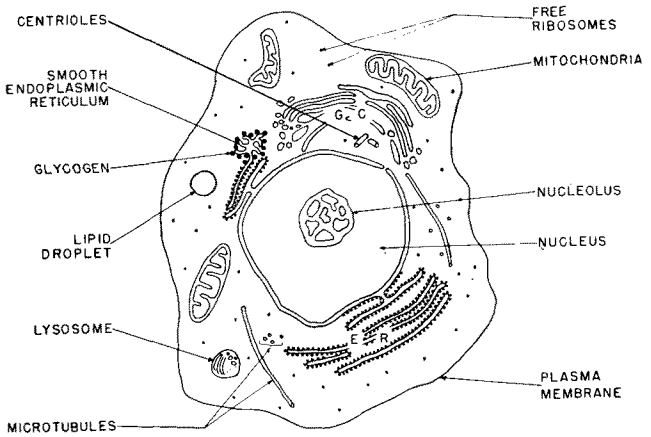
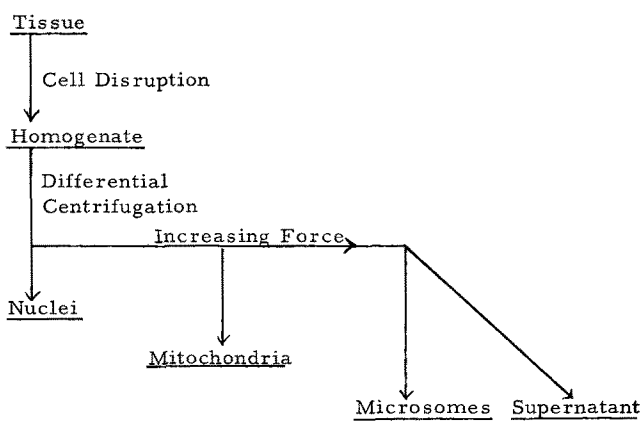


FIG. 3. Diagrammatic representation of the cell. (Provided by Dr. David Slautterback, Department of Anatomy, University of Wisconsin).

the isolation of subcellular particles. The reader is referred to two current reviews on the isolation of subcellular particles (25,99)) for procedures to be used. Cell disruption is a critical manuever that usually involves mechanical shear of the tissue in an artificial medium. Too much shear will damage the organelles while insufficient force will leave many cells intact. A delicate balance must be obtained. The Waring blendor or various types of homogenizers have been used. The amount of shear is regulated with homogenizers consisting of a glass tube fitted with a glass or Teflon pestle by using a carefully defined clearance between tube and pestle and rotation of the pestle at a controlled speed.

Isotonic (0.25M) or hypertonic sucrose solutions have been used widely as media for cell disruption and fractionation. These may be fortified with various stabilizers including dextran, ficoll, salts, and ethylenediamine tetraacetate. The medium employed depends upon the aims of the isolation, e.g. preparation of cell membranes is accomplished by bursting cells in distilled water. When the organelles have been released from the cell their separation can be accomplished.

Differential centrifugation is the most widely employed procedure for separating subcellular particles from homogenates. Separations can be obtained based on differences in sedimentation rate of particles which is in turn determined by the size, shape, and density of the particles. The classical scheme (below) sediments sequentially at increasing forces of gravity fractions containing nuclei, mitochondria, and microsomes leaving a supernatant fraction (5,6,13). Nuclei, mitochondria, and microsomes represent the main mass



of most cells. Cell fractions obtained by the classical centrifugation scheme are frequently purified further by more refined differential centrifugation procedures. Density gradient centrifugation, a relatively new procedure, has proved invaluable as an additional approach (26).

In density gradient centrifugation cell particles are separated on the basis of differences in density. The cell particles may be layered over a gradient of densities encompassing those of the particles to be separated. Centrifugation forces may be applied and allowed to continue until the particulates reach their layer of density equilibrium. The time (frequently 1.5 to 6 hr) required for equilibration depends on the force applied, the density of the medium, and the nature of the fraction.

Many practical difficulties are encountered in the isolation of highly purified subcellular particles. The shearing force used for the cell disruption must be delicately controlled to minimize damage to the organelle structures, and enzymatic degradation caused by the release of autolytic enzymes after cell disruption must be minimized by working rapidly in the cold. Density gradient centrifugation which requires more time is usually used subsequent to differential centrifugation to decrease the effects of autolytic enzymes. Membrane fragments derived from smooth endoplasmic reticulum, Golgi complex, and the cell membrane are difficult to distinguish morphologically. In the intact cell there is an association of some of the membranes of the organelles with each other. There is confluence of the nuclear envelope with the endoplasmic reticulum (14) and the endoplasmic reticulum with the cell membrane (94). Perhaps there are distinct changes in properties at the point of contact or fusion of the different membranes, but there is no definite information on this point. It must be stressed that no universal recipe exists which can be used for isolating subcellular components in all tissues, but rather that only a framework of procedures and techniques are available that must be applied to new systems with care.

The criteria for purity and morphological integrity of subcellular particles rests to a great extent on examination by electron microscopy. Once an enzymatic activity or component has become identified with a single organelle it can be used as an additional criterion of purity. This is especially important since electron microscopy is not available to all investigators. A number of components and activities are frequently associated with cell fractions: DNA with the nucleus; cytochrome *c* and succinoxidase with mitochondria; glucose-6-phosphatase and cytochrome b_5 with microsomes; acid phosphatase, aryl sulfatase, and ribonuclease with lysosomes. Care must be exercised in using such secondary criteria. They are based necessarily on experience which may not be refined or complete. Thus extranuclear DNA has now been clearly shown to exist in mitochondria (91) and chloroplasts (75), glucose-6-phosphatase is not present in all tissues (48), and some acid hydrolases are found outside of lysosomes (12,42).

The discussion of the morphology, function, and isolation of subcellular particles given below is necessarily limited. References to the literature have been selected and discussions confined to the better known organelles. The types of organelles and subcellular particles that can be purified continues to increase as methods for cell fractionation and preparation of samples for electron microscopy improve. Particles from specialized tissues such as those from nerve endings and synaptic vesicles of brain (82) or the rod outer

segments from retina (28), although important, are not considered.

Nucleus

The nucleus is the most prominant organelle the discovery of which dates back to the light microscopy studies of Robert Brown (1). Electron microscopy reveals that the nucleus is bounded by the nuclear envelope, a double membrane structure (two unit membranes) (15). This nuclear envelope contains circular pores which appear to permit contact between the nucleus and cytoplasm. The outer nuclear membrane is also observed to be continuous with the membranes of the endoplasmic reticulum (14,94). The nucleus contains the genetic information in the form of DNA (9,11,19,31,37,46,72,84,96).

The literature for the isolation of nuclei is quite extensive (73). It dates back to 1871 with the isolation of nuclei by Miescher (2). The nuclei are found in the first (low speed) sediment in the classical differential centrifugation technique (5,6,13). Such nuclei are usually grossly contaminated by whole cells. An auxiliary step is thus required for purification (73).

The procedures of choice for isolation of nuclei are modifications of the method described for liver nuclei by Chauveau et al. (17). They take advantage of the high density of nuclei, which is due to the large amount of nucleic acid contained therein. The nuclei are sedimented to the bottom of a tube in 2.2 M sucrose whereas the lighter components are floated upwards (68,83,102). A pure preparation of thymus nuclei is shown in Figure 4.

The Nucleolus

One to several dense bodies (nucleoli) are readily visible within nuclei with the light microscope. Electron microscopy reveals that nucleoli have a granular texture which is pock-marked with less dense areas (Fig. 2). There does not appear to be a limiting membrane.

The nucleoli undergo cyclical disappearance and reappearance at cell division (8). The biological importance of the nucleolus is currently being clarified. It appears to be the site for the synthesis of ribosomal RNA. The RNA methylases, the apparent function of which is the alteration of the structure of transfer RNA at the macromolecular level by methylation of the component bases, are also concentrated in the nucleolus (55,59,60).

The isolation of fractions enriched with respect to nucleoli has been reported. Purified nuclei are first isolated and then disrupted to release the nucleolus. The nucleolar fraction is then separated by centrifugation in a density gradient (7,69). The nucleolar fraction from guinea pig liver contained 86% protein, 4% RNA, and 10% DNA. The amount of RNA is about the same as found in nuclei, the % DNA about half that of the isolated nucleus, and the amount of DNA is about one half the amount found in nuclei (69).

The Golgi Complex

The Golgi complex (Golgi apparatus), originally detected by light microscopy on the basis of it preferential fixation with heavy metals (3), has become equated to a characteristic network of membrane bound elements which are observed by electron microscopy (27). It consists of parallel arrays of flattened sacs at the periphery of which lie clusters of small vesicles. The latter range in size from 0.03–0.05 μ in diameter and appear to be derived by the pinching off of the flattened discs. In some cells the network also includes vacuoles which are probably distended discs (33). The Golgi complex is confluent with the endoplasmic reticulum and can be classified as a type of smooth-surfaced endoplasmic reticulum (14).

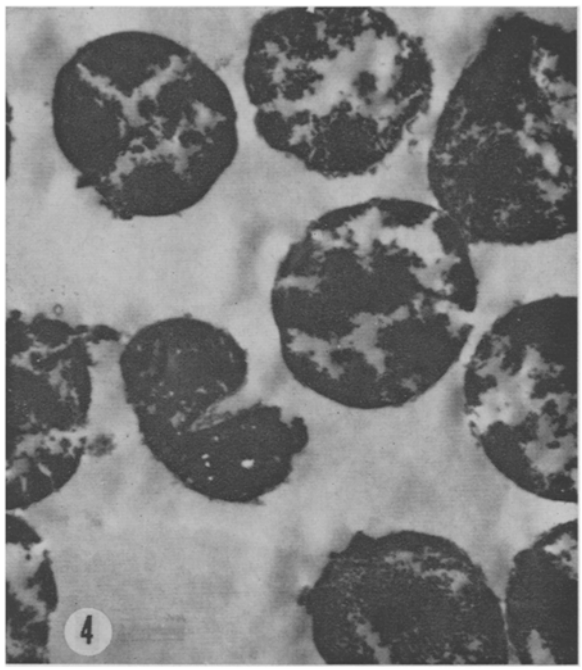


FIG. 4. Electron micrograph of isolated cell nuclei (x 6000) prepared according to Allfrey, Litton and Mirsky (83). This photograph was provided by and used with their permission.

A special role of the Golgi complex can be demonstrated in secretory cells. In experiments using high resolution radioautography, the path of newly synthesized protein was traced from the rough-surfaced endoplasmic reticulum to the Golgi complex. There it is concentrated and condensed as granules (79,85).

Attempts to isolate the Golgi complex have met with limited success. A major difficulty is the lability of the complex to shear. Conventional blending methods used for the isolation of other organelles (nuclei, mitochondria, chloroplasts) result in the complete disappearance from the homogenate of any structures resembling the Golgi complex (27,92). Loose-fitting homogenizers or sharp razor blades for chopping the tissue have been used together with stabilizers added to the medium. The Golgi fraction obtained by density gradient centrifugation was found to be enriched with respect to lipid and acid phosphatase (27).

Endoplasmic Reticulum and Ribosomes (The Microsomal Fraction)

The endoplasmic reticulum is a three-dimensional network in the cytoplasma composed of tubules, vesicles, and flattened sacs (cisternae) which are limited by a single membrane about 70 A wide. The tubules and vesicles are 0.03 to 0.5 μ in diameter, and the cisternae can extend the length of the cell (20 μ or more). The membranes are interconnected and the space which is enclosed forms a single compartment which is separated from the rest of the cytoplasm (24,44,94).

A distinction is made between the rough and smooth surfaced endoplasmic reticulum. To the former are attached many dense granules giving the membrane a rough appearance. These granules, about 150 A in diameter, are the ribosomes which contain approxi-

FIG. 5. Electron micrograph of isolated beef heart mitochondria (x 20,000). This preparation (22) was used in the studies of Fleischer, Fleischer, Casu and Rouser (in preparation). The electron microscopy was performed by Dr. David Slautterback of the University of Wisconsin.

mately equal amounts of RNA and protein. Unattached ribosomes also exist free in the cytoplasm (14). The relative content of the rough and smooth surfaced membranes as well as the total volume occupied by the system varies with the cell type. In addition to protein synthesis, which occurs in ribosomes (53,100) the endoplasmic reticulum is the key center of various metabolic interconversions including triglyceride and steroid biosynthesis (48,73).

