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# Biochemical Aspects of Lipid Storage and Utilization in Animals

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SYNOPSIS. The high energy content of lipids makes these compounds attractive to active metazoans as a light form of fuel storage. Animals have evolved complex systems of enzymes and transport proteins in order to utilize lipids for the generation of energy. While the enzymatic systems responsible for lipid synthesis and oxidation are probably similar in all metazoans, mechanisms for control of the rates of these processes differ with taxonomic group. The means by which lipids are transported through the body fluids of metazoans also show phylogenetic variation. Complete, quantitative data on the mechanisms and rates of lipid digestion, synthesis, transport and utilization are available for only a very few mammals, birds and insects. This would seem to be a very fertile field for comparative physiologists.

## INTRODUCTION

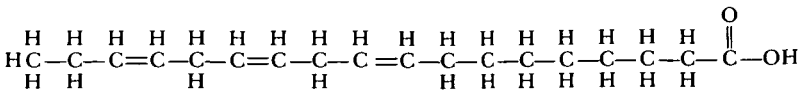
Animals put lipids to a multiplicity of uses ranging from the relatively mundane (*e.g.*, insulation in mammals; structural components of biological membranes—Vandenheuvel, 1971; anti-wetting agents produced by the preen glands of birds—Jacob, 1975; possible pheromone and antimicrobial action of mammalian skin lipids—Nicolaides, 1974; surface anti-desiccant properties of insect cuticular lipids—Gilbert, 1967; bouyancy control in marine fish—Lee *et al.*, 1975) to such truly exotic roles as bioacoustic lenses in cetaceans (Ackman, 1975). Unquestionably, however, the function of lipids as energy reserves of high free energy content per unit weight (9 kcal/g) is one of the most general and significant functions of these compounds in animals. An overview of the chemistry and biochemistry of those lipids important in energy metabolism will be presented. A comparative physiological approach is taken toward lipid metabolism in accordance with the interests of the author and the impossibility of reviewing briefly the voluminous literature on mammals and birds. Space is also devoted to the analytical biochemistry of lipids, as new methods in liquid and gas chromatography appear to be on the threshold of automating the quantitative determination of lipid classes and fatty acids.

## CHEMISTRY OF LIPIDS IMPORTANT TO ENERGY METABOLISM

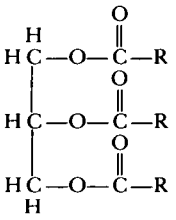
Lipid energy metabolism centers upon the anabolism and catabolism of long-chain fatty acids and the esters which these acids form with various alcohols. Such lipids as sterols, phosphoglycerides, sphingolipids, and glycolipids serve principally as structural components of membranes rather than as energy reserves and will not be discussed further. The structures of lipids of proven or possible significance in energy metabolism are summarized in Table 1.

The long-chain fatty acids of animal lipids have predominantly even-numbered, straight carbon chains of from 14 to 24 carbons. Depending upon the source of the lipid, small proportions of fatty acids with odd-numbered chains, branched chains, and hydroxy substitutions may be present. Fatty acids may be saturated or unsaturated. In the latter case the double bonds have the *cis* configuration and from one to six double bonds may be present. The structures of monounsaturated fatty acids may be indicated by one of two shorthand systems specifying the position of the double bond. Thus, 9-18:1 (Mead, 1968) and 18:1$\omega$9 both designate oleic acid. The former system indicates the position of the double bond from the carboxyl terminus (*i.e.*, double bond is between carbons 9 and 10), the number of carbon

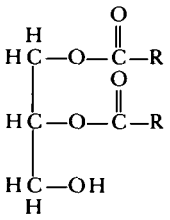
TABLE 1. *Lipids important to energy metabolism.*



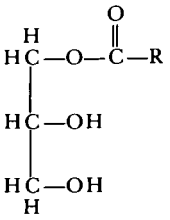
Fatty acid (linolenic acid, 18:3ω3)



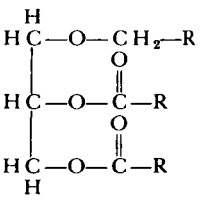
Triglyceride



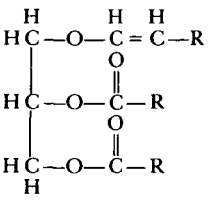
1,2-Diglyceride



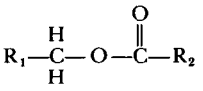
1-Monoglyceride



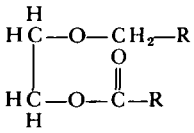
Alkyl diacyl glycerol



Alk-1-enyl diacyl glycerol



Wax ester



Acyl ethanediol ether

atoms in the chain (18), and the total number of double bonds (1). The latter system specifies carbon atoms in the chain (18), number of double bonds (1), and the number of carbons between the center of the double bond and the methyl (or ω) terminus of the chain (1). In polyunsaturated fatty acids (*i.e.*, those acids with more than one double bond) the spacing of the double bonds is methylene-interrupted. The double bonds are neither adjacent nor conjugated (both of which cases would favor oxidation) but, rather, separated by three carbon atoms. Exceptions to methylene-interrupted spacing of double bonds do, however, occur (Paradis and Ackman, 1975).

Triglycerides comprise the principal form in which fatty acids are stored as depot lipid. Triglycerides constitute a family of compounds which vary according to the nature of the fatty acid residues esterified to the three glycerol carbons. Hübscher (1970) has reviewed the metabolism of triglycerides and Litchfield (1972) has described in great detail methods for their analysis. Fasting experiments with most animals indicate that triglycerides are the lipid class most rapidly depleted. Diglycerides and monoglycerides are usually present in only small amounts in animal tissues and in most instances function as intermediates of triglyceride metabolism.

Wax esters (esters between fatty acids and long-chain, monohydric alcohols) have received considerable recent study as a result of their major participation in marine food chains (Benson and Lee, 1975). Notably, copepods in many oceanic regions store

lipid in the form of wax esters rather than triglyceride. In at least one instance depletion of wax ester reserves in copepods has been shown to result from fasting (Lee and Barnes, 1975).

Triglyceride analogs in which a long-chain alcohol (alkyl diacylglycerol, Table 1) or aldehyde (alk-1-enyl diacylglycerol) is bound in ether linkage to C-1 of glycerol occur in small quantities in many mammalian tissues and in considerably larger amounts in such marine organisms as starfish and sharks. Recent reviews on the biochemistry and enzymology of these lipids have been provided by Snyder (1969, 1972). Analytical methods for glyceryl ethers are described by Viswanathan (1974). The similarity in structure of alkyl diacylglycerols to triglycerides suggests that they may serve as fuel depots, but this function has not been demonstrated. Neutral diol lipids (for review see Bergelson, 1969) constitute a final lipid class which may function as a lipid energy reserve. These compounds are composed of dihydric alcohols (frequently ethylene glycol) in which one hydroxyl group binds a long chain monohydric alcohol in ether linkage, while the remaining hydroxyl is esterified to a fatty acid residue. Neutral diols occur in high proportions in lipids of many marine invertebrates (Vaver *et al.*, 1971; Vaver *et al.*, 1972).

