CAROLINE M. POND

The Fats of Life

With drawings by Mat Cross and Sarah Sutcliffe

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Introduction to fats

Fats and oils are a varied group of chemicals that do not readily dissolve in or mix with water. They dissolve in 'organic' liquids such as chloroform, benzene and acetone. These solvents are familiar as ingredients of nail polish removers and home dry-cleaning fluids: much of their efficacy in these roles derives from their capacity to dissolve the fatty components of stains caused by food, sweat and other biological materials. Fuel oil and its derivatives such as lubricating oils, paraffin (kerosene) and petrol (gasoline) share many of the properties of biological lipids, but they are chemically different, and, not being components of living organisms, are outside the scope of this book. So-called essential oils that are extracted from plants and used for perfumes, flavourings and aromatherapy are not oils in the chemical sense of the term.[1] They evaporate readily, forming 'essence' to which human noses are very sensitive.

By convention, 'oils' are liquid at room temperature, and 'fats', such as butter and lard, are solid or nearly so. Although impressive and important for their roles in living organisms, this distinction does not reflect a fundamental difference in chemical composition: it is merely a reversible change of physical state, just as ice, snow, and rain are different physical states of the same chemical substance, water. Fats are frozen oils, so to avoid confusion, hereafter the term 'lipid' will be used to describe both fats and oils, regardless of the physical state in which they are most usually found.

Basic discoveries

Until the end of the eighteenth century, living organisms, and most substances known to emanate from them, were regarded as too complex, or too sacred, to be amenable to chemical analysis in the same way as non-living 'minerals'. Almost all oils and waxes used as food, fuels or lubricants were derived from plants or animals, and so were rarely regarded as suitable subjects for scientific investigation. The distinction faded after the English nonconformist minister and schoolmaster, Joseph Priestley (1733–1804) observed that a candle burned and a mouse lived for longer if they were confined separately in sealed jars than if held together in a similar vessel, suggesting that mice and candles were competing for the same component of air.

A few years later, in 1783, the French civil servant and landowner, Antoine Lavoisier (1743–1794), in collaboration with the mathematician and astronomer, Pierre Laplace (1749–1827), demonstrated that the amounts of ice melted and 'air used' by a guinea-pig were equivalent to the heat produced and air 'consumed' by burning charcoal, and concluded that the chemical processes of respiration and combustion were fundamentally similar. Biological materials became 'fair game' as subjects for chemical analysis, although their complexity and their tendency to rapid decomposition made them technically difficult to study.

The basic structure of biological fats was elucidated by another enterprising and imaginative Frenchman, Michel-Eugène Chevreul. He was born in the Loire valley in 1786, when King Louis XVI and Queen Marie-Antoinette still ruled France, the aristocrats seemed safe in their nearby châteaux, and the basic design and construction of most kinds of machinery and vehicles had hardly changed since Roman times. As a young man, Chevreul moved to Paris, where his long and varied career included service at the Jardin des Plantes, directing the synthesis and applications of the dyes used in the manufacture of tapestries at the famous Gobelin factory, and being Professor of Organic Chemistry at the University of Paris.

The celebrations for Chevreul's hundredth birthday recorded over 500 publications between 1806 and 1885 on an immense range of topics in palaeontology, botany, zoology, physiology, chemistry and history of science. Such longevity was even more exceptional then than it is now, especially for a man (most centenarians are women), and suggests that a lifetime of exposure to dyes, oils and other 'chemicals' can't be that dangerous. By the time he died, which was not until 1889, exploitation of mineral oil (petroleum) was well under way, the electric motor, the internal combustion engine (used in cars, etc.) and the modern 'safety' bicycle had been invented, and would shortly revolutionise industry, homes and transport.

When Chevreul was young, what we now call organic chemistry was practised as an eccentric hobby by wealthy gentlemen such as Lavoisier, who funded his research out of his huge personal fortune until he was arrested and guillotined by revolutionaries at the age of 50. He lived to see it transformed into an important profession that serves major industries. His research on lipids underpinned the development of the protective coatings and the lubricants upon which all such machines depend.

During much of the first 30 years of Chevreul's life, France experienced political and social changes on a scale not seen for centuries, and, by the time Napoleon Bonaparte came to power in 1799, it was also embroiled in major wars with several other European states. The resulting loss of international trade, land reforms and the new Republic's active encouragement of rapid population growth[2] made food supplies a major issue during the first two decades of the nineteenth century. Napoleon was, from an early age, personally interested in many branches of science and archaeology, and was impressively knowledgeable on certain topics. He recognised the importance of science in military operations, civilian prosperity and national prestige, and befriended and financed its practitioners. Armies need good rations and hunger fuels discontent among civilians, so the newly established French Republic vigorously promoted research into what we would now call food technology, enlisting the help of its most promising scientists.

Starting in 1811, just before Napoleon's ill-fated invasion of Russia, and continuing until after his devastating defeat at Waterloo in 1815, Chevreul devoted himself to identifying the 'immediate principles' in mutton fat. Borrowing procedures long used in making soap (which was then still a luxury item, manufactured only on a small scale), he heated fats with alkalis (what we call potash or caustic soda) and purified the resulting mixtures. He named the clear, syrupy, sweet-tasting liquid that he extracted 'glycérine' (from the Greek word, γλυκυς, sweet).[3] In spite of its sweet taste, glycerine, now called glycerol,[4] is an alcohol, not a sugar, chemically similar to ethanol, the main active ingredient of beer, wine and other 'alcoholic' drinks. Glycerol contains three carbon atoms (and their associated hydrogen and oxygen atoms) while ethanol has only two, and it does not affect the nervous system and behaviour in the way that ethanol does. Both substances are produced in significant quantities by a wide variety of organisms, and can be broken down to release energy in a form that can be used by living cells.