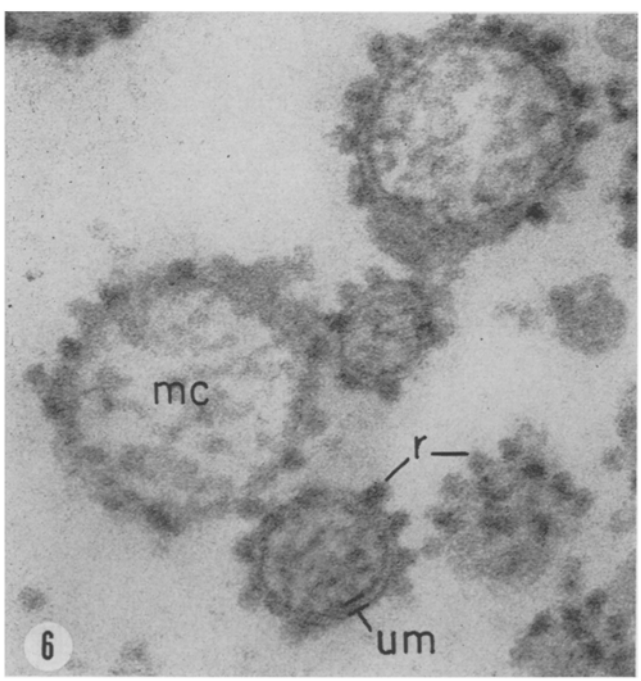


FIG. 6. Electron micrograph of microsomes isolated from guinea pig pancreas (x 220,000). Ribosomes (r), subspherical particles of ribonucleoprotein, 150 A in diameter, are attached to the membranes. The unit membrane (um) and the microsomal content (mc) are clearly visible. This photograph was provided by Dr. George Palade of the Rockefeller Institute.

The membranes of the endoplasmic reticulum are found in the microsomal fraction on differential centrifugation of a homogenate. These appear as vesicles which range from 0.05 to 0.3 μ in diameter. The larger membranes of the endoplasmic reticulum seem to pinch off and form these smaller vesicles in the process of handling. Both rough and smooth surfaced vesicles are obtained (24). It must be recognized that microsomes are only defined operationally: they are the sediment which results from high speed centrifugation of the mitochondrial supernatant. Under carefully controlled conditions to prevent contamination with mitochondria and lysosomes the microsomal fraction may be relatively free of contaminants. Other components normally obtained in the microsomal fraction are the Golgi complex and possibly the cell membrane fragments. Furthermore, since the proportions of the components of the cytoplasmic membrane system may vary with the tissue, the microsomal fraction may be different from different tissues (56). Figure 6 illustrates the appearance by electron microscopy of microsomes isolated from guinea pig pancreas.

Ribosomes can be released from the isolated microsomal (rough) fraction by treatment with deoxycholate and isolated free from the membrane to which they were attached to give a preparation of the type shown in Figure 7 (54). Ribosomes occurring free in the cytoplasmic matrix are enriched by prolonged sedimentation of the supernatant remaining after sedimentation of microsomes.

Rough and smooth surfaced microsomes can be separated since their densities differ. Membranes containing ribosomes are denser due to their increased amount of protein and RNA (74). Enzymic differences between rough and smooth microsomes have been reported (34). A novel procedure for the separation of microsomes is based on the unequal binding of cations. Three fractions can be obtained: 1) rough-surfaced vesicles which bind both Cs^{+} and Mg^{++} ions; 2) smooth-surfaced vesicles which bind only Mg^{++} ions; and 3) smooth-surfaced vesicles which bind neither Mg^{++} nor Cs^{+} ions. The enzymes in fraction 3 were found to be quite different from the enzymes in fractions 1 and 2 (62).

Mitochondria

The mitochondrion is the most thoroughly studied subcellular particle. Investigations from many laboratories have yielded very important information regarding the structure and functions of this organelle (10,16,39,45,52). The mitochondrion continues to fascinate investigators as the number of basic principles obtained from its study continues to increase.

While mitochondria from different sources may differ widely as to ancillary enzymic capacities, their basic function is to provide energy for the cell. The mitochondrion catalyzes the complete combustion of pyruvate; the energy derived from the oxidation is conserved with the formation of ATP. The overall process can be divided into three parts: 1) the citric acid cycle; 2) the mitochondrial electron transfer chain; and 3) the "coupling" enzymes. Citric acid cycle intermediates derived from various foodstuffs are oxidized by the primary dehydrogenases of the citric acid cycle. The electrons are then transferred to oxygen via the electron transfer chain, a complex assemblyline of oxidation-reduction components. During the stepwise oxidation-reduction sequence, "high energy" intermediates are formed. The "coupling" enzymes then transfer this "trapped energy" to the

synthesis of ATP (oxidative phosphorylation). The electron transfer chain contains coenzyme Q, a lipid oxidation-reduction component (20,23,40). The first conclusive demonstration of a functional role for phospholipid in an enzyme system has been with the electron transfer chain and its complexes. The phospholipid can be removed with loss of electron transfer properties that are restored by addition of phospholipid (50,65,88).

One powerful method of studying this complex entity has been to disassemble the mitochondrion into its component parts which can be separated from one another and studied independently (52). The mitochondrial electron transfer chain has been further separated into four component complexes, and then reassembled with recovery of overall electron transport (51).

Mitochondria are generally rod-shaped and vary in size (usually from 0.3 to 0.7 μ in diameter and 1 to 7 μ long). Morphologically the mitochondrion can be likened to two membranous balloons, one of which is contained within the other and the two are separated by a space, the "intracristal space." The "inner membrane" contains a number of infoldings into the center. These infoldings are the "cristae" and they enclose the "intercristal space" (10). Negative staining of mitochondria reveals new fine structure in the form of "elementary particles" (70–90 A in diameter) which line the inner membrane and which are connected to the membrane by cylindrical stalks (71,78, 87).

The crude mitochondrial fraction obtained in the classical scheme of differential centrifugation has been purified by resedimentation in a buffered medium of slightly higher pH (7.8). Two distinct layers are obtained and can be separated mechanically from one another. The upper or "light mitochondrial" fraction consists of swollen mitochondria and any contaminating lysosomes and microsomes. The lower "heavy mitochondrial" fraction represents highly purified mitochondria as shown in Figure 5 (22). Small amounts of highly purified mitochondria can be prepared by sucrose density gradient sedimentation after nuclei and debris have been removed by preliminary sedimentation (67).

The Chloroplast

It is common knowledge that chloroplasts of plants utilize light energy for the endergonic synthesis of carbohydrates. The principal photochemical event involves the absorption of light by chlorophyll to form an activated state. The next step which is poorly understood, involves the displacement of an electron and results in the reduction of ferridoxin. Ferridoxin has the lowest redox potential found in any biological material. Electrons are then transferred along a series of oxidation-reduction components including plastoquinones and cytochrome *f*. In the process of electron transport, ATP is formed (photosynthetic phosphorylation). Photosynthetic phosphorylation energizes the synthesis of carbohydrates which can occur in the dark (dark reactions) (35,47,58,95).

The fine structure of the chloroplast in the alga *Chlamydomonas* can be seen in the electron micrograph in Figure 1. The organelle is contained within a double-membraned envelope. The interior, the matrix, is crowded with double-membraned flattened lamellar (membranous) sacs which run more or less parallel to each other. In the center of the chloroplast is the nonlamellar body, the pyranoid, about 1.5–2μ in diameter, which is associated with starch synthesis

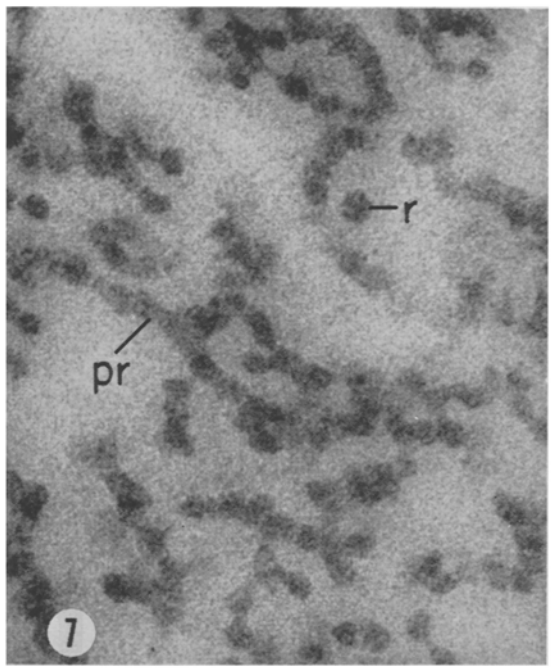


FIG. 7. Electron micrograph of ribosomes (x 225,000). The ribosomes have been detached from the microsomal membranes (cf Fig. 6) by treatment with deoxycholic acid. They exist singly as ribosomes (r) or in strands, termed polyribosomes (pr). This photograph was provided by Dr. George Palade of the Rockefeller Institute.

(21). In higher plants the chloroplast is usually a disc-shaped body 4–6 μ in diameter and 2–3μ thick and contains no pyranoid body. The chloroplast is particularly rich in lipids (30–45%). Polar lipids predominate with the major lipids being galactolipids.

Chloroplasts are frequently isolated from spinach or Swiss chard leaves. The leaf structure is first disrupted, e.g. by controlled grinding with sand in isotonic salt (0.35 M NaCl). Whole cells, debris, and sand are removed at very low speed. The chloroplasts are then sedimented with slightly higher force (81).

In the isolation of chloroplasts from algae, the cells were disrupted with a French Press in a medium containing a number of additives including sucrose, ficoll, and dextran. The mixture was then separated by centrifugation in a discontinuous sucrose gradient (75).

Lysosomes

Lysosomes contain hydrolases (63,64) and are particularly plentiful in cells that perform digestive tasks (white blood cells and macrophages) (89). Studies of the intracellular localization of acid phophatase disclosed the existence of lysosomes (12). Refined sedimentation techniques showed that this enzyme was localized in a distinct fraction intermediate in sedimentation between mitochondria and microsomes and hence both of these fractions usually have contaminating lysosomes (12,64). Over a dozen known hydrolytic enzymes are contained within the structure bounded by a single limiting membrane which protects the rest of the cell from autolytic destruction. These enzymes are capable of digesting proteins, nucleic acids, and polysaccharides, but not lipids. Lysosomes have not been reported in plant cells (63,64). Identification of lysosomes in situ by electron microscopy is difficult as their size (0.25–0.5 μ) and appearance is quite variable. This may be a reflection of the metabolic state of the lysosomes. Lysosomes may fuse with a food vacuole and various states of autolysis of the material in the vacuole may be observed (63,93).

Tissues differ widely in the degree of stress that can be applied to disrupt cells and yet maintain lysosomal integrity (64). When the lysosome is damaged, hydrolytic enzymes are released and autolysis of the homogenate proceeds.

Lysosomes have been prepared by sucrose density centrifugation from the "light" fraction of mitochondria. The presence of ribonuclease, acid phosphatase, and aryl sulfatase is usually an indication of the presence of lysosomes. The activities are latent, i.e. they can be measured only after disruption of the lysosomal membrane (12,64,98).

Centrioles

These organelles are usually positioned in the cytoplasm just outside the nucleus on one side and the Golgi complex on the other. Centrioles are usually found in pairs which lie at right angles to each other. Electron microscopy has indicated that the centriole is "a characteristic body composed of nine tubules in cylindrical array. The tubules are each about 200 A in diameter and about 5000 A long and the cylinder which they circumscribe is about 1500 A in diameter" (41). Centrioles have not been detected in higher plants.

The first event visible in cell division (mitosis) is the division of the centrioles. Pairs of centrioles migrate to opposite sides of the nucleus and establish the poles along which the cell components line up and divide. This "mitotic apparatus" has been isolated, although the centrioles per se have not yet been isolated (41).

Microtubules

The cytoplasmic microtubules have been documented only recently (66,77,101). These are long straight tubules with an outside diameter of about 180 A and an inner diameter of 80 A. They have been found to be as long as 2 μ. The microtubules are morphologically identical with the mitotic spindle fibers (101). In the early stages of cell division, the spindle fibers and chromosomes become oriented between the centrioles to form the mitotic apparatus. It has been speculated that when the nucleus is not dividing the spindle fibers play an active role in determining the shape of cells and are involved in the transport of water or ions in the cell.

The Plasma (Cell) Membrane

The plasma membrane is the outer barrier of the cell and performs the vital function of regulating the flow of materials into and out of the cell. The membrane is far too thin to be observed with light microscopy, although its existence had long been inferred from osmotic considerations as well as from its semipermeable character.

The plasma membrane is only about 75 A in width. With appropriate staining it can be resolved into three layers: two dark (electron opaque) layers approximately 20 A wide sandwich a central light layer about 35 A wide. This trilaminar fine structure is referred to as the "unit membrane" (30). The first studies on cell membranes were limited to membranes ("ghosts") from mammalian red blood cells. Such cells are normally devoid of nuclei, mitochondria, and endoplasmic reticulum. Upon hemolysis such "ghosts" can be recovered by sedimentation (43).

Of the solid tissues, plasma membranes derived from liver have been studied most extensively (29,36,86). The cell is disrupted quantitatively by homogenization in hypotonic medium. Under these conditions most of the nuclei and probably the other membrane-limited organelles are disrupted as well. The crude membrane fraction is collected by sedimentation at low speed and is then purified by density gradient centrifugation.

The appearance of these membranes as viewed by electron microscopy is suggestive of a cell but devoid of its contents (29,86). The membrane is not contaminated by nuclei, mitochondria, or lysosomes, although a number of smooth vesicles are visible. These cell membranes were found to contain about 0.3 mg phospholipid per milligram protein and about 4 molecules of cholesterol per 10 molecules of phospholipid (86).