## ANALYTICAL BIOCHEMISTRY OF LIPIDS AND LIPOPROTEINS

Comprehensive treatises on the extraction, separation, and quantitative determination of lipids include Lowenstein (1969), Nelson (1972), Kates (1972), Johnson and Davenport (1972), Perkins (1975), and Christie (1973). Procedurally, the analysis of tissue lipids requires adequate techniques for accomplishing the following: (1) complete extraction of lipid with minimal oxidative or hydrolytic deterioration (Bligh and Dyer, 1959; Folch *et al.*, 1957); (2) quantitative determination of total lipid by gravimetry or colorimetry (Frings *et al.*, 1972); (3) chromatographic separation of lipid classes by liquid chromatography (LC) or thin-layer chromatography (TLC); (4) quantitative determination of the separated lipid classes by colorimetry, gravimetry, or densitometry for the case of TLC; (5) analysis of the fatty acid composition of lipid classes by gas chromatography (Ackman, 1969); (6) radioassay of total lipid and chromatographically fractionated lipids for the case of metabolic studies employing radioisotopes. A combination of methods which have worked adequately in my laboratory for the quantitative determination and radioassay of lipids at the microgram level is outlined in Fig. 1 (Allen, 1974). This procedure has enabled the relatively rapid determination of both the mass and specific activity of the body fluid lipid classes of marine invertebrates which were fed $^{14}C$-labeled lipids in order to measure rates of flux of lipid classes through the body fluid.

Scientists interested in the role of lipids in the energy metabolism of animals must, perforce, remain current in the rapidly evolving chromatographic methods for these compounds. High-performance liquid chromatography (HPLC) is the most rapidly developing area in chromatography. HPLC employs high pressure pumps for delivery of eluting solvent, precision designed chromatographic media, and continuous detection of compounds in the eluant stream to dramatically improve on the resolution and time requirements of older column chromatographic procedures (Done *et al.*, 1974). The development of methods for lipid analysis by HPLC has proceeded slowly relative to other classes of compounds due to the lack of adequate detectors. As most lipids lack UV-absorbing chromophores, the UV-detector most commonly used in HPLC systems is unsuited to their analysis. The refractive index detector can be used with isocratic elution of lipids, but good resolution of lipid mixtures generally requires gradient elution. The wire-transport flame-ionization detector (WTFID) has emerged as the detector best suited to lipid analysis by HPLC. Commercial versions of this detector are expensive, somewhat cumbersome to operate, and have a detection limit of approximately one microgram (Kiuchi *et*

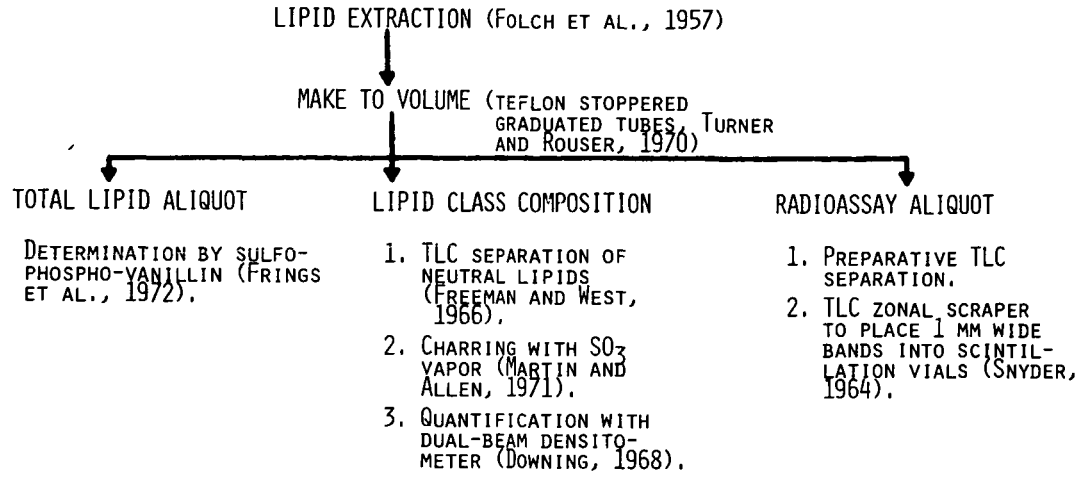



FIG. 1. Flow chart for micro-determination of lipid classes and their specific activities.

*al.*, 1975). They do, however, yield complete resolution of the neutral lipid classes within 30 minutes (Aitzetmüller, 1975) and a response linear with carbon content of lipid (Kiuchi *et al.*, 1975), the latter feature assuring excellent quantification. Stolyhwo *et al.* (1973) have described a WTFID which can be laboratory constructed and have investigated its application to lipid class analysis (Stolyhwo and Privett, 1973).

The coming revolution in chromatographic instruments is the development of microprocessor controlled chromatographs (Crockett and Mikkelsen, 1976). Microprocessors are microcomputers built into the chromatograph. A microprocessor can be programmed to operate a chromatograph automatically (*i.e.*, for the case of HPLC the microcomputer can inject a sample, determine the shape of a solvent gradient, and reduce the data to the form desired by the analyst). It seems probable that within a very few years lipid class analyses which are presently performed, somewhat tediously, by TLC and column chromatography will be rapidly and routinely accomplished by automated HPLC instruments. A recent report describes a complete lipid class analysis of human plasma within one hour by a novel approach employing an automated gas chromatograph (Kuksis *et al.*, 1975). Automated analysis of fatty acids by temperature-programmed gas chromatography has also been reported (Slover and Mason, 1975).

Electrophoresis and ultracentrifugation are the methods used to isolate, quantify and characterize circulating lipoproteins. Applications of preparative and analytical ultracentrifugation to the analysis of lipoproteins are described in detail by Lindgren *et al.* (1972). Paper electrophoretic methods for the separation of lipoproteins have been supplanted by agarose (Noble, 1968) and polyacrylamide (Frings *et al.*, 1971; Hall *et al.*, 1972) electrophoresis. Noble *et al.* (1969) have shown a good correlation between human serum lipoprotein analyses by analytical ultracentrifugation and agarose gel electrophoresis, thus establishing the latter method as an inexpensive alternative to the former. Detection limits for the electrophoretic visualization of lipoproteins can be improved by using polyacrylamide gels (the protein bands are narrower and more distinct than is the case for agarose gels) and by staining lipoprotein samples with Sudan black B prior to electrophoresis (Hall *et al.*, 1972). Prestaining of human serum lipoproteins does not alter electrophoretic mobility. To illustrate the importance of increasing detection sensitivity for lipoproteins when screening previously unstudied animals, I can cite experience with the crab *Cancer magister* (Allen, 1972) and the sea urchin *Strongylocentrotus purpuratus* (Allen, 1974). Agarose electrophoresis failed to reveal lipoproteins in the hemolymph of male *C. magister* or the

coelomic fluid of either sex of *S. purpuratus*, whereas lipoproteins were detected in both cases by polyacrylamide electrophoresis of prestained samples.

