Plant lipids specialists hold 10th symposium in Tunisia

High-quality olive oil is an important economic factor in Tunisia. From the air, an observer can see a regular pattern of tree plantings covering many square miles; about 60 million olive trees are cultivated. It is therefore not surprising that in this country an active research is focused on plant lipids, covering economic, nutritional, ecological and biochemical aspects.

Thus, it was logical to come to the 10th International Symposium on the Metabolism, Structure and Utilization of Plant Lipids, held April 27-May 2, 1992, in Jerba, Tunisia, marking the first time the biennial meeting has been held in Africa.

This report on the 10th International Symposium on the Metabolism, Structure and Utilization of Plant Lipids was prepared by INFORM Associate Editor Thomas J. Bach of CNRS-IBMP, Département d'Enzymologie Cellulaire et Moléculaire, Université Louis Pasteur, Institut de Botanique, Strasbourg, France. The meeting was held April 27-May 2, 1992, in Jerba, Tunisia, marking the first time in its history to present their newest data on plant lipid research.

This biennial meeting, now with a history spanning two decades, is devoted exclusively to plant lipids, and every aspect—biochemistry, molecular biology, biotechnology and ecology—has been represented by highly interesting communications. As is traditional, the proceedings of this meeting will be published (details will be printed in INFORM when available) and, in our experience, the citation rate of articles in this series is quite high.

A considerable number of groups worldwide are engaged in research on plant lipid synthesis and function, and interest is increasing rapidly (as shown by recent articles and features in INFORM). But still, these meetings have a nice “family” character, and for regular attendants it is particularly interesting to monitor scientific progress made in the special domain of certain research groups and how modern developments—key words: molecular biology and genetic engineering—create new directions, interests and expertise.

The meeting was organized into seven major sessions: (a) metabolism of glycerolipids and its regulation, (b) fatty acid biosynthesis and catabolism, (c) biochemistry and biosynthesis of isoprenoid compounds, (d) structure, function and physical properties of lipids, (e) plant lipid metabolism changes under environmental aspects, (f) biotechnological and molecular aspects of plant lipids, and (g) transfer of lipids and proteins.

Each session included one or two plenary lectures of 40 minutes, oral short communications of 20 minutes, as well as posters plus discussions. This article is an attempt to put things together that are sometimes apparently out of joint, not always clearly attributable to a specific session mentioned above, and to give an idea of where considerable progress has been made or where areas may be especially interesting to INFORM readers.

Thanks to newly developed radio-HPLC (high-performance liquid chromatography) methods, the group of Mazliak (Paris) could pursue the metabolic fate of each molecular species of phosphatidylcholine as the major species of phospholipids, which are constantly modified by either desaturation or deacylation/recylation cycles. However, species of phos-
Phatidylinositol remained virtually unchanged after their biosynthesis. Phospholipid synthesis must be looked at as a dynamic process with different turnover, comparable to the situation found with proteins.

Because there is always a search for new lipids that might have some nutritional or technical importance, the findings of Eichenberger (Bern, Switzerland) are noteworthy. He has studied the occurrence of betaine lipids, e.g., in ochromonas (Chryso-phyceae) and cryptomonas (Chryto-phyceae). In ochromonas the diacylglyceroltrimethylhomoserine (DGTS) also acts as a primary acceptor for exogenous fatty acids, as a substrate for fatty acid desaturation, and as the acyl donor in the redistribution of fatty acids among different lipids.

Dubacq and his colleagues (Paris) question the hitherto widely accepted concept of "prokaryotic" (C_{18}/C_{16}) and "eukaryotic" (C_{18}/C_{18} or C_{16}/C_{18}) lipids. However, the occurrence of eukaryotic lipids in a cyanobacterium sheds new light on evolutionary metabolic relationships between cyanobacteria and chloroplasts.

Gawer and her colleagues (with Mazliah) investigated choline kinase (CK) and CTP-choline phosphate cytidylyltransferase (CT) in two strains of tobacco cells, either resistant or sensitive to high-choline concentrations. Phosphate, sucrose or choline seem to modulate both enzyme activities. CT appears to be reversibly associated with ER (endoplasmic reticulum) membranes.

Several contributions concerned the formation of lipids in olives. In contrast to other species of oil-bearing plants, olives have an outer layer of the pericarp (epicarp) that contains chloroplasts capable of CO_{2} fixation and of active fatty acid synthesis (Sanchez et al., Sevilla, Spain, in collaboration with Harwood, Cardiff, United Kingdom). This synthesis might contribute some percentage to total fatty acid accumulation. The main function of olive fruits is the storage of photosynthate coming from leaves in the form of neutral glycercerolipids (group of Abdelkader Cherif, Tunis). Linear incorporation of ^{14}Cbicarbonate for at least three hours by tissue slices of olive fruits was strictly dependent on light; radioactivity was found to be located in the triacylglycerol, diacylglycerol and phosphatidyl-

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Choline fractions [Cuvillo and Sanchez (Sevilla) and Harwood (Cardiff)]. Microsomes obtained from olive cultures, when incubated with ^{14}C-glycerol-3-phosphate in the presence of palmitoyl-CoA and oleoyl-CoA esters, resulted in good labeling of the various Kennedy pathway intermediates. Microsomes from tissue cultured at 35°C incorporated significantly more radiolabel into lipids and were also able to synthesize proportionally more triacylglycerol [Rutter and Harwood (Cardiff) and Sanchez (Sevilla)].