The isolation of empty tubular-like cell membranes from rat skeletal muscle has been described as well. The method consists of 1) mincing rat skeletal muscle in hypotonic medium, 2) extracting the cell fragments with salt, and 3) separation of the fragments by sedimentation (38).

The "unit membrane" is the basic arrangement found in all membranes. In addition to the cell membrane, it has been observed in membranes of mitochondria and chloroplasts (both outer and inner) as well as in the limiting membranes of the nucleus, lysosomes, endoplasmic reticulum and Golgi complex. The unit membrane arrangement is clearly visible in Figure 6. A double membrane refers to two unit membranes.

The trilaminar arrangement of the unit membrane can be interpreted on a molecular level according to the model of Danielli and Davson (4), i.e. it consists of a bimolecular layer of lipid between two layers of protein. All unit membranes are not identical; they differ both in width and in fine structure (76). No unit membrane has been observed in the microtubules or the centrioles but they are the exceptions.

A membrane, whether at the limiting surface of a cell or surrounding organelles, encloses a compartment. Nuclei, mitochondria, microsomes, and lysosomes as well as microsomal vesicles all respond to changes in osmotic conditions (80). Selective transport through the limiting membrane (18) has been demonstrated in nuclei (32), mitochondria (39,61,97), and microsomes (49).

II. Quantitative Analysis of Lipid Class Compositions (References 103–134)

Modern analytical methods are based on chromatographic separations, although these may be combined with older procedures such as solvent partition and colorimetric assay. The chromatographic approaches are of two distinctly different types: chromatography of hydrolysis products or of intact lipids. Chromatography of hydrolysis products was widely used prior to the advent of satisfactory analytical separation procedures for intact lipids.

Determination of Hydrolysis Products

It must be appreciated at the outset that methods of analysis based on hydrolysis are subject to limitations that cannot be overcome with additional work (see references 103–126 for the factors involved and a general definition of the problem). The most serious deficiency is that the fatty acid composition of individual lipid classes cannot be studied. Hydrolytic procedures are all based on assumptions that may not be valid in every case. It is usually assumed in the determination of hydrolysis products that each lipid class yields products by which it can be distinguished from other lipid classes, although it is appreciated that diacyl and monoacyl (lyso) phosphatides both yield the

same water soluble hydrolysis products. With hydrolytic procedures it must be assumed that uncharacterized lipid classes are not present that could give the same hydrolysis products as those classes known or suspected to occur. The validity of this assumption can be established only by isolation and characterization of all lipid classes of a mixture by other procedures. A quantitative accounting of all lipids of a mixture must be available before the presence of uncharacterized lipid classes can be completely excluded. The hydrolytic procedures are based upon the further assumption that, under carefully controlled conditions, one lipid class will give characteristic and reproducible hydrolysis products. This is frequently difficult to attain in a strictly quantitative manner since lipids can hydrolyze by more than one mechanism and different procedures may give different ratios of hydrolysis products.

Hydrolytic techniques are commonly used with the assumption that a spot in the correct position on a chromatogram or a peak from a column emerging at the proper place represents a certain substance without confirmation by other means. This can be very misleading. A recent example of this type of difficulty is found in work on sea anemone lipids. Early work on this organism based on hydrolytic techniques without chromatography indicated sphingomyelin to be the only lipid present, but a subsequent study utilizing column, paper, and thin-layer chromatography as well as infrared spectroscopy and hydrolytic techniques disclosed that sphingomyelin was entirely absent, many other polar lipid classes were present, and a new type of sphingolipid, ceramide aminoethylphosphonate, was isolated and characterized (130). Subsequently Hori et al. (131) isolated the same lipid from another organism, but similarity of migration to authentic compounds by paper chromatography alone was used to identify hydrolysis products and 2-aminoethylphosphonic acid was erroneously identified as ethanolamine-O-phosphate. A later study (132) corrected the first report when it was realized that 2-aminoethylphosphonic acid and ethanolamine-O-phosphate could be distinguished readily by infrared spectroscopy (130).

Acid hydrolysis is most commonly used to degrade polar lipids (glycerol lipids and sphingolipids) relatively completely to the most stable products. Thus diacylglycerylphosphorylcholine (lecithin) is hydrolyzed to fatty acids, glycerophosphates (a mixture of the 1- and 2-phosphates), and choline. Choline plasmalogen (plasmalogen form of lecithin) that contains one ester linked and one α, β-unsaturated ether linked hydrocarbon chain hydrolyzes to yield fatty acid, fatty aldehyde and the same water soluble products as the diacyl form, but the recently discovered form of the phosphoglycerides that contains one ester and one ether linked hydrocarbon chain will give either a phosphorylated glyceryl ether or a glyceryl ether and orthophosphate depending upon the conditions employed. Provision must be made for these differences. Acid hydrolysis may be associated with some decomposition of products such as choline, ethanolamine, and sugars and a correction factor may be necessary in some cases to take into account decomposition of the more labile products.

Mild alkaline hydrolysis has been used in lipid chemistry for many years to split the ester linked fatty acids of glycerol lipids and leave intact the sphingolipids with amide linked fatty acids. Methods of analysis have been based on this principle. The alkaline hydrolysis procedure is complicated by several factors. The plasmalogen forms of glycerol lipids give lyso compounds since the α, β-unsaturated ether linkage is stable under the usual hydrolysis conditions. A lyso compound is formed also from the true ether linked type of phospholipid. While these differences can be used to determine the relative amounts of the different forms, the existence of the different forms of the ethanolamine and choline-containing lipids presents difficulties when it is desired to determine the total amounts of these lipid classes (e.g. total lecthin including diacyl, plasmalogen, and glyceryl ether forms).

Despite many uncertainties and difficulties, the hydrolytic procedures have provided valuable data before other methods were available. The approach introduced by Dawson (126) is particularly noteworthy and has provided a great deal of valuable information. In its present form, the Dawson approach to analysis of hydrolysis products is still useful. Some studies of subcellular particles by hydrolytic procedures were carried out prior to the time that difficulties with these procedures were appreciated and the results are thus of a preliminary and less precise nature.

Paper Chromatography

Marinetti et al. (127) introduced a quantitative procedure for the determination of individual phospholipid classes based upon separation by one-dimensional silicic acid impregnated paper chromatography, detection with rhodamine 6G, elution, and phosphorus analysis. This method has been used widely and provided information before improved column and thin-layer chromatography procedures were available. The combined experience of the authors made possible comparison of the Marinetti technique with other paper, thin-layer, and column chromatographic methods. The Marinetti paper chromatography procedure suffers from the lack of resolution of components characteristic of all one-dimensional chromatographic techniques. Some lipids decompose in the Marinetti system. This is particularly serious since altered lipids formed either during handling prior to chromatography or during chromatography migrate in the areas attributed to other phospholipids and are erroneously quantitated. This behavior is noted particularly for cardiolipin and phosphatidyl ethanolamine, each of which can give rise to several altered products. The findings are presented in more detail in Part III of this report.

Identification of substances by paper chromatography have been made largely by relative migrations (R_f) and are thus subject to the usual difficulties of interpretation. Additional methods are usually desirable for characterization of substances separated by one-dimensional chromatography. The presence of a substance in the lysolecithin region on paper chromatograms is a good example. In our experience lysolecithin is not encountered in tissues (although seen in plasma) and the "lysolecithin" spot sometimes seen with the Marinetti system does not migrate to the lysolecithin position with the chloroform/methanol/ammonia paper chromatography system (128) or by TLC (133) nor does it appear in the proper fraction from DEAE cellulose columns. Oxidation products of lipids can be mistaken for lysophosphatides. These difficulties must be borne in mind when results are evaluated.

Column and Thin-Layer Chromatography

Silicic acid column chromatography has been used extensively in the study of subcellular particle lipids.

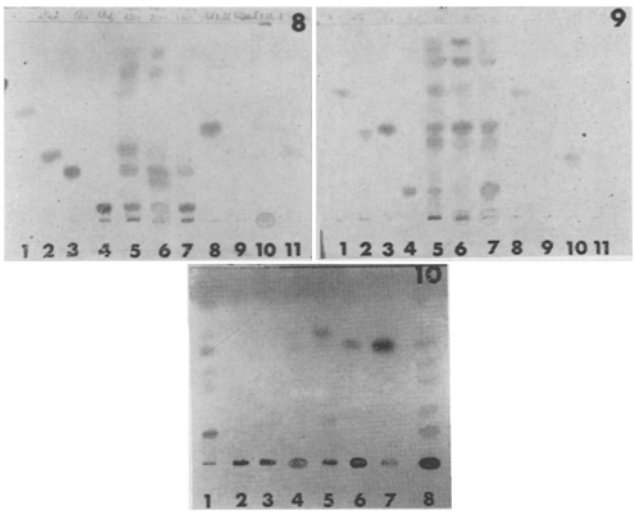


FIG. 8. Thin layer chromatogram to illustrate neutral lipid components of beef heart, liver, and kidney mitochondria. The neutral lipid fraction was eluted from a DEAE cellulose column with chloroform. The chromatogram was developed with benzene (Silica Gel G as adsorbent) and spots developed with 5% phosphomolybdic acid in 95% ethanol and heat (120C). Applications are: 1) 10 μg vitamin K_1, 2) 10 μg α-tocopherol, 3) 10 μg coenzyme Q, 4) 10 μg cholesterol, 5), 6), and 7) 50 μg each of neutral lipid from beef liver, heart, and kidney mitochondria respectively, 8) 10 μg cholesterol acetate, 9) 10 μg tripalmitin, 10) 10 μg oleic acid, and 11) 10 μg tristearin. Note presence of lipid in the tocopherol, coenzyme Q, and cholesterol regions as well as the very small amount of lipid in the triglyceride region. Unknown substances are present. Compare with Figure 9 (chromatogram developed with hexane/ether/acetic acid).

FIG. 9. Thin layer chromatogram of neutral lipid components of beef liver, heart, and kidney mitochondria. The chromatogram was developed with n-hexane/diethyl ether/ acetic acid 70/30/1 and stained with iodine vapor. Applications as noted for Figure 8.

FIG. 10. Thin layer chromatogram of beef heart mitochondrial components of the neutral lipid fraction (defined by silicic acid column chromatography) eluted from a DEAE column. The chromatogram was developed with n-hexane/diethyl ether/ acetic acid 70/30/1 and stained with the phosphomolybdic acid reagent (see legend Fig. 8). Applications 1–7 were 50 μg each of fractions from the column eluted with 1) chloroform; 2) chloroform/methanol 9/1; 3) chloroform/methanol 7/3; 4) methanol; 5) chloroform/acetic acid 3/1; 6) acetic acid; 7) chloroform/methanol 4/1 containing 20 ml/liter 28% aqueous ammonia and made 0.01 M with ammonium acetate; 8) 200 μg of mixed liver mitochondrial lipid. The presence of two uncharacterized acidic lipids migrating near the solvent is demonstrated and a small amount of free fatty acid is shown migrating just up from the origin.

Several difficulties with silicic acid column chromatography have been discussed previously (129) and various thin-layer and column chromatographic procedures have been compared (134). The most pertinent observations with regard to subcellular particle work are that cardiolipin (50) and phosphatidyl ethanolamine (129,130) tend to decompose on silicic acid columns and may not be eluted as a single well-defined peak. DEAE cellulose columns and TLC appear to give the best resolution of components and decomposition during chromatography has not been encountered with these techniques in our laboratories. Recently both one- and two-dimensional TLC procedures and a DEAE column-TLC procedure for quantitative determination of components of lipid mixtures have been presented (133,134). Mitochondrial lipids were studied with these systems and it has become apparent that they do not possess the defects observed with other chromatographic systems.

III. Lipid Class Composition of Subcellular Particles (References 135–182)

Total Lipid and Percent Phospholipid

All values reported for total lipid as percentage of the dry weight and the proportion as phospholipid are in relatively good agreement for mitochondria. Thus Swanson and Artom (135) found rat liver mitochondria to contain 29% lipid with 79% of the total lipid being phospholipid. Kretchmer and Barnum (136) reported 27.4% lipid in mouse liver mitochondria, while Marinetti et al. (144) reported 28.6% lipid in pig heart of which 91% was phospholipid. Redfearn (158) found 90% of the total lipid of pig heart was phospholipid while Fleischer et al. (154) found 27% lipid in beef heart mitochondria with 92% of the lipid being phospholipid. Schwarz et al. (160) reported 28.2% lipid in rat liver mitochondria with 85% as phospholipid. It is clear that pig and beef heart and mouse and rat liver mitochondria all have very similar total lipid (about 27%) and that about 90% of the total lipid is phospholipid.

Nuclei have not been studied adequately for total lipid content. Levine and Chargaff (138) and Spiro and McKibben (143) reported values of 11 and 16% for rat liver nuclei. Phospholipid was reported to represent 93% of the total lipid by Spiro and McKibben (143). It would appear, however, that the amount of phospholipid in a purified nuclear preparation is actually much lower. When the values presented by Gurr et al. (173) are recalculated, phospholipid is found to represent only about 3% of the dry weight of a pure preparation of nuclei.