## BIOCHEMICAL MECHANISMS OF LIPID ENERGY METABOLISM

Biochemical processes important to the processing of lipids for the eventual generation of energy are summarized in Figure 2. A complete definition of the participation of lipids in the energy metabolism of a given animal would require elucidation of the mechanism and rate of each process and knowledge of the controls exercised by diet and environmental variables via endocrine and nervous systems. Figure 2 applies to vertebrates and insects in which alimentary and lipid storage organs are distinct. In many invertebrates the alimentary system also serves as a lipid depot. Detailed knowledge of all eleven processes enumerated in Figure 2 is available for a very few species of mammals, birds and insects. There follows a brief consideration of the biochemistry of these processes as each has been delineated for mammalian systems with an eclectic and noticeably less than comprehensive discussion of the comparative physiology of each. The text by Masoro (1968) serves as an excellent and concise introduction to mammalian lipid metabolism.

### DIGESTION AND ABSORPTION OF DIETARY LIPID

Triglyceride comprises the major dietary lipid of most omnivorous and carnivorous animals. In mammals other than ruminants triglycerides are emulsified to particles of 1000 nm diameter with the aid of bile components and subsequently hydrolyzed to free fatty acids (FFA) and 2-monoglycerides (2-MG) by the action of pancreatic lipase (Masoro, 1968). Bile micelles comprised of bile salts, cholesterol and phospholipid take up the FFA and 2-MG forming mixed micelles of approximately 10 nm diameter. FFA and 2-MG diffuse from the mixed micelles into the mucosal cells of the jejunum. FFA are activated by formation of fatty acyl-CoA derivatives on the smooth endoplasmic reticulum of the mucosal cells and then reesterified to 2-MG to reconstitute triglycerides. Electron microscopy (EM) of intestinal micelles has played a major role in resolving mechanisms of lipid digestion and absorption (Strauss, 1966). Stein and Stein (1971) have written a fascinating de-

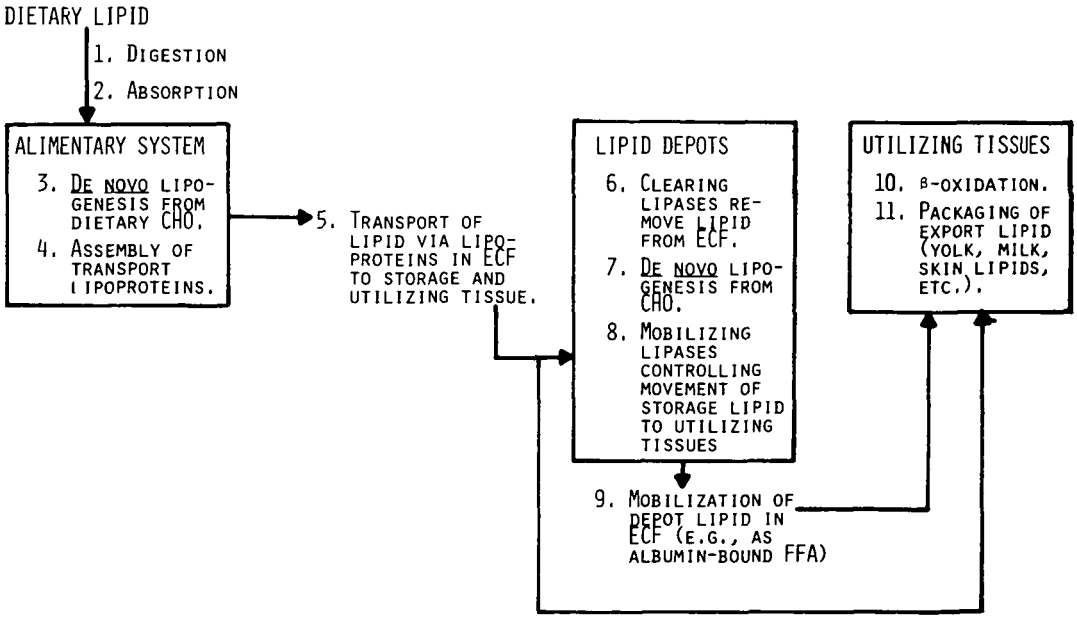



FIG. 2. Biochemical processes important to lipid energy metabolism.

scription of the application of EM-autoradiographic methods to the study of such problems in lipid metabolism as absorption and interorgan translocation.

Digestive lipases have received considerable study in insects (Gilbert, 1967; Weintraub and Tietz, 1973) and crustaceans (Brockerhoff *et al.*, 1967). Scattered reports exist on digestive lipases and esterases (enzymes specific for the hydrolysis of esters of short-chain fatty acids) in other invertebrate groups (Barnard, 1973). The importance of emulsifiers to lipid digestion and absorption has stimulated research on the comparative biochemistry of these compounds. Higher vertebrates have $C_{24}$ bile acids, while many lower vertebrates produce $C_{27}$ bile acids and bile alcohols (Hoshita and Kazuno, 1968). Compounds related to the bile acids in structure have yet to be found in invertebrates although crustaceans produce acylsarcosyltaurines which are thought to function as lipid emulsifiers (van den Oord *et al.*, 1965).

### *DE NOVO* SYNTHESIS OF LIPID

Depot lipid in animal tissues must originate from dietary intake of lipid or synthesis from non-lipoidal substrates, largely carbohydrate. Important aspects of *de novo* lipogenesis include: the synthesis of long-chain, saturated fatty acids from C2 precursors; desaturation and chain elongation of saturated fatty acids; the incorporation of fatty acids into such neutral storage lipids as triglycerides and wax esters. Rates of lipogenesis in mammals and, most probably, all animals are subject to manifold nutritional, endocrine, and developmental controls (Volpe and Vagelos, 1976). Studies on relative or absolute rates of lipogenesis generally employ isotopically labeled lipid precursors, most commonly acetate or glucose. Lowenstein *et al.* (1975) and Jungas (1968) have described methods for the measurement of absolute rates of lipogenesis using deuterated or tritiated water as a labeled precursor of fatty acid hydrogen. While these methods employ quantities of tritium which many laboratories might not wish to handle, or require access to a mass spectrometer, they have considerable advantages over procedures utilizing $^{14}$C-acetate or glucose. When measuring rates of lipogenesis, cognizance must be taken of the fact that *in vitro* tissue preparations other than perfused organs synthesize lipid more slowly than do *in vivo* preparations (Brunengraber, 1975).