As new techniques become available to purify and stabilize enzymes involved in lipid biosynthesis, careful kinetic studies are now possible without dealing with substrates being intrinsic in membranous preparations. In this way the group of Douce (Grenoble, France) has purified by 500-fold UDP-galactose:1,2-diacylglycerol galactosyltransferase (or MGDG synthase) from spinach chloroplast envelope membranes and determined its reaction pathway. In contrast to earlier publications, it was shown that the binding of the two substrates diacylglycerol and UDP-gal, respectively, occurs randomly and at distinct sites of the enzyme. Malherbe and colleagues (also from the Grenoble group) showed that phosphatidate phosphatase from spinach chloroplast envelope membranes is feedback-inhibited by diacylglycerol (apparent Ki 70 μM, apparent Km for phosphatidate 600 μM). Since several plastidic glycerolipids (phosphatidylglycerol, galactolipids and sulfolipid) are synthesized from phosphatidic acid within the envelope membranes, this type of regulation makes functional sense.

The substrate specificity of the first two acylation reactions of the Kennedy pathway had been studied using microsomes from avocado mesocarp. The active site of glycero-

3-phosphate acyltransferase has been localized on the outer surface of the microsomal vesicles. Both enzyme activities could be solubilized and partially purified (Eccleston and Harwood, Cardiff). Stymne and his colleagues (Uppsala, Sweden) have studied the specificities of diacylglycerol acyltransferases in microsomal fractions from developing oil seeds (Cuphea procumbens, rape, sunflower and safflower) demonstrating that the enzymes depend on the acyl quality of both the diacylglycerol (a very hydrophilic substrate) and the acyl-CoA species.

The presentation by Lichtenthaler, Focke and their associates (Karlruhe, Germany) was concerning the de novo fatty acid biosynthesis, starting from acetate. The first enzyme in this series, acetyl-CoA synthetase, is inhibited by alkyladenylates which might mimic intermediates in the reaction. The next enzyme, acetyl-CoA carboxylase, a biotin-containing enzyme catalyzing the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA, appears to be the key enzyme in fatty acid biosynthesis. This enzyme is the molecular target of a series of highly efficient and selective herbicides, and work is in progress to assess the mechanisms of sensitivity versus resistance. Further biotin-enzymes such as methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase have been identified. Finally, derivatives of cerulenin have been synthesized and their effect on 3-oxoacylsynthetases has been studied.

Jaworsky (Oxford, Ohio), in collaboration with Post-Breitenmiller and Ohlrogge (East Lansing, Michigan), provided clear evidence that it is
acetyl-CoA and not acetyl-ACP that functions as the primer for fatty acid biosynthesis, another example of how widely accepted “dogmas” exist—until experimental proof shows they are invalid. By neatly designed incorporation studies, Post-Breitenmiller and Ohlrogge, in collaboration with Roughan (Auckland, New Zealand), have determined the regulation of substrate pools for plastidic fatty acid synthesis. Practically all CoA in chloroplasts freshly isolated from light-grown spinach was found as acetyl-CoA, representing up to 77% of total leaf acetyl-CoA. Furthermore, they showed that the malonyl-CoA:ACP transacylase reaction is near equilibrium in both light- and dark-incubated chloroplasts, whereas the acetyl-CoA:ACP transacylase reaction comes nearer to equilibrium in light-incubated chloroplasts. Malonyl CoA and malonyl-ACP could be detected in chloroplasts only during fatty acid biosynthesis, in contrast to acetyl-CoA and acetyl-ACP that were also detectable in the absence of fatty acid synthesis. Thus, in situ evidence has been provided that acetyl-CoA carboxylase plays a regulatory role. The same group showed that acetyl-carnitine does not serve as a substrate for fatty acid biosynthesis, in contrast to acetyl-CoA and acetyl-ACP, which were also detectable in the absence of fatty acid synthesis. Thus, in situ evidence has been provided that acetyl-CoA carboxylase plays a regulatory role. The same group showed that acetyl-carnitine does not serve as a substrate for fatty acid biosynthesis by density-gradient purified chloroplasts.