Microsomes may contain more lipid than nuclei or mitochondria. Microsomes from mouse liver were reported by Kretchmer and Barnum (136) to contain 35.1% lipid, from rat liver Spiro and McKibben (143) found 32% lipid, and from pig heart Marinetti et al. (144) reported 24.6% lipid. Phospholipid was reported by these three groups to represent 62.7% (mouse liver), 94% (rat liver), and 68% (pig heart) of the total lipid of microsomes. More work is needed before a clear picture is obtained of the total lipid and percent phospholipid of microsomes from different organs and species.

The possible role of redistribution of lipid during in vitro manipulation reported by Rosenfeld and Lang (167) has not been considered in most studies. The redistribution of neutral lipids observed by these authors may give rise to misleading results.

Components of the Neutral (Least Polar) Lipid Fraction

The composition of the neutral lipid fraction of subcellular particles has not been studied as extensively as phospholipid class composition. Rat liver mitochondria were reported to contain 5.4% total and 2.4% free cholesterol by Swanson and Artom (135), while Spiro and McKibben (143) found 5.5% total cholesterol and Schwarz et al. (160) reported 3.0% cholesterol. Spiro and McKibben (143) reported 4.5 and 5.8% cholesterol in lipid of rat liver nuclei and microsomes, respectively. Redfearn (158) reported 3.4% cholesterol in pig heart mitochondrial lipids. The data, largely for rat liver, indicate that cholesterol is found in nuclei, mitochondria, and microsomes and that it makes up from 3 to 6% of the total lipid. Kritchevsky et al. (148) reported that a larger proportion of liver cholesterol is recovered in microsomes than in mitochondria of several species (mouse, rat, chicken, rabbit, monkey, and man).

Neutral lipids other than cholesterol have been stud-

ied to a very limited extent. Spiro and McKibben (143) reported 2.5, 1.4, and 0.0% neutral fat in lipids from rat liver nuclei, mitochondria, and microsomes respectively, and Redfearn (158) reported 3.7% of the lipid of pig heart mitochondria as neutral fat. The actual identification of the component(s) designated as neutral fat has not been reported, but it is usually assumed to be glycerides.

Jayaraman et al. (157) reported the presence of coenzyme Q and tocopherol in nuclei, mitochondria, and microsomes from rat liver, heart, and kidney, and rabbit liver. Our studies confirm the presence of coenzyme Q in microsomal preparations. The contamination of these preparations by mitochondria or derived fragments was evaluated enzymologically and did not exceed 3% in heart and 8% in liver. Expressed as μM/gm protein the values obtained were 1.43 and 0.68 in heart and liver microsomes, respectively. The minor contamination of the preparations by mitochondria precludes this as the source for coenzyme Q in these fractions since the values for mitochondria of beef heart (3.94) and beef liver (0.89) are incompatible with this interpretation.

The less polar components of mitochondria are of considerable interest and deserve more careful study. Figures 8–10 show TLC findings from our laboratories. Figures 8 and 9 illustrate components detected in chloroform eluates from DEAE columns. Chloroform elutes the less polar, nonionic lipids from DEAE, but acidic lipids such as fatty acids are not eluted with chloroform in contrast to their elution from silicic acid columns as "neutral" lipids. TLC of fractions from DEAE columns with beef heart, liver, and kidney mitochondria as samples are shown after development with benzene (Fig. 8) and hexane/diethyl ether/acetic acid 70/30/1 (Fig. 9). The presence of cholesterol, coenzyme Q, and material in the tocopherol area is clearly indicated. Several uncharacterized substances are visualized. (One of these migrates just behind coenzyme Q and others, particularly in liver mitochondria, migrate near the solvent front.) The chromatogram developed with the acidic solvent shows particulraly well that there is almost no material in the triglyceride region from heart and kidney mitochondria and only a small amount from liver mitchondria. This suggests that triglycerides are almost entirely absent from our mitochondrial lipid preparations. The chromatogram shown in Figure 10 illustrates components of other fractions from the DEAE column. From the chromatogram it is clear that two substances, eluted in part in several fractions, were retained by DEAE and eluted as acidic lipids. The two acidic lipids migrate in the triglyceride-vitamin K region on TLC with hexane/diethyl ether/acetic acid as solvent.

Polar Lipids of Subcellular Fractions

Discussion of the data is facilitated by consideration of the literature pertaining to a given organ together (regardless of species).

Liver. The early reports (135,136,138,143) are primarily of historical interest as far as polar lipid composition of subcellular particles are concerned since the methods of analysis are inadequate by present standards. Swanson and Artom (135) reported rat liver mitochondria to contain 45% lecithin, 47% noncholine containing phospholipid (mostly phosphatidyl ethanolamine) and 8% sphingomyelin. The presence of cardiolipin, now known to be a major component of mitochondria was not recognized and the large amount of sphingomyelin reported does not appear to be characteristic of mitochondria. The results of Kretchmer and Barnum (136) indicated that rat liver mitochondria and microsomes contained 38.2% and 54.5% choline phospholipids, respectively. The work of Levine and Chargaff (138) indicated rat liver mitochondria and microsomes to contain lecithin, phosphatidyl ethanolamine, and phosphatidyl serine. Spiro and McKibben (143) indicated the presence of lecithin, sphingolipid (measurement of sphingosine) and inositol lipids (measurement of inositol) in rat liver nuclei, microsomes, and mitochondria. The early studies thus indicated nuclei, mitochondria and microsomes to be similar in composition with lecithin and phosphatidyl ethanolamine being the major components of each fraction. The presence of phosphatidyl inositol, phosphatidyl serine, and sphingomyelin was also indicated, but the presence of cardiolipin was not detected.

TABLE I

Phosphatides of Subcellular Particles[a] (% of total phosphorus)

	Lec	PE	CL	PI	PG	PS
Rat liver mitochondria	49	30	12	8	1	
Rat liver lysosomes	45	24	20	8	2	
Rat liver microsomes	68	18,12		12	2,5	
Rat kidney mitochondria	41	30	9	14	3	1
Rat kidney microsomes	+++	++		++		+
Sheep heart mitochondria	46	35	10	5	4	
Beef heart mitochondria	38	37	16	6	1.1	+
Rat brain mitochondria	55	32	2	5	2	4

[a] From Strickland and Benson (150).
Abbreviations: Lec = lecithin, PE = phosphatidyl ethanolamine, CL = cardiolipin, PI = phosphatidyl inositol, PG = phosphatidyl glycerol, and PS = phosphatidyl serine.

Collins and Shotlander (153) studied the composition of rat liver microsomes and mitochondria. They separated products of dinitrophenylation and methylation by countercurrent distribution and reported mitochondria to contain phosphatidic acid (2%), cardiolipin (5%), phosphatidyl ethanolamine plus serine (20%), uncharacterized phospholipid (37%), lecithin plus sphingomyelin (31%) and inositol and minor phospholipids (5%). Microsomes were found to be similar except that cardiolipin was a very minor component. The presence of a new type of phospholipid that was suggested to be a phosphotriester unstable to other separation procedures is questionable. It seems probable to the authors that the dinitrophenylation and methylation procedures produced some unexpected artifactual component from other phospholipids indicating the apparent presence of a new type of phospholipid. We have not detected uncharacterized components using DEAE cellulose column chromatography and TLC. (Neither technique when used properly appears to alter even relatively labile lipids.)

Strickland and Benson (150) reported on the lipids of rat liver mitochondria and microsomes. They used mild alkaline hydrolysis (deacylation) and determined hydrolysis products by neutron activation analysis after paper chromatography. The values obtained are shown in Table I. In agreement with earlier reports, lecithin and phosphatidyl ethanolamine were found to be major components of both liver mitochondria and microsomes with phosphatidyl inositol being a significant component in both. Cardiolipin was present in mitochondria only, and phosphatidyl glycerol was indicated to be a minor component of both mitochondria and microsomes. (The large value for phosphatidyl glycerol of beef heart mitochondria entered in the table presented in the original paper appears to be a typographical error.) The presence of phosphatidyl serine was not detected in most preparations. Gray

TABLE II
Phospholipids of Mitochondria and Microsomes of Rat Liver [a]

	Mito	Micro
Phosphatidyl serine and phosphatidyl ethanolamine	42.0	27.0
Lecithin	38.6	62.6
Polyglycerol phosphatides	8.4	0.8
Inositol phosphatides	9.9	9.3
Polar lipids	2.1	3.3
Lysophosphatides	3.0	23.7

[a] From Getz, Bartley, Stirpe, Notton, and Renshaw (163).
Abbrev. Mito, mitochondria; micro, microsomes.

(179) later reported 0.4% phosphatidyl glycerol in rat liver mitochondria.

Schwarz et al. (160) using silicic acid column chromatography reported cardiolipin to represent 10.1% of the total lipid of rat liver mitochondria. This value corresponds to 11.8% of the total phospholipid as cardiolipin and is in agreement with the value of 12% reported by Strickland and Benson (150). Later Schwartz et al. (175) using a deacylation procedure found rat liver mitochondria to contain (as % total phospholipid) 49.6% lecithin, 26.4% phosphatidyl ethanolamine, 4.6% phosphatidyl serine, 4.4% phosphoinositide, 7.7% cardiolipin, and 1.7% phosphatidyl glycerol.

Macfarlane et al. (149) determined the relative distribution of phospholipids of rat liver mitochondria to be 51% lecithin, 31% phosphatidyl ethanolamine plus serine, 9% cardiolipin, and 6% phosphatidyl inositol. The authors noted that sphingomyelin and plasmalogens were not found in rat liver mitochondria, but the presence of phosphatidyl serine was indicated. The values for microsomes were: 62% lecithin, 26% phosphatidyl ethanolamine, 10% phosphatidyl inositol, and less than 1% cardiolipin plus phosphatidic acid. Plasmalogens were absent. The microsomal values are similar to those of Strickland and Benson (150) except in the case of phosphatidyl ethanolamine.

Getz et al. (163) reported on the composition of rat liver mitochondria and microsomes. Total lipid recoveries (expressed as g/100 g original liver) were 2.4 for mitochondria and 13.0 for microsomes (total in original liver 21.0) demonstrating more of the total liver lipid to be present in the microsomal fraction. Phospholipid class composition was determined by a combination of silicic acid column chromatography and deacylation. Some of the values reported are shown in Table II. The value for phosphatidyl ethanolamine is much higher than values reported by Strickland and Benson (150) and Macfarlane et al. (149). The presence of lysophosphatides in liver mitochondria was indicated by Getz et al. and lysophosphatide was reported to be a major component (23.7%) of rat liver microsomes.

The only detailed analysis of the phospholipids of rat liver nuclei where purity of the fraction was documented was reported by Gurr et al. (173) who used a deacylation procedure for analysis. The relative distribution of phospholipids was found to be: 52.2% lecithin, 25.1% phosphatidyl ethanolamine, 5.6% phosphatidyl serine, 6.3% sphingomyelin, 4.1% phosphatidyl inositol, 1.2% unidentified. Only 1.1% of the total phosphorus was reported as in the plasmalogen form, and a questionable trace of phosphatidic acid and the absence of cardiolipin were reported. Except for the sphingomyelin value calculated from the value for phosphorus stable to both mild acid and alkali, a procedure that may give misleading results, the values appear to be relatively reliable and were obtained from relatively pure nuclei.

TABLE III
Phosphatides of Pig Heart Cell Fractions [a]
(% total phosphorus)

	Mito	Micro
Origin	1.95	2.66
Unidentified	0.70	.....
Inositol phosphatide	14.32	3.92
Lysolecithin	.....	8.04
Sphingomyelin	5.58	16.45
Lecithin	36.20	41.80
Phosphatidyl serine	2.75	3.59
Phosphatidyl ethanolamine	25.30	21.20
Acidic glycerol phosphatide	13.30	2.00

[a] From Marinetti, Erbland, and Stotz (144).
Abbr.: Mito, mitochondria; Micro, microsomes.

Figure 13 shows some TLC results obtained in our studies on highly purified preparations of mitochondria and microsomes from beef organs. The absence of cardiolipin from beef liver microsomes is demonstrated in agreement with reports in the literature on rat liver microsomes. In contrast, a small amount of cardiolipin is detectable in heart microsomes. This may be related to contamination with nonmicrosomal particles. No evidence for the presence of cerebrosides in liver mitochondria as reported by Lovtrup and Svennerholm (174) was obtained by TLC. Lovtrup and Svennerholm appear to be the only investigators to have reported cerebrosides in liver subcellular particles and it is probable that their method of analysis was unreliable.

Conclusions Regarding Liver Subcellular Particles. The values reported in the literature for phospholipids of liver subcellular particles are quite variable and are contradictory in many cases. Lecithin, phosphatidyl ethanolamine, and phosphatidyl inositol have invariably been reported as components of all liver subcellular particles. The values for lecithin and phosphatidyl inositol have generally been in relatively good agreement, but the values for phosphatidyl ethanolamine have been variable and significantly different. It is apparent, particularly in the case of rat liver mitochondria, that different methods of analysis give different results. There is general agreement that cardiolipin is a component of mitochondria, and that cardiolipin is low in or absent from microsomes and nuclei. The quantitative values for cardiolipin are in relatively good agreement. Whether or not phosphatidyl serine, phospatidyl glycerol, phosphatidic acid, sphingomyelin, lysophosphatides, and a major uncharacterized phospholipid occur in liver mitochondria cannot be judged from the reports in the literature since there is no general agreement. These have been identified as components and determined in some studies but not in others.