Lipogenesis confers upon an animal the advantage of storing 8 times as many calories per unit weight as energy storage in the form of hydrated carbohydrate (Weis-Fogh, 1952). The enzymology and regulation of saturated fatty acid biosynthesis have been reviewed most recently by Volpe and Vagelos (1976). Lowenstein (1969, 1975) is an excellent source of detailed information on methods for the assay, purification, and characterization of enzymes involved in fatty acid synthesis. Two multienzyme complexes, acetyl-CoA carboxylase and fatty acid synthetase, catalyze the formation of long-chain, saturated fatty acids. Acetyl-CoA carboxylase is located in the cytosol of animal cells. It catalyzes the biotin-dependent formation of malonyl-CoA via carboxylation of acetyl-CoA (1). BCCP is a subunit of acetyl-CoA carboxylase

$$\text{ATP} + \text{HCO}_3^- + \text{acetyl-CoA} \xrightarrow[\text{(biotin carboxyl carrier protein, BCCP)}]{} \text{ADP} + \text{P}_i + \text{malonyl-CoA} \quad (1)$$

which serves as an intermediate in the transfer of $HCO_3^-$ to acetyl-CoA. In animal cells acetyl-CoA carboxylase exists as either an inactive protomer (mol wt 2-4 $\times$ $10^5$) or an active polymer (mol wt several million). Conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase is the first enzymatic step commiting acetyl-CoA to lipogenesis. Mechanisms (allosteric and endocrine) effecting short term changes in lipogenic rate may act by altering the equilibrium between protomeric and polymeric forms of acetyl-CoA carboxylase (Volpe and Vagelos, 1976).

Fatty acid synthetase is also a multien-

zyme complex in the cytosol fraction of animal cells. Attempts to dissociate animal fatty acid synthetases into active individual enzymes have been only partially successful. Most detailed information on the enzymatic components of fatty acid synthetase has been obtained with the *E. coli* enzyme which can be dissociated into active subunits (Volpe and Vagelos, 1976). Acyl carrier protein (ACP) plays a literally pivotal role in the function of fatty acid synthetase in that it binds to its 4′-phosphopantetheine prosthetic group the growing acyl chains which undergo sequential hydrogenation and dehydration. A scheme for the operation of pigeon liver fatty acid synthetase (Fig. 3) stipulates the presence of two acyl binding sites ($B_1$ and $B_2$) on β-ketoacyl synthetase. Acetyl-CoA is transferred successively from $B_1$ to ACP to $B_2$. In turn, malonyl-CoA is transferred to ACP via $B_1$. The priming acetyl group at $B_2$ is then condensed with malonyl-ACP to commence the cyclic operation of the fatty acid synthetase complex:

$$\text{malonyl-ACP} + \text{acetyl} - B_2 \xrightarrow{\beta\text{-ketoacyl synthetase}} CO_2 + \text{acetoacetyl-ACP} \quad (2)$$

$$\text{acetoacetyl-ACP} + \text{NADPH} + H^+ \xrightarrow{\beta\text{-ketoacyl reductase}} NADP^+ + \beta\text{-hydroxyacyl-ACP} \quad (3)$$

$$\beta\text{-hydroxyacyl-ACP} \xrightarrow{\beta\text{-hydroxyacyl dehydrase}} H_2O + \alpha,\beta\text{-unsaturated acyl-ACP} \quad (4)$$

$$\alpha,\beta\text{-unsaturated acyl-ACP} + \text{NADPH} + H^+ \xrightarrow{\text{enoyl reductase}} NADP^+ + \text{acyl-ACP} \quad (5)$$

The acyl group produced by one turn of the cycle is temporarily bound to $B_2$ while a fresh malonyl group binds to ACP. The malonyl-ACP is condensed with the acyl group by β-ketoacyl synthetase to start a new turn of the cycle, which continues to operate until palmityl-ACP is formed. Palmityl deacylase then releases free palmitate (16:0), which is the major end product of fatty acid synthesis. Long-term regulation of the rate of fatty acid synthesis may be effected by changes in the concentration of the fatty acid synthetase complex (Volpe and Vagelos, 1976).

With the exception of cestodes (Meyer *et al.*, 1966), the capacity to synthesize, *de novo*, fatty acids other than linoleate and linolenate is probably general amongst metazoans. A variety of echinoderms (Allen, 1968), molluscs (Voogt, 1972) and arthropods (Gilbert and O'Connor, 1970) can incorporate $^{14}C$-substrates into fatty acids. A subject of greater interest to comparative physiologists than the mere demonstration of lipogenesis is the study of mechanisms by which diet and environment alter the rate of lipogenesis. In contrast to a wealth of data on the control of mammalian and avian lipogenesis, little information exists for invertebrates. Bollenbacher *et al.* (1972*a*, 1972*b*) noted increased rates of fatty acid synthesis (as measured by $^{14}C$-acetate incorporation) by the hepatopancreas of crabs and crayfish in which the premolt condition was induced by eyestalk removal. Eyestalk removal results in increased secretion of ecdysterone by the Y organ, but ecdysterone injection of intermolt animals did not accelerate lipogenesis. These workers concluded that an eyestalk factor other than molt-inhibiting hormone or hyperglycemic hormone was regulating lipogenesis.

Palmitic acid produced by *de novo* synthesis can be desaturated and/or elongated by the stepwise addition of C2 units. Enzyme systems catalyzing the elongation of palmityl-CoA are associated with both mitochondria and the endoplasmic reticulum. The former system employs acetyl-CoA as C2 donor, while the latter uses malonyl-CoA (Wakil, 1970). Desaturation of saturated fatty acids is effected by a microsomal enzyme system requiring