Medium-chain fatty acid (MCFA) synthesis has been studied in plastids from Cuphea wrightii embryos (Heise and Fuhrmann, Göttingen, Germany). Colorless embryos accumulated capric (C10:0) and lauric acid (C12:0) in their storage lipids. MCFA synthesis, exclusively bound to intact plastids, required ATP, which could not be substituted for by C3-intermediates of the glycolytic pathway, but the reduction of the pyridine nucleotide pool was dispensable for optimum MCFA formation. MCFA synthesis was driven by substrates of the pentose pathway, especially glucose-6-phosphate. In a complementary study, developing seeds of Cuphea lanceolata (Spener and Schuch, Münster, Germany) were used to localize ACP1 and ACP2, differentially expressed in the embryo (ACP1 and ACP2) and in the seed coat (ACP1). This corresponds to the finding that in the seed coat mainly C18 acids are present, but no decanoic acid. Determination of reactions of fatty acid synthesis in cell-free seed extracts, i.e., incorporation of acetate from (1-14C)acetyl-CoA and malonate from (2-14C)malonyl CoA into intermediate acyl-ACPs, showed the presence of ceruleni-insensitive condensing enzyme ketoacyl-synthetase III (KAS III). Analysis of the products of the KAS III reaction revealed the dominance of C9- and C6-ACPs. KAS III was shown to be 25 times more active than acetyl-CoA:ACP transacylase, competing for the same substrate, thus KAS III must be responsible for the first condensation step in fatty acid synthesis.

Fatty acid elongation activities in subcellular fractions of developing seeds of Limnanthes alba have been studied by Lardans and Trémolières (Gif-sur-Yvette, France). These seeds accumulate the very long chain fatty acids (VCFA) Δ9 cis-eicosenoate (20:1Δ9) and Δ13 cis-docosadienoate (22:2Δ9,13), produced by chain elongation of stearate (18:0) and oleate (18:1Δ9). Using partially purified enzymes from 15,000 g x g and 100,000 g x g fractions the authors provide evidence for the existence of two distinct elongation systems: one specific for saturated acyl-CoAs, the other for monounsaturated acyl-CoAs. The biosynthesis of VLCFA by subcellular fractions isolated from developing rapeseed has been studied by Créach, Lessire and Cassagne (Bordeaux, France). The C18:1-CoA elongase was mainly localized in a 15,000 g particulate fraction, with C18:1-CoA and malonyl-CoA being the best substrates for the synthesis of C20:1, C22:1 and C24:1, which are then esterified to triglyceric glycerols. Time course experiments and the effect of malonyl-CoA concentration support the existence of two elongation systems, one leading to the synthesis of C20:1 and the other responsible for the formation of C22:1 and C24:1. René Lessire and his colleagues (Bordeaux, France) have characterized the intermediate reactions involved in the leek icosanoyl-CoA synthase, catalyzing the formation of C20:0 via the elongation of C16:0-CoA by addition of malonyl-CoA. The elongation products released as acyl-CoAs have been isolated and suggest that the overall mechanism involves four different intermediate reactions (condensation, first reduction, dehydration and second reduction). Antibodies against β-hydroxyacyl-CoA dehydrase from rat liver reacted with one of the constituting proteins of the complex, indicating a molecular weight of 65 kDa.

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The biosynthesis of storage oil and petroselenic acid in embryos and cell cultures of Umbelliferae has been studied by the group of Murphy (Norwich, United Kingdom). In the presence of high osmotic media, large quantities of storage triglyceric glycerols (TAG) are accumulated, deposited in oil bodies surrounded by a proteinaceous annulus of oleosins of 15 and 19 kDa (see below). Although carrot cell cultures and somatic embryos produce similar oil bodies with similar oleosins to those made by zygotic embryos, the fatty acid content of
their oil is totally different. Although the latter contain up to 80% petroselenic acid (cis Δ⁶ isomer of 18:1), the former contained mostly linoleic and linolenic acids. This implies that, although TAG and oleosin accumulation can be induced in carrot tissues by an osmoticum, the Δ⁶ stearate gene is under a different form of regulation. Cahoon and his colleagues (East Lansing, Michigan) showed that petroselinic acid is synthesized via an acyl-acyl carrier protein (ACP) desaturase. Several Umbelliferae species have been probed for cross-reaction with an antibody against avocado Δ⁹ stearoyl-ACP desaturase, yielding strong signals of 38 and 36 kDa in Western blots, whereas in extracts of four non-Umbelliferae species only the 38 kDa band appeared, as in roots and leaves from Umbelliferae (which do not synthesize petroselinic acid).

A concerted effort of three groups—Bristol, United Kingdom (Smith and Stobart); Benin City, Nigeria (Bafor); and Uppsala, Sweden (Johnston and Stymne)—has led to the characterization of ricinoleic acid biosynthesis in microsomal preparations from castor bean endosperm via a NADH-dependent hydroxylation of oleate esterified to position sn-2 of phosphatidylcholine (PC) and the subsequent hydrolysis of the ricinoleyl-PC by the action of a microsomal phospholipase A₂ to yield free ricinoleic acid. Antibodies raised against plant cytochrome β₅ inhibited both Δ₁₂ desaturation and Δ₁₂ hydroxylation. Together with similar substrate, cofactor and electron transport requirements, this suggests an evolutionary relationship between the two enzymes.

Murphy’s group (Norwich, United Kingdom) has shown that the hydroxylase activity, responsible for the conversion of the oleoyl residue in PC into the corresponding ricinoleoyl residue, is localized in the endoplasmic reticulum. This primary reaction is followed by the eventual transfer of ricinolate from PC to triacylglycerol.