Heart. Marinetti et al. (144) reported on the phospholipid composition of pig heart mitochondria and microsomes. The analyses were performed by paper chromatography of intact lipids and determination of phosphorus in each spot. The values obtained are shown in Table III. Lecithin, phosphatidyl ethanolamine, phosphatidyl inositol, and cardiolipin were found as principal components of mitochondria with phosphatidyl serine and sphingomyelin being minor components. The analysis is similar to some reported for liver mitochondria. Heart microsomes were found by Marinetti et al. to have lecithin, phosphatidyl ethanolamine, and sphingomyelin as major components. A rather large amount (8%) of lysolecithin was reported and phosphatidyl inositol, phosphatidyl serine, and cardiolipin were found to be minor components. Microsomes differed from mitochondria in having less cardiolipin and phosphatidyl inositol, more sphingomyelin, and in that lysolecithin was present.

Strickland and Benson (150) reported values for sheep and beef heart mitochondria (see Table I) that

are similar to those of Marinetti et al., but the phosphatidyl ethanolamine values are very different (37 and 25%). Strickland and Benson used the alkaline deacylation technique that, while directly applicable to liver lipids that do not contain plasmalogens, should present difficulties with heart mitochondria which are rich in plasmalogens. It is possible that the Dowex 50 resin (used by Strickland and Benson for neutralization of the methanolysate) hydrolyzed lysophosphatides produced from ethanolamine and choline plasmalogens to form glycerylphosphorylethanolamine and choline.

Fleischer et al. (154), using the paper chromatographic technique of Marinetti et al., reported the phospholipid composition of beef heart mitochondria to be 40% lecithin, 34% phosphatidyl ethanolamine, 14% cardiolipin, and 11% "phosphatidyl inositol." The values are in good agreement with those of Strickland and Benson (150) for beef heart mitochondria.

Buahene and Cornatzer (172) reported relatively high values for phosphatidyl inositol, phosphatidyl serine, and sphingomyelin in rat heart mitochondria. The relative proportions of the phospholipids was reported as lecithin 666, phosphatidyl ethanolamine 644, phosphatidyl serine 234, phosphatidyl inositol 266, and sphingomyelin 222.

Paper chromatography with the chloroform/methanol/ammonia-system of Rouser et al. (128), one- and two-dimensional TLC (133), and DEAE cellulose column chromatography (130) applied to beef heart mitochondria by Fleischer et al. (183) failed to disclose the presence of the large amount of phosphatidyl inositol in beef heart mitochondria indicated to be present by the Marinetti paper chromatography system. The discrepancy between the Marinetti paper chromatography system and the other chromatographic systems was traced in part to decomposition of the "altered" form of phosphatidyl ethanolamine recognized in lipid extracts by Rouser et al. (133,134) and in part to decomposition of cardiolipin in the Marinetti system. Since neither cardiolipin nor phosphatidyl ethanolamine (either the native or "altered" forms) decomposes with the TLC system of Rouser et al. (133) and wide separations of mitochondrial lipids are obtained with TLC, the erroneously high value for phosphatidyl inositol with the Marinetti system was explained. Quantitative TLC by the charring technique (133) gave values for beef heart mitochondria of 40.7% lecithin, 41.6% phosphatidyl ethanolamine, and 17.6% cardiolipin (as % of total phospholipids ignoring trace components). When the lower values for phosphatidyl ethanolamine (34%) and cardiolipin (14%) reported previously with the Marinetti system (154) were subtracted from the values obtained by TLC and added together, a value of 11.2% was obtained. This is very near the value of 11.0% previously reported for phosphatidyl inositol by paper chromatography (154). The difference between the paper and TLC systems evidently lies primarily in the fact that decomposition products of cardiolipin and phosphatidyl ethanolamine are erroneously estimated as "phosphatidyl inositol" by paper chromatography according to Marinetti.

Figures 11 through 14 illustrate some of the findings by paper, thin layer, and column chromatography. Figure 11 illustrates a typical paper chromatogram used for quantitation by the Marinetti technique with beef heart mitochondrial lipid as sample. The different lipid classes are not as widely separated by paper chromatography as by thin-layer chromatography.

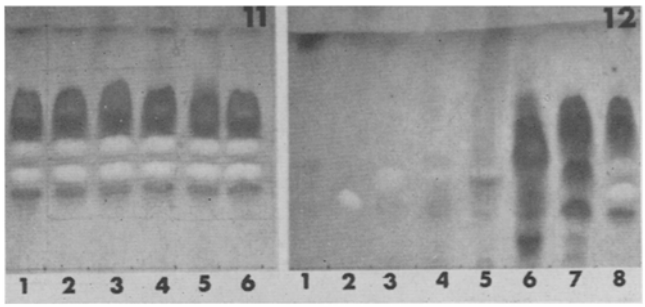


FIG. 11. Paper chromatogram according to Marinetti of beef heart mitochondrial phospholipids. The chromatogram was stained with rhodamine 6G and photographed under ultraviolet light. Cardiolipin gives rise to the dark spread-out spot above while the two light spots were from phosphatidyl ethanolamine and lecithin. The lower spot is in the phosphatidyl inositol region (see text for discussion of the true components of this region of the chromatogram). This is a typical chromatogram for a quantitative determination by phosphorus analysis of each spot. Compare with Figure 13 where the same heart mitochondrial sample was applied at the same concentration for TLC. Note the much more compact spots by TLC (Fig. 13) and the absence of material in the phosphatidyl inositol region (just off the origin) by TLC in contrast to the paper chromatographic system.

FIG. 12. Paper chromatogram according to Marinetti to illustrate composition of fractions eluted from DEAE cellulose columns (compare with Figure 14 when the same fractions are shown after TLC). The chromatogram was stained with rhodamine 6G. Applications were 50 μg each (1–5) and 10 μg (6 and 7) of fractions eluted with 1) chloroform (neutral lipids); 2) chloroform/methanol 9/1 (lecithin); 3) chloroform/methanol 7/3 (phosphatidyl ethanolamine); 4) methanol ("altered" phosphatidyl ethanolamine); 5) chloroform/acetic acid 3/1 (another form of "altered" phosphatidyl ethanolamine); 6) chloroform/methanol 4/1 containing 20 ml/liter 28% aqueous ammonia (cardiolipin and related products); and 7) solvent as for 6 but containing 0.01 M ammonium acetate in addition (cardiolipin and related products). Note the spreading of spots in 4, 5, 6, and 7 indicating decomposition during chromatography of the "altered" forms of phosphatidyl ethanolamine and decomposition of cardiolipin. The results are in contrast to those obtained by TLC of the same fractions (Fig. 14) where decomposition during chromatography is not observed (all spots well-defined and without spreading). The paper chromatogram illustrates how material in the phosphatidyl inositol region of the Marinetti chromatogram can be formed from other substances.

When the sample was applied to a DEAE column and elution carried out as indicated in the legend for Figure 12, a large amount of material migrating in the phosphatidyl inositol region was detected by paper chromatography (Fig. 12) but not by TLC (Fig. 14). DEAE column chromatography (with recoveries of from 99 to 101%) followed by one-dimensional TLC indicated phosphatidyl inositol to be only a trace component of the mitochondrial lipid extract (Fig. 14). Phosphorus analysis after paper chromatography of the DEAE column fractions indicated that the excess phosphorus in the phosphatidyl inositol region on the paper chromatograms came in part from breakdown of phosphatidyl ethanolamine and in part from cardiolipin.

The instability of phosphatidyl ethanolamine to paper chromatography in the Marinetti system appears to be related to the presence in lipid extracts of the "altered" form of phosphatidyl ethanolamine (about 20% of the total amount of this lipid present in the extracts) reported by Rouser et al. (133,134). This form of phosphatidyl ethanolamine is not separated from the native lipid by TLC, but is separated by DEAE cellulose column chromatography. The variations in phosphatidyl ethanolamine values noted above for rat liver subcellular particles may be related to varying amounts of this "altered" form of phos-

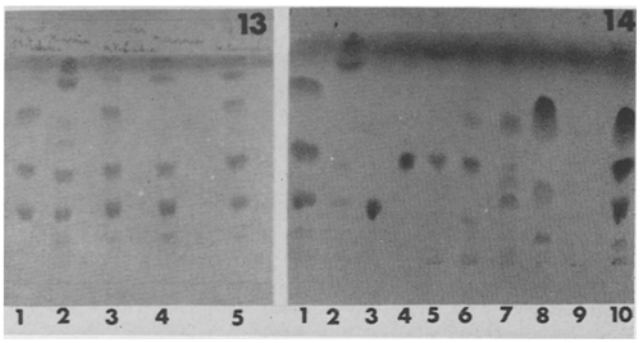


FIG. 13. TLC of beef heart, liver, and kidney mitochondrial and microsomal lipids developed with chloroform/methanol/water 65/25/4 (Silica Gel G adsorbent) and spots localized by exposure to iodine vapor. (1) and (2) from 100 μg each of heart mitochondrial and microsomal lipids. In (1) the upper spot is cardiolipin and the lower spots phosphatidyl ethanolamine and lecithin. In (2) the spot at the solvent front is from a mixture of neutral lipids with cholesterol just behind the solvent front. The more polar components from above down are cardiolipin, free fatty acid, phosphatidyl ethanolamine, and lecithin with material in the sphingomyelin region closest to the origin. (3) and (4) from 100 μg each of beef liver mitochondrial and microsomal lipids, and (5) from 100 μg of beef kidney mitochondrial lipids. Note the presence of the same three major lipid classes in very nearly the same amounts in the mitchondrial preparations with only traces of substances migrating in the phosphatidyl inositol and sphingomyelin areas. Cardiolipin is present in the heart microsomal preparation, but not in the preparation from liver.

FIG. 14. TLC of the same fractions from the same DEAE column for which paper chromatography results are shown in Figure 12. The chromatogram was prepared as described for Figure 13 and spots localized with a phosphomolybdic acid (5% in ethanol) spray and heat (120C). (1) and (10) from 100 μg of beef heart mitochondrial lipid. Material eluted with (2) chloroform (100 μg applied); 3) chloroform/methanol 9/1 (25 μg); 4) chloroform/methanol 7/3 (25 μg); 5) methanol (100 μg); 6) chloroform/acetic acid 3/1 (50 μg); 7) chloroform/methanol/ammonia (50 μg); and 8) chloroform/methanol/ammonia plus ammonium acetate. Of special interest is the presence of the "altered" form of phosphatidyl ethanolamine eluted with methanol (application 5) and chloroform/acetic acid (application 6). The "altered" lipid is separated from the native lipid on DEAE columns but not by TLC. Note that the substances in the chloroform/methanol/ammonia eluates (applications 7 and 8) are well separated and streaking indicating decomposition is absent in contrast to results with paper chromatography (Fig. 12). A trace of material is seen in the phosphatidyl inositol region in application (8) (the spot nearest the origin) demonstrating that this lipid class is only a trace component in our beef heart mitochondrial preparations. No trace at all of sphingomyelin can be seen moving behind lecithin in application (3). The presence of small amounts of phosphatidyl inositol, sphingomyelin, and lysophosphatides may be inferred from one-dimensional TLC of mitochondrial lipids from spots moving behind lecithin (see applications No. 1 and 10) but in our preparations this is from decomposition products of cardiolipin and phosphatidyl ethanolamine formed when the solutions are stored for a prolonged time.

phatidyl ethanolamine in lipid extracts and the extent to which this unstable substance decomposes when different separation techniques are used.

Figure 13 illustrates TLC results obtained in the authors' laboratories on the polar lipid components of highly purified mitochondrial and microsomal preparations of beef heart, liver, and kidney. The presence of lecithin, phosphatidyl ethanolamine, and cardiolipin in mitochondria from the three organs is shown and it is apparent that the relative proportions of the components vary only slightly from organ to organ. The absence of cardiolipin from liver microsomes is in keeping with most reports in the literature for rat liver microsomes and the presence of a small amount of cardiolipin in the beef heart microsomal fraction is in agreement with the report of Marinetti et al. (144) on pig heart microsomes.

Brain. Tyrell and Richter (137) in a very brief communication reported values for polar lipid components of human brain nuclei. The relative amounts of cerebroside was found to be greater in nuclei than in whole brain. The presence of a large amount of cerebroside in human brain nuclei should be confirmed by more modern methods.

Nussbaum et al. (165) determined sphingomyelin in subcellular fractions of rat brain and concluded that this lipid was present in all fractions with both mitochondria and microsomes containing a significant amount.

Petersen and Schou (141) studied rat brain nuclei, mitochondria, and microsomes. For nuclei they reported sphingomyelin, cephalin, and lecithin to be present in the ratio of 1.5/7.7/0.8. The same lipids were reported in mitochondria in the ratio of 3.4/14.6/3.8 and in microsomes in the ratio of 0.9/6.4/2.6. The results are best regarded as preliminary in nature.