Chevreul named the other major 'principles' he found in animal fat 'fatty acids', and showed that they occurred in the proportions of three fatty acids to each glycerol. When separated from the glycerol, fatty acids dissolve in alcohol and, by repeated extraction and precipitation with salts, could be purified sufficiently to form crystals. Chevreul noted that the fatty acids from mutton fat formed two distinct types of crystals, indicating that they were a mixture of at least two different chemicals.

Laboratory techniques and theoretical concepts about the structure of large biological molecules were then so limited that he was unable to take the analysis any further. He reported his finding in his classic book entitled *Recherches chimiques sur les corps gras d'origine animale*,[5] first published in 1823, and then turned his attention to dyes and pigments.

Fatty acids

Following Chevreul's pioneering work, scientists in France, Germany and elsewhere took up the study of natural lipids in a wide range of plants and animals, and their seeds, eggs and embryos. For a while in the middle of the nineteenth century, scientific knowledge of biological lipids was ahead of that of either proteins or carbohydrates (sugars and starches). Glycerol was found in the great majority of biological lipids (but not in mineral oils, including paraffin, motor fuels and lubricating oils), but the fatty acid components proved to be much more variable. By the end of the nineteenth century, dozens of different fatty acids had been described and named. Modern methods of extraction and separation have extended the list to hundreds that occur naturally in micro-organisms, plants or animals, and many more kinds can be synthesised artificially by chemical transformations. A few of the more abundant and physiologically important fatty acids are discussed in Chapter 3.

All fatty acids consist of a chain of carbon atoms, each attached to hydrogen atoms. The epithet 'acid' derives from the —COOH group on one end of the molecule: this H (hydrogen) atom (but not the many others elsewhere in the molecule) readily dissociates from the rest of the molecule, forming an H^+ (hydrogen ion), the hallmark of an acid. The other end consists of a carbon atom and three hydrogen atoms, and is called the methyl end, because of its resemblance to methane (CH_4), familiar as the main component of marsh gas. Figure 1 shows three alternative ways of illustrating the structure of a typical fatty acid with 16 carbon atoms that has the basic chemical formula $C_{16}H_{32}O_2$. A knowledge of the way in which carbon, hydrogen and oxygen combine with each other suggests the linear arrangement shown in Figure 1(a) as the most likely. It is often written as $CH_3(CH_2)_{14}COOH$ to show that the two ends of the molecule differ from each other and from the repeating chain of 14 similar units between them. Most of the carbon atoms are linked to two others and to two hydrogen atoms by a single bond, represented by a line. One of the oxygens is joined to a carbon by a double bond, shown as two parallel lines. The other forms the acid group.

During the first two decades of the twentieth century, Sir William (Henry) Bragg and his son, Sir (William) Lawrence Bragg, working at the Royal Institution in London used X-rays to determine the sizes of atoms and their spatial relationships to one another in various molecules. The common fatty acids were among the first molecules whose structure they and their colleagues worked out by such means. Fatty acids are genuine biological molecules, large enough to be interesting but small enough for the structure to be worked out without the need for the elaborate computing techniques that are used for similar analyses these days. The simpler fatty acids that they chose for study are chemically stable – they do not deteriorate when exposed to air and strong radiation – and, above all, as Chevreul discovered, they form regular crystals.

The model that the Royal Institution researchers built from wooden balls and wire to summarise their findings is shown in Figure 1(b). The carbon atoms (dark balls) link to each other in a zig-zag arrangement (called the bond angle) with their hydrogen atoms (light balls) on opposite sides. They form a repeating chain, terminating in the acid group on the last carbon ($COO^- H^+$), here shown on the right. The shape of molecules is so important to their properties and biological roles that organic chemists have devised ways of drawing them that emphasise it. Figure 1(c) shows the same fatty acid represented in this way: most of the hydrogen atoms are omitted, and the carbon atoms are understood to be at the bends of the zig-zag pattern revealed by the crystallographic discoveries. Only the acid group is shown in detail.

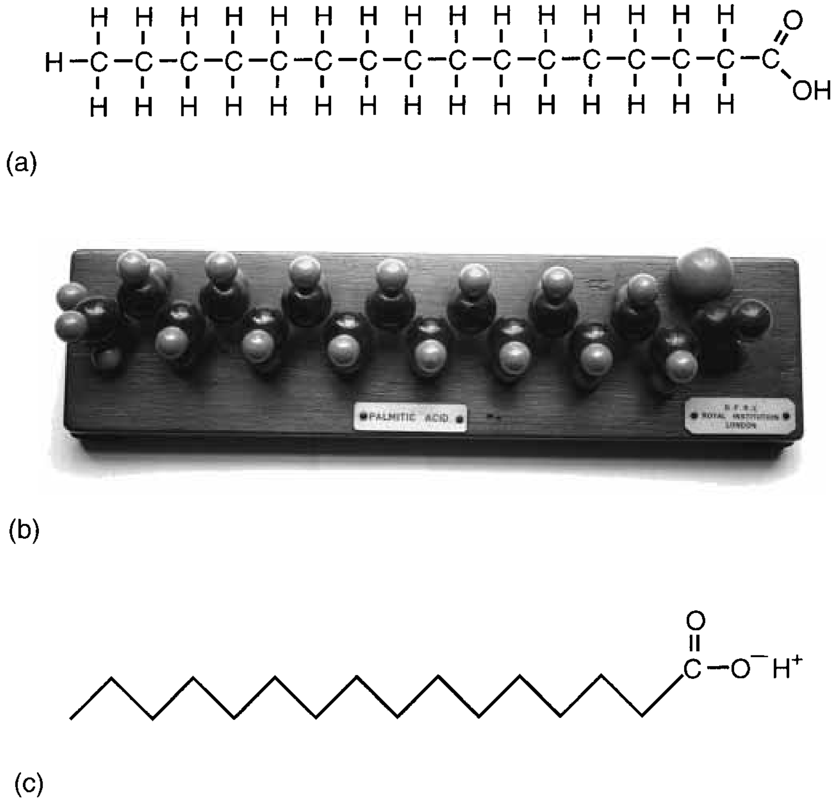

Figure 1. Three ways of illustrating the structure of a fatty acid molecule. (a) The basic arrangement of all the atoms. (b) A model incorporating information about bond angles derived from X-ray diffraction studies. (c) A modern scheme based upon (B), showing only the acid group in detail.