A very helpful contribution in the sense of potential wide biochemical applications in the future came from Rawyler and his colleagues (Neuchâtel, Switzerland), who have introduced cyclodextrins (CDX) as a tool for the controlled lipid depletion of thylakoid membranes (TM). All CDX tested (α-, β-, γ- and dimethyl-β-CDX) removed several lipid classes from TM in a concentration and time-dependent way without affecting their chlorophyll and protein contents. CDX obviously can be used as mild alternatives in the delipidation of membranes more usually achieved by detergent or lipase treatment.

The metabolism of lipids has...
gained considerable interest, e.g., concerning the lipoxygenase pathway in plants. Grechkin (Kazan, Commonwealth of Independent States) has studied the formation of allene oxides and finally their further stereochemically controlled cyclization, leading to C_{18}-cyclopentenone-12-oxophytodienoic acid, the precursor of the plant senesence promoter, jasmonic acid. This pathway is significantly stimulated in response to the action of stress factors such as wounding. Oxygenated fatty acids, generated via the lipoxygenase pathway, exhibit growth-stimulating activity. Gafarova, also from Kazan, provided convincing evidence for a further metabolism of products of the lipoxygenase pathway in corn seeds by action of a hydroperoxide dehydrase specific for hydroperoxide groups at position C-16. The group of Vliegenhart (Utrecht, The Netherlands) has purified lipoxygenase from ungerminated barley and provided evidence for the formation of leukotrienes from arachidonic acid. Two isozymes of lipoxygenase have been purified from the embryos of barley, mainly localized in the scutellum. Lipoxygenase 1 catalyzes the formation of 9-hydroperoxide, whereas the other isozyme exclusively produces 13-hydroperoxide. Monoclonal antibodies have been prepared and three cDNA clones encoding different lipoxygenases have been cloned. In considering the fact that products of the lipoxygenase pathway most probably play similar regulatory roles in plants as they do in animals, these contributions are very important and we eagerly look forward to further progress in this direction.

Lipase activity can cause serious problems during the processing of cereals, namely oat. Scientists from Calgene (Davis, California), in pursuing the aim of genetically manipulating rapeseed for the production of novel oils, have introduced the rape desaturase cDNA in the antisense direction under the control of seed-specific regulatory elements and have obtained a seed with 20-fold higher stearate than is found in standard canola. The stearate is incorporated into triacylglycerols in the sn-1 and sn-3 positions, and the resulting oil is a solid at 4°C.

In order to achieve the biosynthesis of trierucin in developing seeds of *Brassica napus* and thus improve industrial usability of rapeseed oil, Frenzten, Wolter and their colleagues (Hamburg, Germany) want to transform *Brassica* with the gene encoding microsomal 1-acylglycerol-3-phosphate acyltransferase from *Limnanthes douglasii*.

Murata and his colleagues (Okayama, Japan) transformed tobacco plants with the gene encoding chloroplastic glycerol-3-phosphate acyltransferase from squash (sensitive to chilling). The content of cis-unsaturated fatty acids (18:1+18:2+18:3) in phosphatidyglycerol was decreased and chilling sensitivity was markedly increased. By contrast, introduction of the cDNA for the same enzyme from *Arabidopsis* (resistant to chilling) caused a small but significant increase in cis-unsaturated fatty acids and decreased chilling sensitivity. These studies show that it is possible to alter fatty-acid unsaturation of phosphatidylycerol and chilling sensitivity of plants solely by the introduction of an appropriate acyltransferase. Highly sophisticated techniques have been used by Murphy's group. The group has isolated and characterized the oleosome-forming protein, termed oleosin, as well as cloned corresponding genes from various plants. They presented a preliminary model of its three-dimensional structure, thereby explaining how "oil droplets" are prevented from simply fusing due to hydrophobic interaction. In an *in vitro* translation system, it was shown that oleosins were efficiently targeted to the ER, but only small amounts were transferred to oil bodies or to other membranes which were included as controls. Sodium carbonate washing.
of the ER indicated that oleosin remained in the membrane, and proteinase digestion studies revealed that a portion of the oleosins remained outside of the ER, so it is not fully translocated into the lumen of the ER. Ross and Murphy used an immunocytochemical approach to elucidate the function of oleosins in developing fruit and seed tissues of olive. Maturing olive fruits are made up mostly of relatively large cells containing a few large oil bodies that have little, if any, oleosin, and lacking protein storage bodies. In contrast, the endosperm and embryo tissues in maturing seeds contain smaller cells typically filled with many small oil bodies arranged around the periphery of the protein bodies. The data are consistent with the view that oleosins do not play a role in oil synthesis but are important in the stabilization of oil bodies during desiccation.