Strickland and Benson (150) reported rat brain mitochondria (see Table I) to contain large amounts of lecithin and phosphatidyl ethanolamine, smaller amounts of phosphatidyl inositol, phosphatidyl serine, and phosphatidyl glycerol, with cardiolipin as a very minor component. Biran and Bartley (152) reported on rat brain mitochondria and the low value of 1.6% for cardiolipin was recorded. The composition of brain mitochondria was reported as 50.0% phosphatidyl ethanolamine plus serine, 32.1% lecithin, 13.4% phosphatidyl inositol, 15.2% lysophosphatides, 1.6% cardiolipin, and 0.5% uncharacterized polar lipid. The microsomal lipids were reported to be very similar qualitatively and quantitatively to the mitochondrial lipids. The results also appear to be more of a preliminary nature.

Lovtrup and Svennerholm (174) reported on the lipid composition of brain and liver mitochondria (species not specified). The very high values of cerebroside (18%) reported for brain mitochondria and the presence of 7.1% cerebroside reported for liver mitochondria appears to be related to the method of analysis that gives erroneous values for glycolipid, possibly for the reason noted by Eichberg et al. (178). The predominant phospholipids of liver and brain mitochondria were reported to be lecithin and phosphatidyl ethanolamine. Cardiolipin values were not reported and sphingomyelin was reported in mitochondria from both organs. Gangliosides were not detected in brain mitochondria, although gangliosides were detected in guinea-pig brain mitochondria by Eichberg et al. (178).

Eichberg et al. (178) reported a comprehensive study of guinea-pig brain subcellular particle lipid compositions. They reported values for large and small myelin fragments, nuclei, synaptosomes, mitochondria, microsomes, and synaptic vesicles obtained using the hydrolytic technique of Dawson (126) for analysis of phospholipids. Nuclei were judged to be contaminated significantly (with myelin) and microsomes were found to be slightly contaminated with other components. Some of the analytical results are presented in Table IV. Large amounts of cholesterol were found in large and small myelin fragments, a moderate amount was found in nuclei and synaptosomes, and a small amount was found in mitochondria. Values for glycolipid (cerebroside and/or sulfatide) indicated most of the cerebroside and sulfatide to be present in the myelin fragments, a moderate amount in nuclei and microsomes, and traces only in synaptosomes and mitochondria. Values for N-acetylneur-

TABLE IV

Distribution of Individual Phospholipids in Subcellular Fractions of Guinea-Pig Forebrain [a]
(% of total lipid phosphorus)

	Large myelin fragments	Small myelin fragments	Nuclei	Mitochondria	Microsomes	Synaptosomes	Synaptic vesicles
Phosphatidyl choline	25.6	32.1	33.6	40.0	41.4	39.3	40.7
Phosphatidyl ethanolamine	8.9	14.1	14.0	23.3	13.0	17.6	13.1
Phosphatidyl serine	12.8	14.1	11.1	5.9	11.9	12.9	10.2
Phosphatidyl inositol	2.8	2.9	2.6	5.5	4.8	4.0	5.9
Phosphatidic acid	1.9	1.3	0.7	0.6	0	0.7	2.3
Cardiolipin	Trace	0	2.3	11.1	0.4	1.6	0
Choline plasmalogen	Trace	0	0	0	0	0	0.6
Ethanolamine plasmalogen	25.5	24.4	17.4	9.4	14.8	16.0	12.0
Serine plasmalogen	0.3	0	0	0	0	0	0
Sphingomyelin	12.4	7.3	7.1	3.7	7.5	5.3	12.3
Alkyl ether phospholipid	3.8	2.3	2.8	2.2	1.9	2.1	3.2
Recovery	94.6	98.5	91.6	101.7	95.5	99.5	100.3

[a] From Eichberg, Whittaker, and Dawson (178).

aminic acid in the preparations indicated the presence of gangliosides in all particles with relatively more in the microsomal fraction in agreement with the findings of Wherrett and McIlwain (170) for guinea-pig brain microsomes. TLC examination indicated that the proportions of the different gangliosides were almost the same in the various particulate preparations except that a monosialoganglioside was more abundant in large myelin fragments.

The relative proportions of the phospholipids reported by Eichberg et al. is of considerable interest. Lecithin, phosphatidyl ethanolamine, and phosphatidyl serine were major components of all preparations with mitochondria having the smallest amount of phosphatidyl serine (5.9%) and small myelin fragments the highest (14.1%). Sphingomyelin was found in all preparations being highest in myelin (12.4%) and lowest in mitochondria (3.7%). A small amount of phosphatidyl inositol (2.6 to 5.9%) was reported for each fraction and a small amount (about 1%) of phosphatidic acid was reported for all preparations except microsomes. Cardiolipin was essentially absent from myelin and synaptic vesicles, present in small amounts in nuclei and synaptosomes, present as a trace component in microsomes, and present as a major component of mitochondria.

The values reported by Eichberg et al. are in contrast to values reported by Lovtrup and Svennerholm (174), who found 18% cerebroside in brain mitochondria, and the results of Strickland and Benson (150) and Biran and Bartley (152) who found cardiolipin to be a minor component of rat brain mitochondria. Unpublished results from this laboratory (185) are more in keeping with the observations of Eichberg et al.

Roots and Johnston (182) reported the presence of phosphatidyl ethanolamine and phosphatidyl serine in beef neuron cell lipid extracts. This is the first report of lipids of neurons and the technique used offers promise for future studies.

Kidney. Rat kidney mitochondria were analyzed by Strickland and Benson (150). Their values (Table I) indicate kidney mitochondria to be similar to mitochondria from other tissues in agreement with TLC results obtained by the present authors on beef kidney mitochondria (Fig. 13). Biezenski and Spaet (151) reported values for phospholipids of rat kidney subcellular particles, but the procedures used for analysis by hydrolysis are questionable and the results obtained are difficult to compare with those obtained by modern procedures.

General Conclusions Regarding Subcellular Particle Polar Lipid Compositions

Although the literature is contradictory in many respects and it is clear that more data are required to resolve many of the points adequately, some general conclusions can be drawn with regard to the phospholipid composition of mitochondria and microsomes. More data are required before firm statements regarding the lipid components of the nucleus are justified.

It is apparent that mitochondria and microsomes are similar in that lecithin and phosphatidyl ethanolamine are major components of both. It is also clear that mitochondria contain cardiolipin as a major component and that cardiolipin is either a minor component of or absent from microsomes. The low values for cardiolipin reported from two laboratories for brain mitochondria are probably in error, but this must be verified. Some variation from organ to organ is indicated since heart microsomes appear to contain a small amount of cardiolipin, although it appears to be entirely absent from liver microsomes.

There is an indication that mitochondria from different organs are similar. Variations in different studies of the same organ have been as large as the variations from organ to organ suggesting that differences in methodology are at least as important as organ variations. There is little indication of any marked species variations, but again variations in methodology may have obscured some of the differences.

Phosphatidyl inositol has been indicated to be a major component of mitochondria. Despite the fact that there is general agreement in the literature on this point our results show conclusively that this concept is incorrect. The presence of phosphatidyl inositol in mitochondria in relatively large amount probably reflects either contamination with other subcellular particles or deficiencies in analytical procedures or both. Evidence for this is presented above. We conclude that phosphatidyl inositol is a very minor component of mitochondria and that the small amount found in our best preparations may be related to very small contamination from other sources.

The situation with regard to other minor and trace components is complicated and much disagreement exists in the literature. In recent investigations on very pure particulate preparations we have not found phosphatidyl serine, sphingomyelin, lysophosphatides, any uncharacterized phospholipids, cerebrosides, or phosphatidic acid in beef heart, kidney, or liver mitochondria and of these lipids only a small amount of phosphatidic acid was detectable in beef brain mitochondria. We believe the finding of anything more than traces of these components to represent methodological difficulties, either contamination with other subcellular particles or deficiencies in analytical procedures or both. The best available data indicate that mitochondria contain cardiolipin, phosphatidyl ethanolamine, and lecithin as major components and traces of other polar lipids are probably from other sources.

TABLE V

Fatty Acid Composition of Phospholipids of Rat Liver Mitochondria [a] (area percentages)

	PG	CL
14:0	0.7	Trace
16:0	12.0	0.9
16:1	1.8	3.3
16:2	0.5	....
17:1	0.7	Trace
18:0	14.1	Trace
18:1	20.9	10.0
18:2	19.7	83.6
20:2	13.4	0.8
20:3	....	1.3
20:4	6.5	Trace
?	10.0	....

[a] From G. M. Gray (179).
Abbrev. PG, phosphatidyl glycerol; CL, cardiolipin.

Traces of phosphatidyl glycerol cannot be excluded entirely, although we have not encountered this lipid in our preparations.

Data obtained recently by Rouser and Kritchevsky (184) indicate that phosphatidyl ethanolamine becomes altered when it comes into contact with glass or other solid surfaces in the dry state. One of the products formed and referred to by Rouser et al. (133, 134) as "altered" phosphatidyl ethanolamine is not separable from the native lipid except by DEAE cellulose column chromatography and decomposes readily to more polar products. Decomposition of "altered" phosphatidyl ethanolamine takes place during paper chromatography with the Marinetti system giving rise to substances that can be mistaken for lysophosphatides and phosphatidyl inositol. Similar decomposition has been noted on silicic acid columns. These observations explain some of the apparent differences in composition reported from different laboratories.

We conclude that there are two basically different types of membrane structures. Myelin represents one type and is metabolically very stable. Mitochondria represent the other extreme with metabolically labile membrane lipids. The two types of membranes have very different turnover properties and very different lipid compositions. Myelin is characterized by a high content of cholesterol and sphingolipids and contains more long chain saturated and monoenoic fatty acids. Mitochondria are characterized by a low cholesterol content, little or no sphingolipid, and highly unsaturated fatty acids. It is clear that formulations of the myelin type membrane structure such as that of Vandenheuvel (176) requiring cholesterol cannot apply to mitochondria. It also seems probable that membrane structures intermediate between the extremes represented by myelin and mitochondria exist.

TABLE VI

Fatty Acid Composition of Phospholipids from Rat Liver Microsomes and Mitochondria [a] (area percentages)

	Cephalin		Lecithin	
	Mito	Micro	Mito	Micro
11:0	....	....	....	....
12:0	0.4	....	....	....
14:0	....	....	0.3	0.2
15:0	0.2	....	0.2	0.2
16:0	17.5	17.5	13.2	13.2
16:1	1.0	0.7	3.5	3.7
17:0	0.6	0.3	0.6	0.9
17:?	0.3	....	0.1	0.2
18:0	30.4	34.5	21.9	24.9
18:1	4.8	4.4	14.1	12.1
18:2	4.3	4.2	19.6	19.5
18:3	....	....	....	....
19:0	....	....	0.5	....
20:3	0.8	1.3	1.5	1.2
20:4	21.0	23.0	13.8	12.6
20:5	2.4	0.6	1.9	1.4
22:5	2.0	2.0	1.2	0.4
22:6	14.0	11.7	7.9	7.7
Saturated acids	50.2	53.6	38.3	40.8
Monoenoic acids	5.8	5.1	14.7	15.8
C_{20}–C_{22} acids	40.2	38.6	26.3	23.3

[a] From Macfarlane, Gray, and Wheeldon (149).
Abbreviations: Mito = mitochondria, micro = microsomes.

TABLE VII

Fatty Acids of Rat Liver Subcellular Fractions [a] (molar percentages)

	Nuclei	Mito	Micro
14:0	0.50	0.41	0.47
15:0	0.57	0.47	0.53
16:0	25.7	21.4	23.4
16:1	1.66	1.63	1.27
17:0	0.64	0.74	1.01
18:0	16.4	17.5	19.9
18:1	14.0	12.2	13.7
18:2	16.5	20.6	16.0
20:3	0.92	1.84	1.30
20:4	14.1	15.4	15.4
22:5	0.64	0.77	0.78
22:6	5.96	5.12	4.73
Total unsaturated acids	53.8	57.5	53.1
Polyunsaturated acids	38.1	43.7	58.2

[a] From Getz and Bartley (155).
Abbr. Mito, mitochondria; Micro, microsomes.

Fatty Acid Composition of Subcellular Fractions

Only a relatively small number of reports have appeared describing the fatty acid compositions of subcellular particles. Most of the work deals with rat liver mitochondria and microsomes, although the reports of Witting et al. (161) and Biran and Bartley (152) deal with rat brain and Richardson et al. (159, 166) reported on fatty acids of total mitochondrial lipids of chicken liver, beef heart, and of hearts and livers of fish, birds, and the seal.

Liver. Klein and Johnson (139) demonstrated a marked reduction in the essential (polyunsaturated) fatty acids of rat liver mitochondria and microsomes using the alkali isomerization procedure for measurement of polyunsaturated fatty acids. The same authors (140) failed to find any appreciable differences in polyunsaturated fatty acids in liver subcellular particles of growing, mature, or aging rats.

Clement et al. (142) studied rat liver mitochondrial and microsomal fatty acids and reported a relatively high content of unsaturated and particularly polyunsaturated fatty acids. Mitochondria and microsomes were found to be very similar in fatty acid composition.