One way in which fatty acids differ from each other is in the number of carbon atoms in the main ‘skeleton’. Fatty acids with from two to 36 carbon atoms have been found in higher plants or animals, and those of certain unusual micro-organisms can have up to 80, but the commonest have between 14 and 22 carbons. Those with fewer than eight carbon atoms are referred to, somewhat arbitrarily, as short-chain fatty acids, those with 8–12 carbons are called medium-chain fatty acids, and long-chain fatty acids have more than 12 carbon atoms. Fatty acids with even numbers of carbon atoms are generally more abundant in biological materials than those with odd numbers, although fatty acids with almost all possible numbers of carbons within this range have been found in small quantities somewhere in some organism.

Under special conditions such as thunderstorms, short-chain fatty acids with two or three carbon atoms can be produced from ‘inorganic’ sources of carbon such as methane or carbon dioxide, but so far as we know, medium- and long-chain fatty acids are made naturally only in living cells. All known self-propagating living cells (i.e. not viruses) contain some molecules that have a fatty acid component. They are thus key indicators of the presence, or former presence, of life. Together with amino acids (the basic components of proteins) and genetic material, long-chain fatty acids are sought in meteorites and in samples of rocks and dust collected by space probes, as evidence for the existence of life on Mars or other celestial bodies. So far, none has been found.

Larger lipids

Fatty acids are very weakly acidic compared with mineral acids such as nitric or sulphuric acid because the nitrate and sulphate groups repel H^+ ions much more strongly than hydrocarbon chains can, but the $COO^- H^+$ group is acidic enough for a high concentration of fatty acids to disrupt the biochemical workings of the cell. Fatty acids are therefore usually tidied away as part of larger, more complex molecules. They are often attached through their —COOH group of atoms to alcohols, characterised by having —OH groups, via a reaction called esterification. When an ester bond is made, a water molecule (H_2O) is formed from a hydrogen atom and an oxygen atom of one molecule (in this case, a fatty acid) and a hydrogen atom from the other molecule (in this case, glycerol).

Three such reactions form a triacylglycerol molecule (called triglyceride in older literature), as shown schematically in Figure 2. In the reverse reaction, water molecules are split and their components become part of the fatty acids and glycerol. This process can be called hydrolysis because water molecules are split, but it is also called lipolysis (i.e. breaking lipids). Esterification and lipolysis are usually facilitated in both directions by specific enzymes (of which more in Chapter 4). As biochemical reactions go, esterification and lipolysis are simple, involving only small quantities of energy,[6] and they can take place inside or outside cells, in the blood or in the gut.

At body temperature, the triacylglycerol molecules assume many more configurations than this static image suggests: the carbon atoms in both the

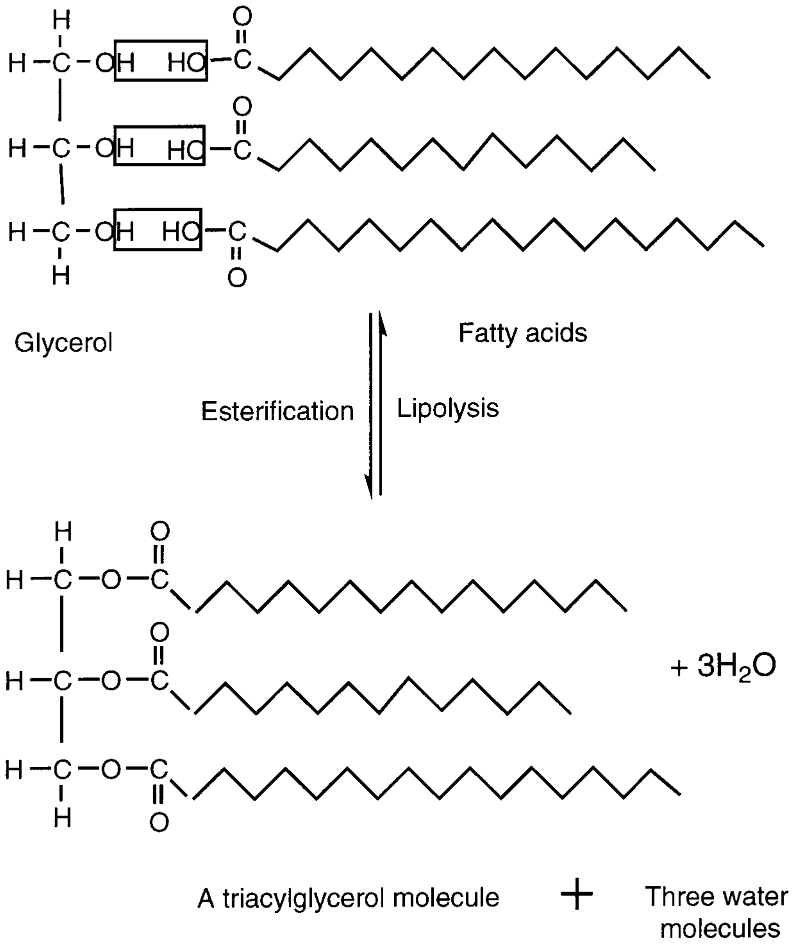

Figure 2. Esterification and lipolysis. Three fatty acids and a glycerol molecule (above) are joined by ester bonds to form a triacylglycerol molecule and three water molecules (below). The fatty acids have different numbers of carbon atoms.