Seeds of the native American tree *Umbellularia californica* accumulate reserve triglycerides containing primarily 10:0 and 12:0 acyl groups. Voelker and his associates (Calgene Inc., Davis, California) have purified a 12:0-ACP thioesterase from this species and have cloned a corresponding cDNA encoding a 382-residue preprotein. Its function was verified by expression of the mature-coding segment in *E. coli*. Transgenic *Arabidopsis* and rapeseed plants have been generated. In *Arabidopsis*, the MCFA is produced at the expense of most of the other acyl species with the exception of 18:3, suggesting a functional/regulatory role for the latter. Kater and his associates (group of Stuitje, Amsterdam, The Netherlands) have isolated a full-length cDNA clone encoding enoyl-ACP reductase from *B. napus*. Southern blot analysis shows that the allo-tetraploid *B. napus* contains two pairs of related enoyl-ACP reductase genes derived from the two distinct genes found in both its ancestors, *B. oleracea* and *B. campestris*. In *Arabidopsis thaliana*, a more distant member of the *Cruciferae* family, enoyl-ACP reductase is encoded by one to two genes. Wissenbach and colleagues (group of Wettstein-Knowles, Copenhagen, Denmark) used 3H-labeled cerulenin as a tagging tool to isolate three highly cerulenin-sensitive proteins from barley chloroplasts, all active as condensing enzyme I (β-ketoacyl-ACP-synthases, KAS, present in the type II fatty acid synthetase (FAS) complex)). Two cerulenin-binding polypeptides (α, β, 45 and 46 kDa, respectively) occur as homodimers (αα, ββ) as well as a heterodimer (αβ) and all three exhibit condensing enzyme I activity. Amino acid sequencing allowed for the construction of degenerate primers for PCR cloning of the Kas 12 gene,
cDNA clone for a putative A9 enoyl (ACP) reductase. These two enzymes catalyze the sequential reductive steps in fatty acid synthesis.

Ray and his co-workers (Gainesville, Florida) have chosen the peanut (Arachis hypogaea L.), one of the most important oil seed crops worldwide, to gain information on the organization and the spatial and temporal regulation of genes for fatty acid synthesis and modification. They reported the partial purification of a \( \Delta^{12} \)-desaturase from peanut. This group has already cloned genes of a family of acyl carrier proteins and the \( \Delta^{9} \)-stearyl-ACP-desaturase present in the same plant. In peanut, evidently multiple genes are encoding acyl carrier protein (ACP), and the seed appears to express these multiple forms. A cDNA clone for a putative \( \Delta^{9} \) desaturase also has been identified.

Verwoert and colleagues (Amsterdam) have transformed Brassica napus, Petunia hybrida and Nicotiana tabacum with the E. coli fabD gene, encoding malonyl-CoA-ACP-transacylase (MCT). To study the effect of over-producing this bacterial FAS component in plants, the coding region of the gene was fused to the napine promoter/plant terminator cassette to accomplish seed- and developmentally specific expression. The leader of the cloned rape enoyl-ACP-reductase was used for chloroplast targeting.

As prenyl lipids such as sterols or carotenoids play important roles in the physiology and biochemistry of plant cells, e.g., as constituents of membranes, considerable interest is given as to regulation and intracellular distribution of their biosyntheses. Yeast is a convenient, simplified system for investigating basic lipid reactions because of its ease of culture and well defined classical and molecular genetics.

Parks (Raleigh, North Carolina) presented work of his group that aims at further elucidating the role of ergosterol and other isoprenoids, studying the biosynthesis of mevalonate, the specific precursor. Heme competency differentially affects the activity of the two structural genes (HMG1 and HMG2) encoding HMG-CoA reductases (HMG, catalyzing the synthesis of mevalonate), suggesting distinctly different roles for the isozymes in the partitioning of mevalonate into the different isoprenoid pathways. The same group has isolated the \( \Delta^{14,15} \) sterol reductase gene by complementation a mutant, erg4, defective in that enzyme. Whereas both yeast HMGs contain seven or more membrane-spanning domains, plant isozymes contain only two. Both radish HMG isozymes have been expressed in a mevalonate-auxotroph yeast mutant (collaboration of Bach's and Boronat's research groups at Karlsruhe/Strasbourg and Barcelona, respectively).

Activity, exclusively induced in presence of galactose since the genes have been placed under the control of the GAL10 promoter, is clearly membrane-associated, indicating a correct targeting to cellular membranes, an observation that raises interesting regulatory questions. Weber and Bach (Karlsruhe, Germany, and Strasbourg, France) have purified a monomeric enzyme of about 55 kDa from radish membranes which catalyzes the double condensation of acetyl-CoA to HMGC-CoA. The reaction is greatly stimulated in the presence of Fell and of a hydrophilic quinone cofactor (best: pyrroquinoline quinone), suggesting catalytic redox-cycling to be involved in the reaction pathway. Plants seem to have developed another mechanism of HMGC-CoA synthesis other than eukaryotes, where two
clearly distinct enzymes, independent of these cofactors, are involved.