Hayashida and Portman (147) studied fatty acids of rat liver mitochondria and microsomes as a function of diet. Particles from animals on a fat-free diet were low in linoleic and arachidonic acid and high in oleic and eicosatrienoic acids. The mitochondria from deficient rats had different swelling properties indi-

TABLE VIII

Fatty Acids in Lipids from Rat Liver Mitochondria [a] (area percentages)

	CL	PE	PI	Lec	Polar lipid
<10:0	0.1	....	....	0.1	....
11:0	0.1	....	....	0.1	....
12:0	....	....	....	0.1	....
13:0	0.1	....	0.3	....	1.8
14:0 br.	....	....	0.1	....	0.7
14:0	0.5	0.1	0.9	0.5	1.2
14:1	0.1	....	....	....	....
15:0 br.	0.1	....	....	....	0.6
15:0	0.2	0.2	1.5	0.5	0.6
16:0 br.	0.1	....	1.1	0.1	0.6
16:0	3.9	20.2	12.1	19.7	33.6
16:1	2.5	0.8	0.8	1.5	2.5
17:uns. br.	0.1	....	....	....	....
17:0 br.	0.1	0.3	1.7	0.3	....
17:0	0.1	0.9	1.4	0.8	1.2
18:0 br.	0.1	....	....	0.3	0.4
18:0	1.4	19.5	32.0	18.7	25.2
18:1	12.8	10.4	6.8	12.2	10.9
18:2	74.0	16.3	16.2	20.0	14.0
18:3	0.1	....	....	....	....
19:0 br.	....	0.2	....	0.2	....
20:0	....	....	0.4	....	....
20:1	0.2	....	....	0.5	....
20:2	0.8	....	1.1	0.3	....
20:3	1.2	0.7	1.8	0.8	0.3
20:4	1.6	21.0	21.5	19.5	7.1
20:5	....	....	....	0.6	....
22:5	0.5	1.0	0.9	0.4	....
22:6	0.4	8.7	3.4	3.4	....

[a] Data from Getz, Bartley, Stirpe, Notton, and Renshaw (163).
Abbrev. CL, cardiolipin; PE, amino phosphatide fraction; PI, inositol rich fraction; Lec, lecithin.

TABLE IX
Fatty Acid Composition of Lipids of Liver Mitochondria [a]
(% of total fatty acids)

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	19:2	20:3	20:4	20:5	22:5	22:6
Fat-free													
Phospholipids	0.54	21.1	6.62	20.0	25.4	1.32	.....	.....	16.5	6.05	.....	1.08[a]	1.23
Nonphospholipids	3.61	31.6	11.8	7.96	39.0	0.96	.....	.....	2.92	1.14	.....	0.23[a]	0.37
18:2													
Phospholipids	0.29	20.9	4.98	20.1	20.1	4.29	.....	.....	7.75	17.1	.....	3.18[a]	0.74
Nonphospholipids	3.42	32.1	12.8	8.61	34.7	2.00	.....	.....	1.74	3.47	.....	0.29[a]	0.49
18:3													
Phospholipids	0.32	25.7	6.50	19.2	21.3	0.91	0.35	.....	3.82	2.36	5.08	1.53[b]	13.6
Nonphospholipids	3.04	32.9	11.8	7.77	35.0	0.78	0.79	.....	1.14	0.80	2.28	0.53[b]	3.17
20:4													
Phospholipids	0.44	23.0	5.61	19.6	18.1	0.62	.....	.....	1.48	25.0	.....	5.29[a]	0.71
Nonphospholipids	3.37	33.0	12.4	7.99	34.1	0.99	.....	.....	0.61	6.37	.....	0.75[a]	0.46
20:5													
Phospholipids	0.42	26.1	6.97	15.5	18.2	0.90	.....	.....	1.31	4.95	7.66	3.68[b]	14.3
Nonphospholipids	3.06	32.5	12.3	7.05	33.6	0.88	.....	.....	0.55	1.48	4.27	1.24[b]	2.72
22:6													
Phospholipids	0.26	27.8	6.18	18.1	21.2	1.09	.....	.....	1.95	2.59	2.84	1.07[b]	17.0
Nonphospholipids	2.53	34.3	11.1	7.17	36.0	1.51	.....	.....	0.70	0.82	1.99	0.37[b]	3.46
19:2													
Phospholipids	0.45	22.7	6.09	18.7	23.1	1.20	.....	4.51	11.93	7.38	.....	2.02[a]	1.97
Nonphospholipids	3.49	31.4	12.6	7.77	35.5	0.64	.....	4.04	2.39	1.28	.....	0.40[a]	0.42
Epoxyoleate													
Phospholipids	0.31	22.4	7.02	19.8	24.8	1.42	.....	.....	16.13	6.13	.....	1.02[a]	1.04
Nonphospholipids	3.91	34.2	11.4	8.29	37.2	1.75	.....	.....	2.08	0.70	.....	0.23[a]	0.25

[a] From Rahm and Holman (180).
Abbrev. a, 22:5ω6; b, 22:5ω3.

cating the importance of fatty acids in mitochondrial structures. The importance of essential fatty acids for mitochondrial structures was further emphasized by the observations of Wilson and Leduc (177) who studied livers of mice on diets deficient in essential fatty acids. Mitochondria were seen to be very much enlarged by light microscopy, and electron microscopy revealed additional cristae to be present.

Hoffsten et al. (164) reported altered swelling properties of rat liver mitochondria when lipid peroxides (measured by a thiobarbituric acid assay) were high indicating changes in properties as fatty acid composition changes.

Getz and Bartley (155) isolated cardiolipin from rat liver mitochondria and found it to contain about 80% linoleic acid as reported for this lipid from heart. Getz et al. (163) and Gray (179) confirmed this finding (see Table V), but the data are in marked contrast to those of Schwartz et al. (160) who reported a very low linoleic acid value for the polyglycerophosphatide (cardiolipin) from rat liver mitochondria.

Macfarlane et al. (149) reported on the fatty acids of lecithin and phosphatidyl ethanolamine of rat liver mitochondria and microsomes (see Table VI). Mitochondria and microsomes were found to be quite similar. The phosphatidyl ethanolamine fraction contained about 40% polyunsaturated fatty acids, mostly arachidonic acid (22%) and docosahexaenoic acid (12%) with only a small amount of linoleic acid (4%). Saturated fatty acids made up about 50% of the total with palmitic (17%) and stearic (32%) acids being the principal components. The lecithin fraction contained less polyunsaturated fatty acid (about 24%) and saturated fatty acids (39%) and more monoenoic acids (15%) than phosphatidyl ethanolamine. Linoleic acid was found to be the major polyunsaturated fatty acid in the lecithin fraction in contrast to the phosphatidyl ethanolamine fraction that contained more arachidonic and docosahexaenoic acids. Plasmalogens were almost completely absent from both the lecithin and phosphatidyl ethanolamine fractions as reported previously by Getz and Bartley (155) for cardiolipin of rat liver mitochondria.

Getz et al. (156) reported on the fatty acid composition of the cell sap and floating fatty layer after centrifugation of subcellular particles and Getz and Bartley (155) reported on the fatty acids of rat liver nuclei, mitochondria, and microsomes (see Table VII). All three particulates were very similar with unsaturated fatty acids representing about 55% and polyunsaturated fatty acids about 41% of the total fatty acids. Linoleic and arachidonic acids were found to be major components of all subcellular particles with palmitic and stearic acids being the predominant saturated fatty acids. The authors reported difficulties in extraction of all the lipid of rat liver and liver subcellular particles. Direct saponification released more lipid than saponification after solvent extraction, but the proportions of fatty acids were unchanged when extraction was incomplete.

Later Getz et al. (163) analyzed the fatty acid compositions of the individual phospholipid classes separated by silicic acid column chromatography from rat liver mitochondria and microsomes (see Table VIII).

TABLE X
Fatty Acid Composition of Liver Mitochondrial Lipids [a]
(area percentages)

	Weanlings at start of expt.	Animals on diets 6 weeks				Animals on diets 21 weeks			
		0.2% corn oil	15% coconut oil	15% corn oil	7% cod liver oil	0.2% corn oil	15% coconut oil	15% corn oil	7% cod liver oil
12:0	0.5	0.9	0.8	0.7	0.6	0.1	0.4	0.3	trace
14:0	1.1	2.6	2.6	2.0	2.1	0.8	2.1	1.2	0.9
16:0	18.5	18.5	19.8	17.3	24.5	22.1	17.5	12.2	21.6
16:1	1.5	7.1	4.5	1.7	5.5	5.5	6.5	2.0	6.5
18:0	21.7	18.3	19.7	20.1	17.5	16.1	14.7	12.1	12.4
18:1	14.7	22.7	19.5	12.9	17.6	20.3	18.5	15.2	14.3
18:2	14.1	6.3	9.5	20.3	5.8	7.7	10.1	20.9	5.0
20:0	1.0	0.4	0.4	0.5	0.4	0.1	0.3	0.3	0.1
20:1	0.4	0.9	0.8	0.9	1.3	0.3	0.4	0.4	1.6
20:?		0.5		1.5		0.1		0.7	0.3
20:3		7.0	5.6	0.7	0.6	4.5	4.4	0.6	0.5
20:4	16.0	9.2	11.4	17.5	5.4	14.8	17.1	25.1	5.7
20:5	0.7	0.7		0.3	6.9	1.6	1.1	0.7	1.6
22:2						0.4	0.3	0.5	0.3
22:5	4.3	1.0	1.1	0.9	2.2	1.8	1.3	2.1	3.8
22:6	5.2	1.8	3.0	1.6	7.9	5.3	4.1	3.5	14.0
24:0		0.5	0.8	1.3	1.2	0.5	0.3	0.8	

[a] From Witting, Harvey, Century, and Horwitt (161).

TABLE XI

Fatty Acid Content of Mitochondria [a] (mole percentages)

	Chicken liver	Beef heart	Rat liver	Salmon heart	Salmon liver	Catfish liver	Carp liver
14:0	0.3	1.6	0.3	0.9	1.2	0.9	0.8
Unknown	tr	2.7	....	....	....	....	....
14:1	....	....	....	....	....	....	0.4
14:2	....	....	0.2	}	}	}	0.2
15:0	....	....	0.3	{0.3	{0.3	{0.2	
16:0	26.0	19.5	20.4	27.3	20.9	19.1	25.5
16:1	1.3	0.5	2.2	1.1	0.9	2.5	2.9
Unknown	0.8	3.2	0.7	....	....	....	....
16:2	....	....	}	}	....	....	0.6
17:0	....	....	{0.8	{0.6	....	1.1	0.2
18:0	7.5	14.4	22.3	2.2	11.2	6.1	6.9
18:1	38.2	5.6	11.6	17.5	19.9	41.2	18.3
18:2	16.1	44.1	16.3	1.1	0.9	....	11.4
18:3	....	....	....	0.6	tr	....	2.6
18:4	....	....	....	1.9	0.9	1.8	1.0
20:2	....	....	....	....	....	....	1.0
20:3	1.0	0.8	0.2	....	....	....	0.8
20:4	6.2	7.5	24.7	1.6	4.0	4.5	9.2
20:5	....	....	....	8.7	16.5	8.4	1.0
Unknown	....	....	....	tr	....	....	tr
22:3	....	....	....	....	....	....	1.6
22:5	....	....	....	5.6	6.5	1.8	3.3
22:6	2.6	tr	....	30.4	15.6	11.8	11.8

[a] From Richardson, Tappel, and Gruger (159).

In mitochondrial cardiolipin linoleic acid was the major fatty acid (74% of the total) with oleic acid being next most abundant (12.8%). Very small amounts of longer chain polyunsaturated acids were detected and stearic acid was a very minor component (1.4%). These values are in good agreement with those of Gray (179) who reported 83.6% linoleic acid and 10.0% oleic acid in cardiolipin from rat liver mitochondria, but Schwartz et al. (160) reported very different values. The phosphatidyl ethanolamine fraction contained 21% arachidonic acid, 16.3% linoleic acid, and 8.7% docosahexaenoic acid. The lecithin fraction was similar to the phosphatidyl ethanolamine fraction. The values reported by Getz et al. (163) are in relatively good agreement with those of Macfarlane et al. (149) except that the latter group found fatty acids of lecithin to be less similar to those found in phosphatidyl ethanolamine.

Rat liver microsomes were reported by Getz et al. (163) to contain cardiolipin with a distinctly different fatty acid composition from cardiolipin of mitochondria in that oleic and linoleic acids were present in smaller amounts in microsomes. Phosphatidyl ethanolamine and lecithin fatty acids from microsomes were very similar to those of mitochondria.

Most investigators have found large amounts of linoleic acid in rat liver mitochondria and in individual phospholipid classes from mitochondria. The data of Schwarz et al. (160) indicated linoleic acid to be very low in cardiolipin of rat liver mitochondria and it is therefore of interest to note that Rahm and Holman (180) found (see Table IX) very little linoleic acid in rat liver mitochondria and the arachidonic acid content reported was also much lower than the values reported by Witting et al. (161), Richardson et al. (159), and Veerkamp et al. (168). The contrasting fatty acid compositions are shown in Tables IX through XII. The data of Rahm and Holman indicate that linoleic acid was a minor component of their liver mitochondrial lipid preparations even when a linoleic acid rich diet was fed. The findings of Schwarz et al. and Rahm and Holman suggest that some strains of rats may be different from others. Some strains may have characteristically low levels of linoleic and arachidonic acids. This interpretation is in keeping with the recent report of Biran et al. (181) on fatty acids of rat liver mitochondrial lipids. The data of Rahm and Holman showed that arachidonic content of liver mitochondria increases markedly when a diet rich in arachidonic acid is fed.