fatty acids and the glycerol 'skeleton' can rotate relative to each other, producing a wide range of conformations. The three-pronged plan of triacylglycerols is fundamentally different from that of the other major category of energy-producing biological materials, the carbohydrates, in which most of the carbon atoms are arranged in rings. The simplest carbohydrates are the sugars, which have only one or two such rings; glucose, the predominant simple sugar in the blood of almost all vertebrates and many other animals, consists of a single ring, but sucrose, the commonest sugar used in cookery, has two rings.[7] Sugars are the basic units of so-called complex carbohydrates, such as starch and cellulose, which may contain thousands of rings, joined together in various different ways.

So-called free fatty acids move in animals' blood on their way between different kinds of cells, but in this state they are always bound to large protein carrier molecules such as albumin. As such, they are not really 'free', so 'non-esterified fatty acid' is a more accurate term than 'free fatty acid' although the latter is still widely used. In other circumstances, fatty acids seldom occur alone, except transiently during chemical reactions. In tissues other than blood, fatty acids are usually esterified to glycerol, forming monoacylglycerols if only one fatty acid is attached, diacylglycerols if two are attached, and triacylglycerols if three fatty acids are attached. All three kinds of acylglycerols occur in insects, vertebrates and probably many other kinds of animals. They have quite different roles, to be discussed in the following chapters.

In principle, triacylglycerols could include any kind of fatty acids, but in practice, we find that almost all the fatty acids in the major storage lipids in most animals and plants, including almost all the species that we use as food, are long-chain. The rows of carbon atoms pack beside each other so well that almost everything else, including water, is excluded. If left to themselves, triacylglycerols segregate from watery components of the cells to form droplets, giving a distinctive appearance to tissues in which they are abundant, as described in Chapter 2.

The triplets of fatty acids esterified to glycerol are not necessarily of the same type: the middle one is nearly always different from the other two, and often the molecule contains three different kinds of fatty acid. The triacylglycerols in edible oils and in lard and other animal fats consist almost entirely of long-chain fatty acids, but many of those in milk, especially that of cows, ewes and nanny goats, contain some medium-chain, and a few short-chain, fatty acids, from where they find their way into cheese, cream and butter. The origins and consequences of this situation are discussed in Chapter 4.

The other major kinds of glycerol-based lipids are phospholipids, which are basically similar to triacylglycerols in structure, but a phosphate group plus various other atoms (often including nitrogen) replaces one of the sites on the glycerol that in triacylglycerols combines with a fatty acid. This component has much greater affinity for water and water-based molecules than the fatty acids, so while they seek each other's company, the phosphate groups reach into the watery regions of cells. This combination of contrasting properties is the main reason why phospholipids are the major components of almost all biological membranes.

Membranes enclose whole cells, and define compartments within cells, keeping proteins, genes, carbohydrates and other large biological molecules together or apart, as required. They also maintain the appropriate concentrations of ions (including hydrogen, sodium and potassium ions), and other small molecules that create the chemical environment required for the larger molecules to function correctly. Although some cells sometimes use the fatty acid components of a few of their phospholipids for the synthesis of certain lipid-based messenger molecules, their primary role is to hold the cell together, so membrane lipids are known as 'structural lipids', in contrast to triacylglycerol 'storage' lipids.

Different enzymes make and break the ester bonds of the two kinds of fatty acid-containing lipids, so within the same animal, or even within the same cell, the mix of fatty acids in the phospholipids is not the same as that in the triacylglycerols. An immense range of different combinations of fatty acids can occur in phospholipids and triacylglycerols, and may change with time, forming membranes and storage lipids with slightly different physical properties such as melting temperature.

Waxes are another important class of biological lipids. They consist of a single long-chain fatty acid, which may have up to 34 carbon atoms, many more than are ever found in the fatty acid components of phospholipids or triacylglycerols, linked by an ester bond to an alcohol, also long-chain, with from 22 to 34 carbon atoms. The components are synthesised inside cells, as are other fatty acids and alcohols, but complete waxes are almost always found outside cells, where their properties of insolubility in water, chemical stability and resistance to decay are put to good use.

Waxes are most widespread as major constituents of the non-living coatings[8] on the outer surfaces of plants. The waxy layer forms the firm, shiny surface of many leaves, flowers and fruits that limits evaporation of water and sometimes, as in the shiny bluish needles of some conifers, protects the leaves from damage by reflecting the harmful components of strong sunlight. As the first point of contact between the plant and herbivorous insects and potentially dangerous fungi, its 'smell' and its resistance to mechanical or chemical attack are integral parts of the plant's defences against disease and destruction. Micro-organisms generally find such large hydrocarbons difficult to break down: butter, lard and suet eventually 'go mouldy', i.e. a fungus manages to establish itself and grow on them, but bees' wax 'keeps' for years, almost as well as hair, shell, tooth or dried bone.

Many animals also synthesise waxes, and as in plants, most are used externally. The skin of reptiles, birds and mammals, including ourselves, produces a mixture of lipids, including waxes (it can become concentrated as ear wax). Many insects, notably cockroaches, feel slightly greasy to the touch because they have a thin outer covering that contains waxes. Honey bees (and certain other insects) have glands that produce large quantities of wax, which they build into their honeycombs and the brood chambers that house the larvae. Some invertebrates, fish and a few mammals, notably sperm whales, synthesise large quantities of liquid waxes which are retained in internal tissues for various specialised roles.