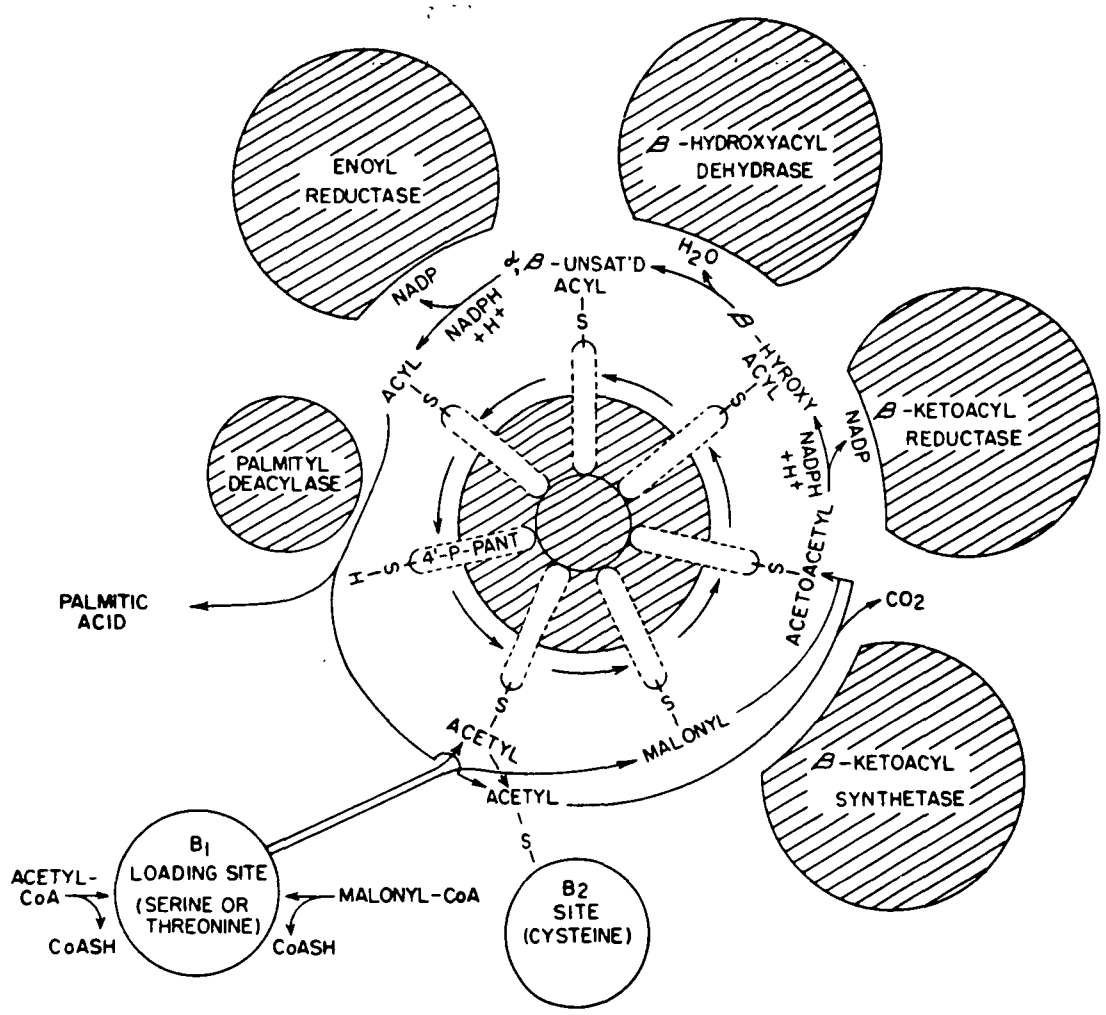



FIG. 3. Model of avian fatty acid synthetase (from Muesing and Porter, 1975).

molecular oxygen and NADH. This enzyme system can introduce several double bonds into one fatty acid chain. In the case of saturated fatty acids, the first double bond is inserted into the 9,10 position relative to the carboxyl group. Thus palmitic acid yields palmitoleate (16:1ω7) and stearic acid gives rise to oleate (18:1ω9). The polyunsaturated fatty acids (*i.e.*, those with two or more double bonds) have considerable significance in animal nutrition as all investigated metazoans have demonstrated a requirement for either linoleate (18:2ω6) or linolenate (18:3ω3). Mead (1968) reviewed the metabolism of polyenes from a historical perspective, stressing the importance of developments in analytical techniques to this field. The polyenoic acids have been grouped into four, non-interconvertible families (ω3, ω6, ω7, ω9) in accordance with the number of carbons between the double bond most distant from the carboxyl group and the methyl terminus of the chain. An important feature of the microsomal fatty acyl desaturase of animal cells is its inability to introduce double bonds more distant than the 9,10 position from the carboxyl terminus. As a consequence, animals are capable of producing polyenoic acids of the ω7 and ω9 families by *de novo* synthesis, but can only manufacture acids of the ω3 and ω6 series from dietary linoleate or linolenate (*e.g.*, dietary 18:3ω3 → 20:5ω3 → 22:6ω3).

Acids of the ω6 series are dietary essentials for mammals, perhaps serving as components indispensable to normal membrane structure or as precursors for the synthesis of prostaglandins (Guarnieri and Johnson, 1970). A characteristic of the microsomal desaturases important to nutritional physiology is their relative lack of specificity. Thus, 18:1ω9 (oleate) and 18:2ω6 (linoleate) can compete for conversion to longer-chain, more highly unsaturated acids of their respective families. A diet containing a normally adequate amount of linoleate but an unusually high oleate/linoleate ratio can result in the prepondererant formation of 20:3ω9 from oleate with a concomitant suppression of 20:4ω6 (arachidonic acid) production from linoleate. Essential fatty acid deficiency syndrome would result.

The comparative physiology of essential fatty acid metabolism merits further study. While acids of the ω6 series are essential to mammals, Lee and Sinnhuber (1972) have found that acids of the ω3 family may be of more importance in the nutrition of trout and salmon. It may prove true that for aquatic metazoans, in which polyenes of the ω3 series predominate, 18:3ω3 is the major essential fatty acid. Another unusual finding pertinent to essential fatty acids is the ability of a terrestrial snail, *Cepaea nemoralis*, to incorporate $^{14}$C-acetate into polyenes of the ω6 series while acids of the ω3 series remained unlabeled (van der Horst, 1973). This result suggests a unique ability to synthesize acids of the ω6 family.

Deposition of fatty acids as energy stores entails their esterification with alcohols to yield glycerides or wax esters. Triglycerides constitute the dominant form of lipid energy storage in animals. Enzymes associated with their synthesis by either the 2-monoglyceride or glycerol phosphate pathways (Fig. 4) are located on the endoplasmic reticulum and have been described in detail by Hübscher (1970).

### TRANSPORT OF LIPID IN BODY FLUID

The hydrophobic nature of lipids enforced the development of mechanisms for rendering these compounds soluble in aqueous body fluids in order to effect interorgan transport. In theory, the solubility problem could be met by transporting lipids in blood cells, but most animals, instead, utilize soluble, circulating lipoproteins. The association between disturbances of lipid transport and atherosclerosis has

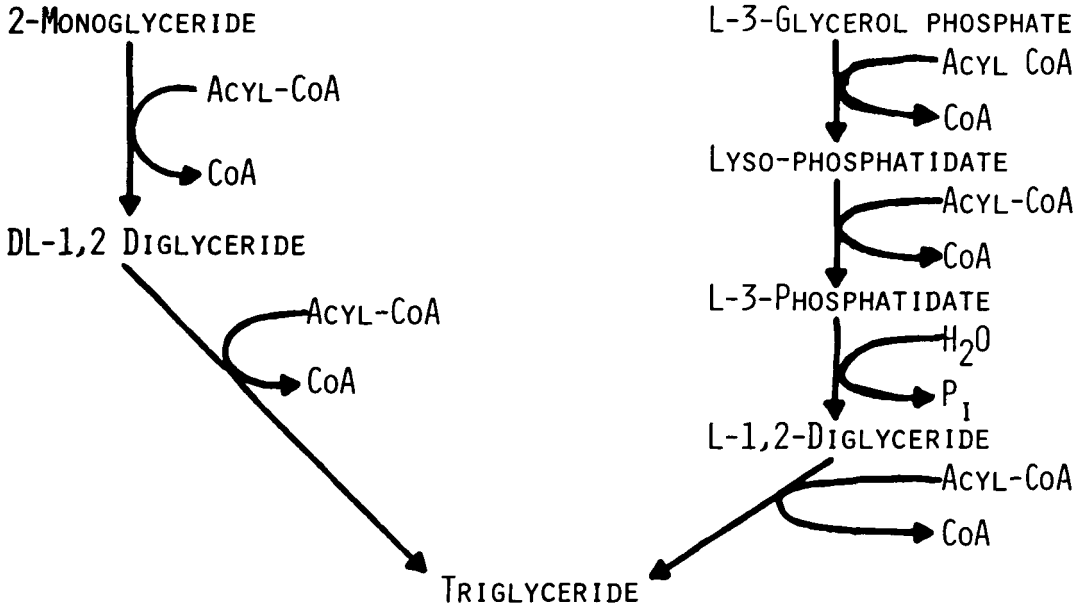



FIG. 4. Pathways of triglyceride synthesis (modified from Hübscher, 1970).