The authors also presented a protocol for the partial purification of radish HMG-CoA lyase, an enzyme that is probably involved in the degradation of leucine and in the so-called mevalonate shunt, a connection between the fatty acid and prenyl lipid biosynthetic pathways. On the other hand, Gerbling and Gerhardt (Münster, Germany) suggest the following pathway for the peroxisomal degradation of 2-oxoisocaproate (formed from leucine) to acetyl-CoA via isovaleryl-CoA, 3-methylcrotonyl-CoA, senecioate, 2-hydroxyisovalerate, 2-oxoisovalerate and isobutyryl-CoA to occur in mung beans. This would imply that HMG-CoA is not an intermediate.

As with other classes of lipids, the composition of prenyl lipids, namely of sterols, is affected by environmental stimuli or during development. Moreau and Preisig (Philadelphia) showed that cellulase treatment of tobacco cells, a condition that elicits phytoalexin synthesis, also induces an increase of sterol esters and acylated sterol glycosides at the expense of free sterols and sterol glycosides. The levels of galactolipids and phospholipids were also found to decrease in treated cells. Treatment with CuCl₂, however, caused a large increase in the levels of acylated sterol glycosides but resulted in a decrease in the level of sterol ester and every other lipid class. Such rapid changes that are thought to occur only in the plasma membrane may have important physiological roles in signal transduction during plant defense.

Brenac, Cerdon and Sauvaire (Montpellier, France) studied developmental changes in the sterol composition in fenugreek pods (Leguminosae). These plants accumulate steroid saponins such as diosgenin in the seeds; steroid saponins are used in the pharmaceutically important hemisynthetic production of corticosteroids as oral contraceptives. Hobbs and his colleagues (Preston and Bristol, United Kingdom) found evidence for a coordinate control of sterol and phospholipid accumulation during floral differentiation in *Brassica campestris*. During early apical development, cholesterol accounted for more than 50% of the total sterols present. As apical development progressed, the proportions of cholesterol decreased and those of sitosterol increased. Prior to bud break, the relative proportions of campesterol increased to 33% from 18%. This might further support the idea of specific roles for individual phytosterols in the regulation of development, similar to the situation in yeast, where ergosterol does not only act as membrane constituent but also has hormonal/regulatory functions (cf. Park’s contribution).
Rashed and Goad (Liverpool, United Kingdom), Burden and Cooke (Long Ashton), and Ralph (Preston) studied the effects of paclobutrazol (a triazole-type plant growth regulator) on celery plants and suspension cultures. Treatment with this sterol biosynthesis inhibitor resulted in a decrease in the major end product sterol, stigmasterol, and an increase in sitosterol, accompanied by a concomitant increase in the 14c-methylsterols such as obtusifoliol, cycloeucalenol and cycloartenol. In addition, the acyl moieties of the phospholipid side chains were seen to become more saturated, and plasma membranes to become less fluid.

From a nutritional point of view, mandarin peel seems worthwhile to be looked at because of its high content of xanthophylls (β-citraurinene epoxide and β-cryptoxanthin, active as provitamin A) esterified mainly with saturated C12, C14, and C16 fatty acids, whereas unsaturated fatty acids, in particular linoleic and linolenic acid, dominated in the lipid fraction of the peel (group of Biacs, Budapest, Hungary). Schindler and Lichtenhaler (Karlsruhe) investigated the high-light induced accumulation of zeaxanthin in aurea mutants of tobacco. The carotenoids violaxanthin (v, 2 epoxy, 2 OH groups), antheraxanthin (a, 1 epoxy, 2 OH groups) and zeaxanthin (z, 2 OH groups) represent the three components of the xanthophyll cycle (v - a - z) of chloroplasts. Zeaxanthin accumulation has a biphasic pattern, a rapid conversion of v to z in the range of minutes, followed by a continuous increase in the z pool by de novo-biosynthesis. This latter process is thought to be involved in the protection of the photosynthetic apparatus through consumption of photochemically produced ATP and NADPH by preventing photoinhibition and associated photooxidation of pigments and lipids.

Williams (London) presented new aspects concerning the role of lipids in determining the stability and function of membranes, applying sophisticated physical techniques such as wide angle X-ray scattering, which allows for studying phase transitions, dependent of concentrations in unsaturated or saturated lipids. In this way the effect of various co-solutes and destabilizing or chaotropic agents has been studied. Siegenthaler, Rawyler and their associates (Neuchâtel) determined the molecular organization and functions of acyl lipids in chloroplast membranes. All lipid classes are heterogeneously distributed in the thylakoid membrane (TM), needed to support vectorial photochemical processes. Monogalactosyldiacylglycerol (MGDG) and phosphatidylglycerol (PG) were enriched in the outer layer of the TM, whereas digalactosyldiacylglycerol (DGDG) is essentially confined to the inner monolayer. Their work points to the control of MGDG packing pressure in the outer monolayer of the TM by activities such as the CF3/C1 complex (plastidial ATPase), thus showing that reciprocal interactions might occur between acyl lipids and functions, here in photosynthesis.

By the use of a mutant of Chlamydomonas reinhardii, unable to synthesize trans-Δ3-hexadecanoic acid containing phosphatidylglycerol, the group of Trémollières (Gif-sur-Yvette, France) could show that these algae were nearly completely unable to form an oligomeric photosynthetic antenna, to regulate light-energy distribution and to form grana stacks. These functional/structural deficits can be overcome by cultivating the mutants in presence of liposomes containing this lipid.