Some properties of lipids

When pure, each triacylglycerol has sharply defined melting and boiling points, but lipids mix very well with each other, and most familiar fatty substances, including lard, butter and salad oil, are mixtures of dozens, sometimes scores, of distinct chemicals (though often one or two major types predominate). These mixtures of molecules do not readily pack neatly together to form crystalline solids as they cool so they freeze over a range of temperatures which differ according to their exact composition. Tallow at room temperature (about +20 °C), butter and lard kept in the fridge (at about +4 °C) or cooking oil stored in an ordinary deep freezer (at about −15 °C) form firm, crystalline solids that crack like ice when struck hard; when heated to about 50 °C, bees' wax and most culinary lipids are clear, free-flowing liquids. Short-chain fatty acids evaporate so efficiently that they are sometimes called 'volatile fatty acids', and people find the smell of many of the common ones unpleasant.

At the intermediate temperatures at which we normally encounter them, such mixtures of lipids are 'greasy', not quite solid, but not really liquid either. Most living processes take place between 0 and 50 °C, the range of temperatures at which most natural lipids are greasy or liquid. Many seeds, spores and even some adult organisms can survive many months at much lower temperatures but under such conditions, they do not actively feed, grow or breed.

By definition, lipids do not dissolve in water, but tiny droplets of liquid triacylglycerols can be dispersed in water to form emulsions, as in milk.

Untreated full-cream milk separates on standing at or below room temperature, to form ‘cream’, which is semi-solid if kept at about 5 °C in the fridge, and whey which remains liquid at this temperature. A few seconds of vigorous shaking re-emulsifies the cream into the whey, and it remains in this state for several hours, especially if the milk is fresh.

Nearly all lipids are less dense as liquids than water, so when not emulsified, they float. When soups and gravy separate, the lipids float on top of the watery phase. Small quantities of lipid, such as a few drops of oil spilt into a puddle, may form a layer thin enough to reflect light differentially, forming patterns of colours. However, while ice is less dense than cold water,[9] so it floats on top of it, frozen lipids behave as normal solids: their density increases as they cool and solidify. So fats always sink in chemically similar oils, though they usually do not become dense enough to sink in water.

The subjective experience of taste of foods that contain lipids depends upon their physical state as well as upon their chemical composition. Exactly how people perceive these properties is still not clear, but it has important implications for how we like our food to be prepared and stored. The taste of skimmed or semi-skimmed milk is less severely impaired by freezing (e.g. in a home deep-freeze) and thawing than that of full-cream milk, partly because there is less lipid in the former, and because it is thoroughly homogenised, i.e. emulsified with the whey.[10] Concentrated forms of milk lipids, such as heavy cream or cheese, should never be frozen: because each component has a different melting temperature, freezing and thawing disrupt the intimate association between the proteins, water and lipids, thereby altering the texture and the flavour. Some cheese connoisseurs even object to Camembert and other full-fat soft cheeses being chilled in a refrigerator.

Lipid roles

The most familiar biological lipids are the triacylglycerols that serve as the principal energy stores of many plants and all terrestrial vertebrates including mammals and birds. In this role, lipids are very efficient, much more so than the water-soluble energy store, glycogen, because large quantities can be packed into a small volume. Since much of the rest of this book concerns storage lipids, we will leave them for now and turn to other functions of lipids.

Skin lipids

Most other biological roles for lipids relate to their most distinctive property, the way they resist mixing with water. Skin, scales, feathers, hair and fur are normally coated with a thin but essential layer of lipids that act as anti-wetting and anti-bacterial agents. Many lipids on the skin of terrestrial vertebrates are acylglycerols, and they often include kinds of fatty acids that are never found in storage or membrane lipids: some have branched chains of carbon atoms, or additional atoms or groups of atoms.

The skin lipids of aquatic birds are particularly effective at repelling water, making it roll off as droplets 'like water off a duck's back', but all reduce the wettability of the body's outer covering. Skin, feathers and fur that are not easily wetted lose less heat in rain or spray, and may also be less susceptible to invasion from foreign bacteria and other tiny organisms that could penetrate the skin and cause disease. The lipids are secreted by special glands embedded in the skin and spread over the pelt or plumage by grooming or preening. Many birds spend a large proportion of their time preening, and if prevented from doing so, by an injury or impediment, their resistance to cold and wet is quickly impaired.

The durability of lipids in a watery world is also an advantage for the many mammals that identify each other by smell. Deer, foxes, large cats and many others have special skin glands from which they rub complex mixtures of odours derived from skin lipids onto trees and rocks. Such chemical messages can be 'read' weeks after they are laid down. Human perspiration consists mainly of water that dissipates body heat as it evaporates, leaving behind small amounts of salts and lipids including, in adults, derivatives of sex hormones. Spontaneous reactions with oxygen in the air, and the action of the many bacteria that live on the skin, convert these secretions into substances that feel and smell unpleasant to many people, so they are removed by frequent bathing and hairwashing.

Soap consists of molecules that form a bridge between lipid-soluble and water-soluble molecules: one end dissolves readily in water, and the other mixes well with lipids. By making lipid-associated particles soluble in water, soap is very effective in removing sweat, food stains and other biological 'dirt' from clothing. Washing the skin or hair with toilet soap removes only the outer layer of lipid, but prolonged contact with powerful laundry detergents makes skin 'dry' and prone to cracking. 'Drying' is a slightly misleading term because water is not the most important material that has been 'washed' away. Detergents remove the thin layer of lipids that holds the outer layer of dead cells in place and protects the living, sensitive tissues underneath. The application of additional lipids, such as Vaseline or hand-cream, immediately rectifies the situation and prevents further drying. Unless badly damaged, the skin continues to secrete its own lipids which build up to normal levels after a few days.