TABLE 2. *Human plasma lipoproteins.*

| Lipoprotein | Composition(%) | | | | Major apoprotein | Mg/100 ml plasma |
|---|---|---|---|---|---|---|
| | Protein | Triglyceride | Cholesterol and cholesterol esters | Phospholipid | | |
| Chylomicron | 1 | 90 | 7 | 2 | B, C-I, C-II, C-III | — |
| Very low density(VLDL) | 10 | 60 | 5 | 14 | B, C-I, C-II, C-III | 173 |
| Low density (LDL) | 25 | 10 | 37 | 20 | B | 380 |
| High density (HDL) | 50 | 3 | 14 | 30 | A-I, A-II | 263 |

From Masoro, 1968 and Volpe and Vagelos, 1976.

resulted in a vast literature on the structure and metabolism of human lipoproteins, a subject which has been reviewed most recently by Jackson *et al.* (1976). Scientists with a casual interest in lipid transport cannot fail to be impressed with the sophistication of contemporary methodology for probing lipoprotein structure and function. Models of the structure of the major human lipoproteins are based on data generated by such techniques as small-angle X-ray scattering, $^{13}C$ NMR spectroscopy, and fluorescence spectroscopy. The apolipoproteins associated with specific lipoproteins have been isolated and the amino acid sequences of four are known. Specific function (other than solubilization of lipid) has been assigned to certain apolipoproteins, *e.g.*, apoprotein A-1 (Apo A-1) is an activator of lecithin: choline acyltransferase (LCAT, Soutar *et al.*, 1975). LCAT is thought to remove phosphatidyl choline and unesterified cholesterol (both of which are surface components of lipoprotein particles) from circulating lipoproteins, thus preserving a balance between the surface lipids and the volume of internal neutral lipids as the latter are removed from lipoprotein particles (Schumaker and Adams, 1969). Of interest to comparative physiologists is the isolation from salmon of an Apo A-1 similar in amino acid sequence and other properties to human Apo A-1 (Nelson and Shore, 1974).

Consideration of the structure and function of human plasma lipoproteins (Table 2) serves as a good starting point for discussion of the comparative physiology of lipid transport. Chylomicra are large particles, rich in triglyceride, which are synthesized in the intestinal mucosa. Chylomicra transport exogenous (dietary) lipid to storage sites (adipose tissue) and other extrahepatic tissues (Sanbar, 1969). Lipoprotein lipase, which is bound to the endothelium of tissue capillaries, catalyzes the removal of triglycerides from the chylomicra with a half-life of less than one hour by hydrolyzing the triglycerides. The products of hydrolysis (diglyceride and FFA) are taken up by extrahepatic tissue and the remnants of the chylomicron particles are removed by the liver (Jackson *et al.*, 1976). VLDL are produced by both liver and intestinal mucosa. VLDL particles released by the liver transport endogenously synthesized lipid to extrahepatic tissues, while the mucosal VLDL serve much the same function as chylomicra. Lipoprotein lipase removes triglyceride from VLDL converting the latter to cholesterol-rich, triglyceride-poor LDL. The specific transport functions of LDL and HDL are less clear. Lipid mobilized from the adipose tissue depots in response to the energy demands of other tissues is transported as FFA bound to serum albumin (Sanbar, 1969). While the FFA content (*ca.* 14 mg%) of human plasma is markedly lower than that of other lipid classes, the half-life of plasma FFA (less than one minute, Baker and Schotz, 1967) is so rapid that in man a flux of 200 g lipid/day is transported through the plasma in this form.

Numerous comparative studies of vertebrate plasma lipoproteins have been reported. Mills and Taylaur (1971) isolated the lipoproteins of 35 vertebrates (including 4 species of fish, 6 reptiles, and one amphibian) ultracentrifugally and determined the chemical composition of the several flotation classes. None of the animals approximated the human lipoprotein profile and no evolutionary generalizations could be made. In a subsequent effort to identify animals with plasma lipoprotein patterns similar to man (such animals might serve as experimental models for human lipid transport), Alexander and Day (1973) employed agarose gel electrophoresis to analyze the lipoproteins of 36 vertebrates (including 3 fish, 3 reptiles, and 2 amphibians). Pigs, opossums and garter snakes most closely approximated man in serum LDL and cholesterol content. Lee and Puppione (1972) conducted a more extensive study on the ultracentrifugal properties and chemical composition of the lipoproteins of the Pacific sardine.

Insect lipid transport has been reviewed quite recently (Gilbert and Chino, 1974). The fat body of insects has functions similar to both liver and adipose tissue of mammals. Depot lipid of the fat body consists mainly of triglyceride. Insects (and perhaps arthropods in general) are unique in that lipid mobilized from the fat body for transport to such tissues as flight muscle is mainly in the form of diglycerides. Van Handel and Nayar (1972) have demonstrated that the rate of hemolymph diglyceride turnover can fully account for the energy metabolism of the moth *Spodoptera frugiperda* both at rest and during flight. Hymolymph lipids of lepidopterans are carried by two major lipoproteins (Gilbert and Chino, 1974). Lipoprotein I plays a metabolic function, carrying glycerides and sterols to sites of utilization and storage. Lipoprotein II carries relatively little lipid and is characterized ultracentrifugally as a very high density lipoprotein (VHDL). Lipoprotein II is believed to be a vitellogenin, a class of glycolipoprotein synthesized extraovarially by animals which produce eggs rich in yolk. Vitellogenins are transported through the blood for eventual incorporation, immunochemically intact, into developing oocytes. The endocrine induction of vitellogenin synthesis by fat bodies of female cockroaches (*Leucophaea maderae*, Engelman, 1974) and mosquitoes (*Aedes aegypti*, Hagedorn, 1974) has been the subject of sophisticated studies. Juvenile hormone induces vitellogenin synthesis in the former insect, while ecdysterone plays the same role in the mosquito.

Lipoproteins have been detected electrophoretically in the hemolymph of many crustaceans (Adiyodi, 1968; Horn and Kerr, 1969; Fielder *et al.*, 1971). Most such lipoproteins appear to be limited to sexually mature females (female-specific proteins) and are probably vitellogenins. Croisille *et al.* (1974) have reviewed the most extensively studied crustacean vitellogenin system, that of the amphipod *Orchestia gammarella*. Males of the Dungeness crab have two electrophoretically resolvable lipoproteins, which probably serve metabolic functions similar to lipoprotein I of lepidopterans (Allen, 1972). As in insects, diglycerides predominate in the neutral fraction of the hemolymph lipids of the Dungeness crab. When crabs were fed $^{14}$C-palmitate, however, the time course of labeling of tissue and hemolymph lipid classes suggested that hemolymph FFA turned over more rapidly than other lipid classes.