TABLE XII

Fatty Acids of Rat Liver Subcellular Particles [a] (weight percentages)

	12:0+ 14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Neutral lipids								
nuclei	2	29.5	6	1.5	31	26	1	3
mitochondria	1	32	5	2	36	22	+	2
Phosphatides								
nuclei	1.5	22.5	2.5	26.5	11.5	17	0.5	18
mitochondria	1.5	21.5	2.5	24	13.5	20	0.5	16.5

[a] From Veerkamp, Mulder, and Van Deenen (168).

The data of Veerkamp et al. (168), some of which is shown in Table XII, indicate the fatty acid composition of phospholipids and neutral lipids in mitochondria and nuclei of rat liver to be very similar. The phospholipids were found to contain much more arachidonic acid than the neutral lipids.

The data of Richardson et al. (159) (see Table XI) indicate that chicken and rat liver mitochondria differ greatly in fatty acid composition with linoleic and arachidonic acids being much higher and oleic acid much lower in rat liver mitochondria. Beef heart mitochondria were intermediate in that linoleic acid was found to be much higher and oleic acid much lower than in the liver mitochondria. A great deal of species variation is indicated, although dietary differences may be responsible for much of the variation. It is clear from these data, however, that mitochondria may have widely different fatty acid compositions.

The additional studies of Richardson et al. (166) are interesting and informative. While the suitability of a single procedure for the isolation of mitochondria from the many sources reported by these authors may be questioned, the findings present an interesting picture that should be confirmed. The authors studied fish heart and liver mitochondria and mitochondria from the same organs of species that normally eat fish (birds, the seal) in order to determine whether the polyunsaturated acids of the linolenate family characteristic of fish replace the polyunsaturated acids of the linoleate type that would be expected to occur on the basis of studies of other animal species whose diet does not contain fish. The results obtained demonstrated clearly that various birds and the seal retain linoleic and arachidonic acids in mitochondria, although acids of the linolenate family also were found demonstrating that the high fish diet did influence the fatty acid composition of mitochondria. The nearly complete absence of arachidonic and linoleic acids in fish mitochondria and replacement with polyunsaturated acids of the linolenate family demonstrates that specific polyunsaturated fatty acids are not required for all mitochondrial structures and functions related to lipid. The absence of polyunsaturated fatty acids other than linoleic acid from mitochondria of a plant (the sweet potato) reported by the same authors confirms this conclusion.

Heart. Holman and Widmer (145) studied polyunsaturated fatty acids of beef heart mitochondria by the alkaline isomerization technique and observed that various electron transport and enzyme preparations had similar contents of polyunsaturated fatty acids and that the preparations were similar to whole mitochondria.

Richardson et al. (159) reported on the fatty acid compositions of mitochondria from beef and salmon hearts and later (166) on heart mitochondria from fish, birds, and the seal. Some of the data are shown in Table XI. Beef heart was found to be high in linoleic acid with a moderate amount of arachidonic acid. Salmon heart was low in linoleic and arachidonic acids

TABLE XIII
Fatty Acids of Beef Heart Mitochondria Lipid Classes [a]
(area percentages)

	15	16	16:1	18	18:1	18:2	18:3	22	20:4
Lecithin	4.45	23.90	.....	4.62	19.22	37.30	4.21	2.60	3.70
Cardiolipin		0.42	2.08	2.34	5.19	83.64	6.33	.....	.....
Phosphatidyl ethanolamine	1.62	1.11	1.54	38.08	3.82	15.32	2.61	2.52	33.38

[a] Analyses performed by P. P. Nair on lipids isolated by the authors.

and high in docosahexaenoic acid. This was the general pattern found for all fish heart mitochondria (166). Mitochondria from the hearts of three different species of birds and the seal contained moderate amounts of linoleic and arachidonic acids as well as docosapentaenoic and docosahexaenoic acids.

The fatty acids of the individual phospholipids of heart do not appear to have been analyzed previously. The results presented in Table XIII were obtained in this laboratory from beef heart mitochondrial lipid classes separated by DEAE cellulose column chromatography. The analyses were performed by Dr. P. P. Nair with a Barber Coleman Model 10 gas chromatograph. Since beef heart mitochondrial lecithin and phosphatidyl ethanolamine exist predominantly in the plasmalogen forms in contrast to the nearly complete absence of these forms in liver mitochondria, it is of great interest that the fatty acids of phosphatidyl ethanolamine of heart are highly unsaturated as reported from other laboratories for liver mitochondria. Linoleic acid is much higher in heart mitochondrial phosphatidyl ethanolamine (15.3%) as compared to the same lipid from liver (Table VI). Stearic acid occurs to about the same extent in phosphatidyl ethanolamine from the two sources (38 and 30%). It appears that the aldehyde residues in heart mitochondrial phosphatidyl ethanolamine replace some of the saturated fatty acids characteristic of liver mitochondrial phosphatidyl ethanolamine since palmitic acid is very low in heart mitochondrial phosphatidyl ethanolamine. The lecithin fractions from heart and liver mitochondria show a similar pattern with linoleic acid being much higher in lecithin than in phosphatidyl ethanolamine. Cardiolipin fatty acids from beef heart (Table XIII) and rat liver mitochondria (Table V) are quite similar in some respects.

Brain. Biran and Bartley (152) reported upon the fatty acid compositions of rat brain mitochondrial and microsomal lipid fractions obtained by silicic acid column chromatography. Since the fractions from columns were mixtures of lipids, much of the potential value of the analyses was lost. The very low level of cardiolipin and the presence of phosphatidyl serine, lysophosphatides, and phosphatidyl inositol reported for mitochondria leads us to question whether the fraction was indeed mitochondria.

Witting et al. (161) reported upon the variations in fatty acid composition of rat brain and liver mitochrondria and erythrocytes with changes in dietary fat (see Tables X and XIV). Liver mitochondrial fatty acids changed extensively with changes in dietary fatty acids. Differences in linoleic, arachidonic, and docosahexaenoic acids were of the order of threefold in liver mitochondria. Smaller changes were noted for erythrocytes and brain mitochondria showed relatively small changes in a few fatty acids only.

The data of Witting et al. (161) provide an interesting comparison of liver and brain mitochondrial composition (see Tables X and XIV). Liver mitochondria from weanling rats were found to contain a relatively large amount of linoleic acid (14.1%) with arachidonic (16%) and docosahexaenoic (5%) acids being abundant as well. Palmitic (18.5%) and stearic (21.7%) acids were the major saturated fatty acids. Weanling rat brain mitochondria were similar in the high content of palmitic (19.6%) and stearic (20.9%) acids. Oleic acid was lower in liver (14.7%) than brain (20.8%) mitochondria as was arachidonic acid (11 and 16%). The most striking differences between brain and liver mitochondria were in linoleic and docosahexaenoic acids. In liver the values were 14.1% and 5.2%, respectively, while in brain the values were 1.7 and 9.1%, respectively. The very low level of linoleic acid in rat brain mitochondria indicates that cardiolipin, a major component of mitochondria, must be very different in brain and contain very little linoleic acid in contrast to liver mitochondria where it is usually reported to be the major fatty acid. The same conclusion can be drawn for lecithin and phosphatidyl ethanolamine of brain mitochondria.

The fatty acid composition data of brain mitochondria from weanling rats and rats on various diets for 6 to 21 weeks demonstrate that maturation changes (myelination, etc.) taking place during this time are not associated with changes in mitochondria. Brain mitochondria thus appear to be quite stable to dietary

TABLE XIV
Fatty Acids Composition of Brain Mitochondrial Lipids [a]
(area percentages)

	Weanlings at start of expt.	Animals on diets 6 weeks				Animals on diets 21 weeks			
		0.2% corn oil	15% coconut oil	15% corn oil	7% cod liver oil	2% corn oil	15% coconut oil	15% corn oil	7% cod liver oil
12:40	0.3	0.3	0.3	0.1	0.3	0.2	0.2	0.2	0.2
14:0	0.7	0.6	1.2	0.6	0.6	0.8	0.8	0.5	0.7
16:al	3.2	2.5	2.0	2.6	2.7	2.5	2.6	2.8	2.2
16:0	19.6	18.1	17.1	16.8	16.6	15.1	14.8	18.5	14.3
16:1	1.5	1.6	1.7	0.8	1.2	0.8	0.9	0.5	0.9
18:al	2.8	4.6	2.7	3.0	3.3	3.3	3.7	3.8	3.4
18:0	20.9	19.4	20.0	17.6	19.0	20.7	20.3	21.2	18.7
18:1	20.8	24.2	23.3	19.5	21.9	24.1	21.7	22.1	22.8
18:2	1.7	0.6	0.5	1.3	0.6	0.5	0.9	1.3	0.8
20:0	1.3	0.1	0.2	0.3	0.3	0.4	0.3	0.3	0.4
20:1		1.7	1.8	1.6	1.6	2.9	3.0	2.0	2.8
20:?						0.2	0.2	0.3	
20:3	0.6	0.5	0.8	0.3	0.4	0.7	0.3	0.3	0.4
20:4	11.4	9.5	9.2	12.8	8.4	9.3	10.7	10.2	8.0
20:5						0.2	0.3	0.3	0.7
22:2						0.4	0.2	0.2	0.4
22:5	1.8	1.6	2.8	2.6	0.8	2.6	2.4	2.1	2.3
22:6	9.1	10.9	9.7	14.9	18.4	9.6	10.9	8.7	16.6
24:0	2.9	2.9	3.3	3.9	2.2	3.2	3.6	3.4	1.3

[a] From Witting, Harvey, Century, and Horwitt (161).

variations and do not appear to participate in the changes taking place during maturation.

Kidney. Richardson et al. (166) reported the fatty acid composition of mitochondria from pelican kidney. The mitochondria were quite similar in composition to those from pelican liver and heart.

Conclusions from Fatty Acid Composition Studies

Mitochondria from different organs and species appear to have a rather characteristic lipid class composition (see above) with lecithin, phosphatidyl ethanolamine, and cardiolipin being the major lipids. It is clear that plasmalogens that occur to such a great extent in heart mitochondrial lipids are not required for the mitochondrial functions dependent upon lipids since liver mitochondria do not appear to contain plasmalogen forms of phospholipids and brain mitochondria contain only a small amount of plasmalogen. In liver mitochondria it appears, for the most part, that saturated fatty acids replace the aldehydogenic residues of lecithin and phosphatidyl ethanolamine characteristic of heart. It is clear also that mitochondria tend to have a high content of polyunsaturated fatty acids, although wide variations in the specific polyunsaturated fatty acids are encountered. Some plants contain only linoleic acid. In animals both linoleic and arachidonic acids may occur in some organs (e.g., the liver), but arachidonic acid and docosahexaenoic acids may predominate in other organs (e.g., brain). In fish the acids of the linolenate family predominate while animals that live on fish may contain acids of the linolenate and linoleate families. It is clear that mitochondrial fatty acids vary from organ to organ and species to species and that large changes may occur with changes in dietary fat. Nevertheless mitochondria always have a relatively high content of polyunsaturated fatty acids when compared to such stable membranous structures as myelin.

The absence of sphingolipids from mitochondria may be related to the fact that the fatty acids of sphingolipids are predominantly of the saturated or monoenoic types and longer chain acids are found. Lipid chemists have been impressed by the low solubility in most solvents of sphingolipids containing long chain saturated fatty acids and the greater difficulties encountered in dispersing these sphingolipids in an aqueous medium. It is thus not surprising to find that myelin, the most metabolically stable membrane unit, contains large amounts of sphingolipids (cerebrosides, sulfatides, and sphingomyelin). Mitochondria are much more flexible in the living cell when viewed through the phase contrast microscope and isotope incorporation is rapid in vivo as compared to that in myelin. In keeping with this more flexible nature, mitochondria are found to be largely if not entirely devoid of sphingolipids and to contain much unsaturated fatty acid. The higher double bond character of fish mitochondrial lipids is in keeping with the fact that fish body temperatures are generally lower than those of warm-blooded animals since fluidity is presumably more readily maintained with increase in unsaturation.

The manner in which the lipids of mitochondria are arranged to form membranes is evidently quite different from that of myelin. Not only are sphingolipids absent from mitochondria, but the cholesterol content of mitochondria is very low while that of myelin is high. The structure proposed for myelin by Vandenheuvel (176) cannot pertain to mitochondria because the lipid composition of the two types of membranous structures are markedly different. The myelin type structure does not appear to be applicable to microsomal membranes for the same reasons.

Studies of fatty acids of other membranous units of the cell (nucleus, microsomes, etc.) are very incomplete, but the data available indicate that nuclei and microsomes are similar in composition to mitochondria.

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