Fats for floating

The fact that biological lipids are less dense than water is of little consequence to land animals such as ourselves, but it is crucially important to aquatic animals and plants, because the type and quantity of lipid in relation to the watery tissues, and mineralised materials such as bone or shell, determines whether they sink or float. Many different kinds of invertebrates and fish use a wide range of lipids, including triacylglycerols and waxes, and various substances derived from lipids for buoyancy, but those of fish have been most thoroughly studied.

A gas-filled swimbladder is the main buoyancy organ of many bony fish, but sharks and rays never have such structures. So lipids often make a major contribution to their buoyancy and that of a few kinds of bony fish that have lost the swimbladder. Lipids that are involved mainly or exclusively in buoyancy accumulate in the liver, probably because of its central location inside the abdomen near the body's centre of gravity, rather than for any metabolic reason. In cod and related fish such as halibut, the lipids are triacylglycerols. Cod-liver oil has a density[11] of about 0.93, so its presence must make the fish only slightly lighter than water, and it may also serve as an energy store.

The liver of the deep sea shark *Centroscymnus* is huge, occupying 30% of the volume of its body,[12] and 80% of its mass is a unique lipid called squalene which has an exceptionally low density (0.86) for an organic liquid. Squalene, whose name derives from *Squalus*, a shark, is a hydrocarbon (a chain of carbon atoms each with two hydrogen atoms attached) similar to the backbone of fatty acids but without the acid group at one end. As far as we know, *Centroscymnus* cannot break down squalene (or any other hydrocarbon) to generate metabolic energy, so providing buoyancy must be the main function of the huge quantities of this material that the fish synthesises. This fish, and a few other kinds of slow-swimming sharks, notably the basking sharks, are neutrally buoyant, i.e. they can float motionless in water. As well as accumulating lighter-than-water lipids, they also reduce the heavier-than-water skeleton, which is poorly calcified in the vertebrae, the gill arches and fins, but not, of course, the jaws. They need the strength and hardness provided by calcium minerals to hold the huge mouth open, or, in the case of predatory sharks, impose a powerful bite.

Lipids in membranes

Although usually not given as much prominence as genes or proteins, membranes are equally fundamental to life. Phospholipids are the major structural components of the membranes of all kinds of cells, from bacteria (except a few very primitive and aberrant types) to mammalian tissues. The key to their biological role is the ability of the phosphate group to mix readily with water and water-loving molecules such as proteins, while the fatty acids prefer the company of lipids, particularly each other. Substances with an affinity for lipids, such as certain hormones and non-esterified fatty acids, can diffuse through the phospholipid membrane, as can water, oxygen and carbon dioxide. Oxygen from the air must pass through the membranes of the cells lining the lungs or gills before reaching the red blood cells that transport it deep into the tissues.

The majority of biological materials, including proteins and sugars, are water-soluble so their passage through cell membranes must be facilitated, and thus controlled, by an assortment of protein-based channels, receptors and transporter systems embedded in or on the membrane. The relative abundance of different types of such structures may confer unique properties on particular tissues or groups of cells within a single tissue. Some channels use chemical energy to ‘pump’ particular kinds of small molecules into or out of the cell, and the properties of others are transiently altered by the electrical voltage across them. Such ‘policing’ of molecular traffic into and out of cells is essential to the integrity of the cell, and to the role they play in the whole organism. Cells can swell and burst if too many small, water-soluble molecules and the water that accompanies them, are allowed to flow through the membrane and into the cytoplasm, the interior of the cell.

The cells of multicellular organisms co-ordinate their activities by a wide variety of chemical and electrical messages passed between them. Scores of messenger molecules have been characterised, and are known collectively as hormones, neurotransmitters, cytokines and other names depending upon their chemical composition, and between what kinds of cells they operate. They all have one thing in common: their action requires specific receptor molecules that are usually located on or in the membrane of the target cells.

As more and more different messenger molecules are discovered, and many are found to act on several different kinds of cells, it is becoming clear that the membranes of many complex cells harbour a wide range of receptor molecules that bind specifically to particular messenger molecules. The binding of messenger molecule to receptor molecule is signalled to the cell's genes and other parts of its interior by a relay of secondary messenger molecules that start at the receiving cell's outer membrane. The composition of the fatty acids in the membrane phospholipids influences their fluidity, where in or on the membrane the proteins are attached, and possibly how 'sticky' it is in attaching to other cells. The outer membrane is constantly turning over, with portions being reabsorbed into the cell, to be replaced by newly assembled membrane, thereby keeping the structure in good working order, and enabling frequent adjustments to the array of receptors, carrier molecules and channels that it supports.

These roles alone make membranes vital to all living cells. Damage to the cell membrane is often even more rapidly lethal than poisoning protein synthesis or altering genes. One of the earliest antibiotic drugs to be developed, gramicidin, kills bacteria by chemically punching holes in their membranes: the cell contents leak out, substances normally excluded or pumped out by the membranes seep in, and the bacteria quickly become so disordered that they die. Even if the membrane itself remains intact, cells can starve to death within minutes if essential nutrients do not get into them because their molecular escorts through the membrane barrier are poisoned, or specific channels are blocked. The main snag with such drugs is that bacterial and mammalian cell membranes are so similar that both are injured almost indiscriminately, so side-effects can be serious. The more modern anti-fungus drug, polymixin, works like a soap, disrupting phospholipids in the cell membranes. Fungi rarely get further than the skin, which can withstand the damage inflicted by the drug very much better than internal organs can, because the outer layers of cells are constantly replaced.