Little information exists on lipid transport in invertebrate phyla other than the Arthropoda. This would seem to be a fertile field for comparative physiologists interested in the quantitative significance and regulation of lipid metabolism in molluscs, echinoderms, and annelids. In the two former phyla body fluid lipid levels are low (ranging from 8-100 mg %; Giese, 1966) relative to arthropods and vertebrates. This fact may have discouraged many workers, who might have assumed from the low body fluid lipid concentrations that lipids were unimportant in the energy economy of echinoderms and molluscs. A preliminary study of lipid transport in a sea urchin indicated, however, that the flux of lipids (apparently transported through the coelomic fluid mainly as FFA contained in coelomocytes) was sufficient to meet the

energy substrate requirements of such peripheral tissues as the gonads and body wall (Allen, 1974).

## MOBILIZATION OF LIPID FROM STORAGE DEPOTS

When food is abundant, excess dietary calories are stored in lipid depots by animals. The comparative anatomy of lipid storage depots has been reviewed by Vague and Fenasse (1965). Fish display a particularly diverse anatomical pattern of lipid storage. In elasmobranchs lipid may comprise 90% of the weight of the liver. Much of the lipid is squalene or diacylglyceryl ether, both of which have lower densities than triglycerides and, accordingly, contribute to the buoyancy of these swim bladder-less fish (Malins and Wekell, 1970). Different teleosts deposit fat in a variety of locations including liver, muscle and, surprisingly, bone marrow (Phleger *et al.*, 1976).

When food intake is low, energy metabolism switches from a balanced combustion of carbohydrate and fat to almost pure reliance upon lipid catabolism. At such times fat must be mobilized into the blood at greater rates from the lipid depots. Mobilization of fat from adipose tissue of mammals (Fig. 5) is increased by several lipolytic hormones which activate adipocyte hormone-sensitive lipase (HSL) via a cyclic AMP-dependent protein kinase (Khoo *et al.*, 1973; Khoo and Steinberg, 1975). HSL catalyzes the rate limiting step (triglyceride → diglyceride + FFA) in the hydrolysis of stored triglycerides to glycerol and FFA with resultant release of the latter into the plasma for transport to utilizing tissues. HSL, thus, is the control point in mammalian lipid mobilization. In contrast to lipolytic hormones, insulin reduces mobilization of FFA by lowering adipocyte cyclic AMP (thus inhibiting HSL) and by increasing the rate of CHO catabolism in adipocytes. This last effect of insulin increases the intracellular level of glycerophosphate, which promotes re-esterification of intracellular FFA to triglycerides.

The central role of depot fat mobilization in lipid energy metabolism and the demonstrated, multiple endocrine regulation of this process in mammals should make its study attractive to the comparative physiologist. Most comparative studies have been restricted to vertebrates. Catecholamines and six different pituitary hormones had quite different adipokinetic actions when tested on rabbit, guinea pig, hamster, rat, pig, and dog (Rudman *et al.*, 1965). For example, epinephrine, which had lipolytic activity in minute doses in the rat, was virtually without effect in rabbits and dogs. Control of lipid mobilization in fish has received considerable recent attention. Thyroxine (McKeown *et al.*, 1975) and prolactin and growth hormone (Narayansingh and Eales, 1975) promote lipolysis in salmonids. The effectiveness of vertebrate adipokinetic hormones in promoting the mobilization of fat body lipid has been studied in insects (Chang, 1974; Bhakthan and Gilbert, 1968). Several of the vertebrate lipolytic hormones were active, but physiological significance could be attached only to the effects of the catecholamines, which do occur in insects. A hyperlipemic factor has been detected in extracts of the corpora cardiaca of locusts (Mayer and Candy, 1969), while the corpora cardiaca of the cockroach, *Periplaneta americana* produce a hypolipemic agent (Downer and Steele, 1972) which may be important in the deposition of dietary lipid in the fat body. No data appear to exist on lipid mobilization in invertebrates other than insects.

## LIPID UTILIZATION

The advantages animals derive from the development of systems for the digestion, transport, and synthesis of neutral lipids are realized when the latter are combusted to $CO_2$ and $H_2O$ with the release of free energy (Dole, 1965). Neutral fats release 9 kcal/g when completely combusted as opposed to a value of 4 kcal/g for carbohydrates and proteins. Furthermore, lipid can be stored in dehydrated form, whereas depot carbohydrates are accompanied by water of hydration thus further reducing their kcal/g value. Oxidation of palmitic acid is achieved by the sequential operation of the $\beta$-oxidation and Krebs cycles. Of the 2,340 kcal/mole of free energy released

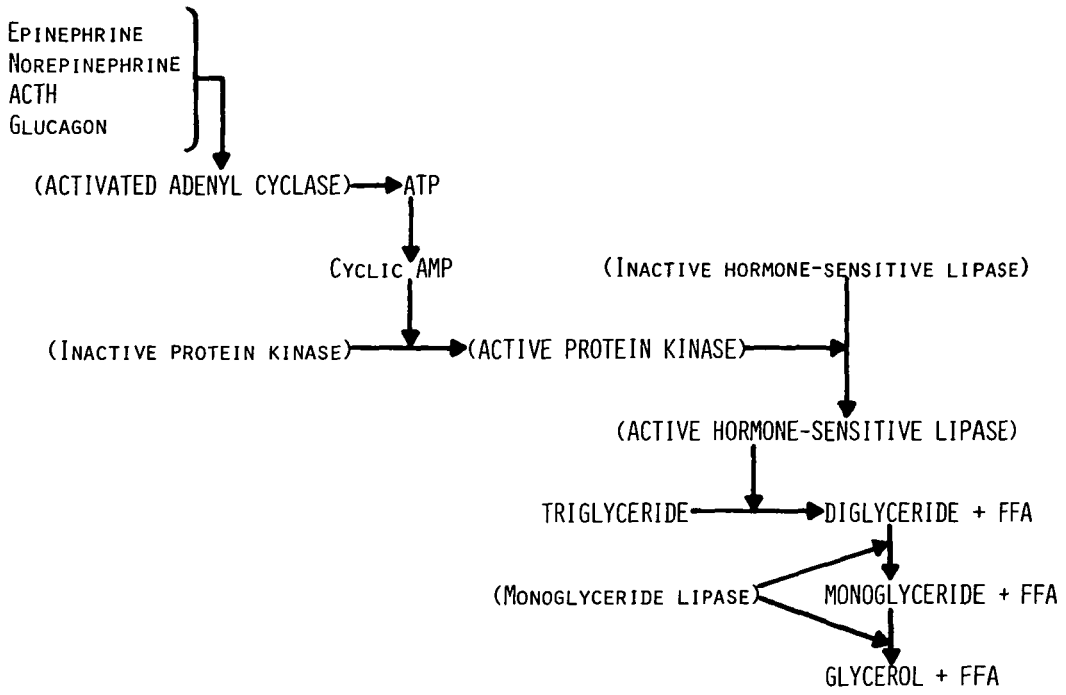



FIG. 5. Endocrine control of fat mobilization from adipose tissue of mammals (from Montgomery *et al.*, 1974).

upon oxidation of palmitic acid (6),

$$\text{palmitic acid} + 23O_2 \rightarrow 16CO_2 + 16H_2O \quad \Delta G^{o'} = -2{,}340 \text{ kcal} \quad (6)$$

$$130\,ADP + 130\,P_i \rightarrow 130\,ATP + 130\,H_2O \quad \Delta G^{o'} = +949 \text{ kcal} \quad (7)$$

approximately 40% is trapped in 130 moles of ATP formed by substrate level and oxidative phosphorylation (7, Lehninger, 1975).