Lilienberg's group (Göteborg, Sweden) demonstrated the dehydration tolerance (acclimation) in oat seedlings by repeated moderate water deficit stress periods. Plasma membranes had drastic changes in the distribution of phospholipids as well as alterations in the level of cerebroside and free sterols, leading to a different phase behavior after acclimation. The research group of Hartmann (Strasbourg) reported on observations using liposomes of soybean phosphatidylcholine bilayers into which various sterols had been incorporated. Phospholipid-sterol interactions had also been studied by 2H-NMR experiments performed on oriented bilayers. Considering their structural and functional data together, sitosterol and 25β-methylcholesterol apparently play roles in plant membranes similar to those of cholesterol in mammalian membranes, and 9,19-cyclopropylsterols (generated after treatment of plants with certain sterol biosynthesis inhibitors such as N-substituted morpholines and azols used as fungicides) behave as good surrogates of sitosterol. Stigmasterol might play a specific role.

Environmental stimuli and stresses and their effects on plant lipid metabolism had been studied by a number of groups. Harwood presented an overview of such stimuli including light, temperature, soil and atmospheric constituents, xenobiotics like several herbicides, physical damage, microbial attack, etc. Changes induced by light included remodelling (positional distribution changes), changes of cis/trans isomerism, changes in unsaturation and chain length of fatty acids, plus other modifications of acyl chains, changes in glycerolipid proportions and in the ratio lipid/protein. Positional changes affect the phase transition behavior of phosphatidylcholine, for example. Little clear information is available hitherto as to how, for example, an increase in unsaturation is achieved—by a change in the turnover of lipids, activation of desaturase gene(s), activation of already existing desaturase or by increase in substrate availability. In Brassica napus, desaturation activity is lost at high temperatures and induced at low temperatures (Williams and Khan, Toronto). In a study conducted by Mancha and his group (Sevilla) it was shown that the oleate content of

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seeds of a high oleic acid mutant of sunflower was only slightly dependent on the growth temperature. In the normal phenotype, low temperature stimulates oleate desaturase activity by inducing enzyme synthesis. Thus this mutation might have changed some control factor or the chain of signal perception and transformation.

Olive oil production in the Mediterranean area is always endangered by a shortage of fresh water. Usage of water having up to 1% salt, e.g., caused by contamination of ground water by instreaming sea water as a consequence of overexploitation too close to the coast line, poses serious problems in the near future (not to mention a scenario of a general climatic change due to elevated CO₂ levels in the atmosphere with possibly dramatic impacts on fragile ecosystems). As expected, the productivity of oil trees depends on many factors. As olive trees represent a slowly growing species, however with a great longevity, selection of individuals having a higher salt resistance or a higher productivity is difficult. Zarrouk, Marzouk and Cherif (Tunis) showed that water stress led to a decrease in galacto- and phospholipids and an increase in neutral lipids in olive leaves. Dimethoate, a pesticide used against olive fly, one of the most economically important pests affecting Mediterranean olive culture, exerts an inhibitory effect on the biosynthesis of fatty acids from ¹⁴C-malonyl-CoA and of storage triacylglycerol in olive fruits (De la Vega and Sanchez, Sevilla; and Harwood, Cardiff). Several studies of Cherif and his associates (Tunis) concerned salt stress effects on lipids of crop plants such as Gossypium hirsutum and Medicago orbicularis as the increase in salt concentration in soil poses serious problems in Tunisian agriculture.

Hegazy (Zagazig, Egypt) and Lösel (Sheffield, England) presented a comparative study of alterations in lipid metabolism of host plant tissue infected by Pseudomonas phaseolicola, P. solanacearum and A. tumefaciens. In general, phosphatidylcholine remained essentially unchanged, phosphatidylglycerol decreased and phosphatidylinositol increased. However, there was a sharp increase in sterols and incorporation of label from fatty acids into sterol esters.

Of considerable economic interest is also the flowering capacity of tulip bulbs (Walch and van Hasselt, Haaren, Holland) in response to an essential cold period of 5°C during 12 weeks. The data suggest that phospholipids play a role in the flowering process. Olsson and Liljenberg (Göteborg) studied the interrelationships between aging, water deficit stress, superoxide dismutase activity (scavenging perox-
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...ide radicals) and glutathione/ascorbate content and lipid peroxidation in pea plants. Stressed plants appear less endogenously protected against peroxidative degradation of polar lipids. Norman and Pillai (USDA, Beltsville, Maryland) reported that tolerance against nitrodiophenyl ether- and ultraviolet-B-induced lipid peroxidation in cucumber seedlings was positively correlated with the glutathione content, as shown by cotreatment with inhibitors or enhancers of glutathione biosynthesis. The effects of ozone exposure (at realistic levels or 65 ppb) on membrane-lipid turnover in Pisum sativum has been studied by Carlsson and his colleagues (Göteborg). Comparative studies were reported on the effects of drought stress on membrane lipids of Cajanus cajan leaves (group of Vieira da Silva, Paris) and of Vigna unguiculata, this in collaboration with Mazliak (Paris). Besides osmotic adjustments, the stability of membrane lipids accounts for drought tolerance in resistant cultivars of C. cajan. The main targets for degradative processes in susceptible cultivars of V. unguiculata are evidently lipids, which are rich in linolenic acid, especially those of galactolipids in the thylakoids.