As well as the outer cell membrane, plant and animal cells and more complex unicellular organisms have several distinct kinds of internal membranes that segregate compartments, assemble enzymes in functional order and form surfaces on which biochemical reactions can take place. The layers and chambers formed by internal membranes of chloroplasts and mitochondria are clearly visible in electron micrographs such as that in Figure 3. They are as important to cell function as the external membrane, and even minor changes in their structure or arrangement can severely disrupt essential biochemical processes. In many cases, key enzymes are attached to

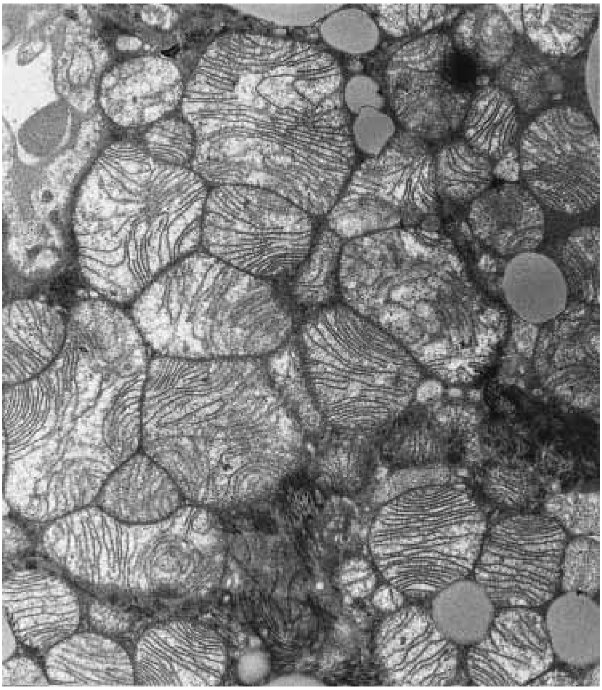

Figure 3. Internal membranes in mitochondria in an animal cell. This electron micrograph is magnified 9000 times, so the field of view is about 4 μm (0.004 mm) across. (Courtesy of Heather Davies.)

internal cell membranes, thereby enabling series of reactions, such as those involved in respiration, to take place in the right order. In brief, genes contain the information to build the cell, proteins catalyse the necessary chemical reactions, but phospholipids act as the marshals, holding the biochemical machinery together and helping to maintain the right chemical environment.

The maintenance of structural order in lipid membranes is critically dependent upon temperature. In many animal and plant cells, death from cold is due mainly to irreversible disorder of the lipid membranes. Almost all structural proteins and the majority of enzymes are perfectly preserved at temperatures far below zero. Biochemists routinely freeze tissues to as low as −80 °C for weeks before thawing them for further study. The blood of humans and other mammals also survives freezing, at least as far as its role in carrying oxygen is concerned, but, except in the cases of some species that are specially adapted to survive extreme cold, most cells and whole organisms are killed by freezing.[13] Their membranes have lost their selective permeability to different kinds of small molecules, and no longer maintain sequences of enzymes in the correct alignment in which they can work together to form a metabolic pathway. Being too hot, which in mammals and birds can mean just a few degrees above normal body temperature, is also lethal, because it disrupts the ordered structure of both proteins and lipids.

In 1816, Chevreul, motivated by little more than simple curiosity, extracted a lipid from gall stones (taken from human or animal cadavers) that would crystallise readily, but could not be broken into glycerol and fatty acids. He named this atypical lipid ‘cholestérine’, from the Greek words for bile, χολη, often used metaphorically to mean angry or embittered, and στερεος, which means stiff, firm or solid and, metaphorically, stubborn. Later research showed that cholesterol and other sterols consist of several contiguous rings of carbon atoms and thus have a fundamentally different structure from triacylglycerols (see Figures 1 and 2, pages 10 and 12).

Chevreul’s choice of name, now altered to cholesterol, is apt in one way: cholesterol is more stable than most other cell components and endures for a long time in living tissues and after death. However, the name is misleading in other ways: cholesterol is most concentrated in bile, but that is by no means its only biological role. Sterols are essential components of the membranes of all kinds of nucleated cells, though their exact function there is still not very clear. Sterols probably affect membrane fluidity, which in turn determines what and how much molecular traffic enters or leaves the cell.

In most animals including all vertebrates, the membrane sterol is cholesterol, while that of insect cells is slightly different. The cell membranes of green plants contain yet another sterol. These differences between plants and groups of animals are probably very ancient: certain lineages of organisms ‘got started’ on particular sterols and the structure and roles of their membranes are adapted to that composition. For reasons that are still not fully explained, the outer membranes of almost all kinds of bacteria manage without any sterols.

Research into the origin and early evolution of life indicates that lipid membranes were essential to cell integrity and basic biochemical processes right from the beginning. Membranes were almost certainly the earliest roles of lipids in the evolution of primitive cells, billions of years ago. We have little direct information about the structure or habits of the first kinds of cells, since very few ancient rocks in which their fossils might be found have survived and are accessible to us now, but laboratory experiments can suggest how lipids assumed their fundamental role in primitive cells.

Pure phospholipids isolated from cells (or even synthesised artificially) assemble themselves into the double layer observed in natural cells when they are spread over water. The molecules line up side by side in two layers with their fatty acids meeting end to end in the middle, forming two sheets, one upside down relative to the other. The tendency of phospholipids to remain associated in the typical bilayer array is so strong that fragments of membrane remain after excess heat or chemical attack has rendered them useless to the cells that made them. The great, grey, green and greasy appearance of the Limpopo River (and other warm, slow-flowing waters) is due to the membranes and membrane fragments of millions of tiny algae, living and dead. Lakes and reservoirs often acquire a similar appearance after hot, sunny spells lead to 'blooms' of algae, which die a few weeks later.