Long-chain fatty acid catabolism (Fig. 6) commences with the activation of FFA in the form of fatty acyl-CoA, a reaction catalyzed by microsomal fatty acid thiokinases. Approximately 70% of the fatty acyl-CoA is formed outside the mitochondria, whereas the enzymes of the β-oxidation cycle reside within the inner membrane of the mitochondria. This latter membrane is impermeable to fatty acyl-CoA. Transport of fatty acyl-CoA into the mitochondrion is effected by transfer of fatty acyl residues to carnitine forming fatty acyl-carnitine derivatives which can cross the mitochondrial inner membrane thus reaching the β-oxidation compartment. Once inside the mitochondrion, fatty acyl-CoA is reformed and the sequential steps of the β-oxidation cycle degrade fatty acyl-CoA to acetyl-CoA. The first dehydrogenation is catalyzed by an FAD-linked dehydrogenase to yield a *trans* double bond in the 2,3 position of the acyl chain (Wakil, 1970). Hydration of the $\Delta^{2,3}$-*trans*-enoyl-CoA intermediate is catalyzed by enoyl hydratase to produce, stereospecifically, the L isomer of 3-hydroxy acyl-CoA. Enoyl hydratase does not act upon $\Delta^{3,4}$-unsaturated fatty acyl-CoA and hydrates $\Delta^{2,3}$-*cis*-enoyl-CoA derivatives to the D stereoisomer of 3-hydroxy acyl-CoA. As the second dehydrogenation of the β-oxidation cycle is catalyzed by an enzyme which is absolutely specific for the L stereoisomer of 3-hydroxy acyl-CoA, the β-oxidation of monounsaturated fatty acids (which have *cis* double bonds exclusively) requires an auxiliary enzyme ($\Delta^{3,4}$-*cis*-$\Delta^{2,3}$-*trans*-enoyl-CoA isomerase) to shift the double bond from the 3,4 to the 2,3 position (to permit action of enoyl hydratase) and to convert the double bond from the *cis* to the *trans* configuration in order to allow

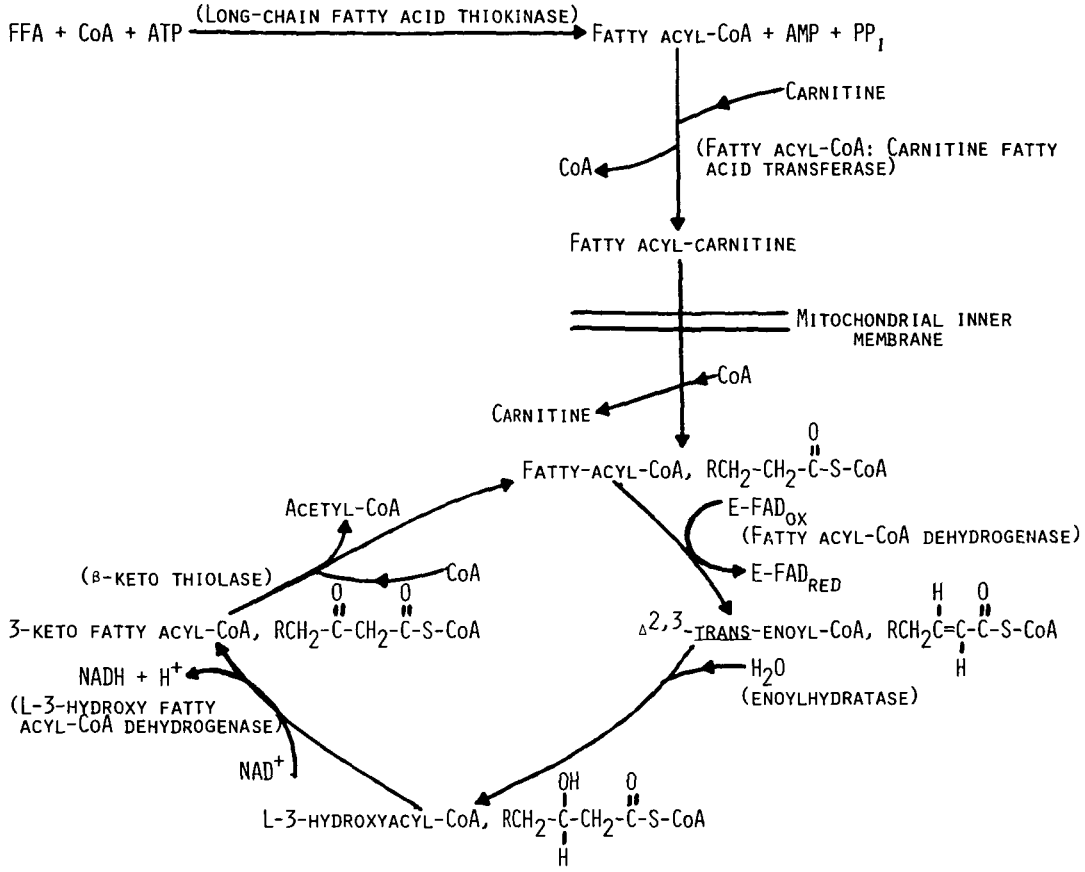



FIG. 6. Long-chain fatty acid catabolism.

attack by L-3-hydroxy fatty acyl-CoA dehydrogenase following hydration (Wakil, 1970). The complete β-oxidation of polyunsaturated fatty acids requires an additional auxiliary enzyme, 3-hydroxy fatty acyl-CoA epimerase (Wakil, 1970). One cycle of β-oxidation is completed by the thiolytic cleavage of 3-keto fatty acyl-CoA to acetyl-CoA, which enters the Krebs cycle, and a fatty acyl-CoA with two fewer carbons than its predecessor.

Information on the assay, purification, and characterization of enzymes of the β-oxidation cycle is provided by Lowenstein (1969, 1975). A complex interrelationship exists between fatty acid catabolism and carbohydrate catabolism. High rates of the former process inhibit the latter and promote gluconeogenesis and ketogenesis in mammalian liver (Bressler, 1970). Conversely, active CHO catabolism suppresses fatty acid catabolism, principally by reducing FFA mobilization from fat depots. The β-oxidation of fatty acids appears to be an unremarkable process in the context of comparative physiology. All metazoans, with the possible exception of obligately anaerobic parasites, appear to have the enzymes of the β-oxidation cycle.

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