Membranous tissues

The properties of membranes become so important to the function of some cells that their surface is enormously expanded. Many kinds of immune cells, including those that form pus after they have completed their job of killing invading bacteria, have an elaborately frilled outer membrane. The greatly increased surface area supports more receptors that enable these cells to detect and track their minute prey, and is also essential to the killing process: the cells engulf bacteria, or any other debris, dead or alive, in a pocket of cell membrane which then moves into the interior of the cell, where digestive enzymes break it down. A severely infected wound can produce literally kilograms of pus over several weeks: supplying the fatty acids necessary for the synthesis of enough phospholipids for all these cells that eventually die and are expelled as pus can impose a significant drain on the body's lipid reserves.

The outer surface of the cells that line the small intestine, the part of the gut that absorbs nutrients from the digested food into the body, is also deeply folded. The area of membrane that actually makes contact with the food is thus enormously extended, greatly speeding up the rate of absorption. Like some immune cells, these intestinal cells have short but busy lives. Even in mammals as large as ourselves, they are functional for less than a day before being shed and replaced. Many of their constituents are digested and reabsorbed, but some are lost in the faeces, representing one of the few ways, other than being oxidised to produce energy (see Chapter 4), or expelled as pus or as ingredients of eggs or sperm, that the body can lose lipids.

By far the most extensive and chemically sophisticated membranes are those of the nervous system, whose functions include transmitting signals in the form of electric currents. Metals, graphite and most watery solutions including those inside and outside of cells, conduct electric currents, but paper, rubber, polythene and oils do so only weakly or hardly at all, so they can be used as insulators. Phospholipid membranes also pose a barrier to the movement of electrically charged ions, which pass, if at all, though channels specialised for the purpose. Controlling the magnitude, timing and location of the flow of currents carried by different kinds of ions, notably sodium, potassium and calcium, is a major function of biological membranes, especially, but by no means exclusively, those in the nervous system.

Peripheral nerves that innervate muscles and send back information from sense organs are usually greatly elongated, branching cells, so a high proportion of their mass is membrane, just as a fair proportion of a twig is bark. Brains consist of a huge number of tiny cells, most of which are branched, though not necessarily elongated. So the whole nervous system, and major sense organs such as the eye and the inner ear, contain far more membrane than tissues such as muscle or bone, that consist of fewer, much larger cells.

In gross composition, the mammalian brain is around 60% lipid, almost all of it various kinds of phospholipids and cholesterol. In lean animals, the nervous system may be by far the largest concentration of lipids in the body, and so from a predator's point of view, one of the most nutritious parts. Fatty tissues are often regarded as the simplest, dullest, most expendable tissue, and the brain as a fabulously complex, glamorous and inviolate organ, but chemically they have a great deal in common. Indeed, one of the reasons for studying how the body handles lipids is its implications for the growth and functioning of the brain and the eye.

The fatty nervous system has little affinity for the water-soluble stains and dyes that attach readily to proteins and thereby make the internal structure of tissues like muscle, liver, gut and skin visible under the light microscope. The brain and the eye were among the last of the major tissues to yield to microscopical examination, after the Italian biologist Camillo Golgi developed (in the 1880s) a lengthy and never very reliable staining process involving potassium bichromate and silver nitrate. Although 'silver staining' remained standard for almost a hundred years, its chemical mechanism has never been fully explained, and it did not work well enough to reveal the outline and internal structure of entire cells. Until well into the twentieth century, it was not even certain that all nerve cells were discrete entities, each entirely enclosed in its own membrane.

The composition of cell membranes means that lipid-soluble molecules, even quite large ones, such as fatty acids, usually need less assistance in getting into cells than water-soluble molecules such as glucose. This property is one reason why alcohol so readily traverses the stomach wall and acts on nerves and muscles (whose membranes are rich in lipids) within minutes of being ingested. Anaesthetics such as chloroform, ether and halothane work fast and effectively because they are lipid-soluble, passing quickly through the membranes of the cells lining the lungs, and thence through further membranous barriers and into the brain, where they disrupt nerve function enough to induce unconsciousness. The liver immediately sets about extracting the foreign substances from the blood and inactivating them, eventually removing enough for the brain to recover, and the patient wakes up again. The solvents in glue and varnish alter mood by similar processes, and, if inhaled repeatedly, cause liver damage.

Potentially toxic foreign substances can easily follow the same route. One class of lipid-soluble substances that is causing concern are organophosphate insecticides that work by penetrating the fatty outer layer of insects' cuticle and disrupting their nervous systems. Unfortunately, they are chemically stable, so they persist for years in and on plants and animals and in soil and ground water, where other animals take them in. They accumulate in fatty tissues, including the nervous system and liver of vertebrates such as fish, birds and mammals, where they can cause mental disorders. These and perhaps other foreign substances have been implicated in the development of bovine spongiform encephalopathy, or mad cow disease, which might cause neurological disorders in people who eat contaminated beef. The main cause of the disease is a prion, which is a protein, but disturbances of lipid membranes may assist, or hinder, an infection becoming established and producing serious symptoms.

Final words

Biochemistry has long emphasised specificity: only one enzyme or receptor or antibody will do, and just one wrongly placed amino acid out of thousands is enough to spoil the whole molecule. Uniquely arranged, highly specific molecules such as proteins and those that form genes tend to be regarded as 'more important' than lipids, whose strengths are their ability to mix with each other, in the case of triacylglycerols to the exclusion of almost everything else. Phospholipids have 'a foot in both camps', enabling them to marshal themselves and proteins into arrays, and to control the passage of other molecules. There is no single 'right' composition for either triacylglycerols or phospholipids, even within a single kind of cell: their fatty acid composition and with them their biological properties, change from time to time, according to temperature and other factors. As will be described in the following chapters, they are often built from whatever happens to be available to the organism. The impression that emerges from the study of the biology of lipids is of plasticity, variability and adaptability.