Selstam and her colleagues (Umeå, Sweden) reported that frost tolerance in Norway spruce of southern and northern provinces is correlated with an increase in the total amount of membrane lipids, a decrease in galactolipid-to-phospholipid ratio, and an increase in needle dry weight.

There remains the question of how a cell regulates the assembly of a membrane, viz., the journey of lipids from the site of synthesis to the nascent membrane. And what signals tell the biosynthetic machinery to stop its activity once the membrane is saturated? How are the syntheses of various lipid classes co-ordinately regulated? Cassagne and his colleagues (Bordeaux, France) in collaboration with Mörre (West Lafayette, Indiana) made a detailed analysis of plasma membrane (PM) biogenesis using etiolated leek seedlings. As to lipid transfer, three hypotheses exist: (a) a cytosolic lipid transfer protein (LTP) mediates the transfer of monomers, (b) a vesicular transfer, and (c) a non-protein lipid transfer. In vivo pulse-chase and double-labeling experiments suggest that a vesicle-mediated pathway involving the ER and Golgi apparatus (GA) could be one of the routes taken by certain lipids destined to the PM. Presence of a perturbant of membrane trafficking (monensin) led to an accumulation of labeled lipids at the level of the GA and to a related decrease in the PM, specifically of very long chain fatty acyl (VLCFA)-containing lipids. This suggests some sorting of lipids according to their chain length, the VLCFA-containing lipids being preferentially transferred through the ER-GA-PM vesicular pathway, in accordance with the intracellular location of the C\textsubscript{18}-CoA elongase in the ER and the C\textsubscript{20}-CoA elongase in the GA. The transfer of VLCFA-containing lipids to the PM was selectively blocked at 12°C, in contrast to that of C\textsubscript{16} and C\textsubscript{18}-containing lipids. At 12°C, label accumulated in the ER and to a lesser extent in GA, with a related decrease in the PM, indicating that a temperature block exists somewhere between GA and PM. Taken together, these data establish the occurrence of at least two vesicle mediated lipid transfer pathways, dependent on acyl chain length.

The control of lipid transfer protein (LTP) activity by oxido-reducing conditions has been examined by Grosbois and colleagues (Paris, group of Kader). Isolation of corresponding genes has led to surprising aspects, e.g., the presence of a signal sequence that resembles those of secretory proteins. As the protein can only bind its substrate if its three-dimensional conformation is maintained by S-S bridges, whereas in a plant cell usually the concentration of glutathione and of cysteine are kept high, sufficient to reduce disulfide...
bridges, full activity of the LTP can only be achieved in a microcompartment with a low reduction potential, as is most probably true for the lumen of the ER. I would speculate here that a high concentration of active LTP in vesicles derived from the ER is needed to maintain membrane integrity and to correct lipid composition just of these vesicles in the presence of an "overload" of lipid molecules to be transported to the PM, where they, for example, serve as precursors for the formation of epicuticular waxes.

Sandelius and Ränt fors (Göteborg) studied the reconstitution of acyl lipid transfer from ER to chloroplasts by an interesting, yet simple, technique. As donor membranes, they used an ER-enriched fraction from hypocotyl segments of etiolated soybean seedlings which had been incubated overnight with \(^{14}\text{C}\)-acetate to label lipids. As acceptors, either chloroplasts or envelope vesicles immobilized to nitrocellulose strips were used. Donor and acceptor were incubated together under various conditions. ATP and cytosolic proteins stimulated the transfer from ER to chloroplasts. Essentially the same technique was applied by Harryson, Sandelius (Göteborg) and members of Kader's group (Paris) to follow more closely the transfer or \(^{3}\text{H}\)-labeled phosphatidylglycerol (PG). Inclusion of a nonspecific LTP, isolated from maize coleoptiles, stimulated the transfer of PG from liposomes or from ER vesicles in a concentration-dependent manner.

Perry and Harwood (Cardiff) and Bligny and Douce (Grenoble, France) examined pool sizes of intermediates in the biosynthetic pathway to triacylglycerols to evaluate possible points of flux control in developing embryos of oilseed rape. Diaacylglycerol amounts increased during maximum triacylglycerol synthesis. In agreement with data from radiolabeling experiments this suggests that diacyl glycerol acyltransferase might exert significant flux control. Mackender (Belfast, North Ireland, United Kingdom) incubated greening oat seedling leaves in the presence of \(\text{NaHCO}_3\) and sucrose, either one of which was \(^{14}\text{C}\) labeled. After incubation in continuous light at 20°C, segments were analyzed, and a balance sheet has been produced for the distribution of photosynthetic to the major biosynthetic pathways in relation to plastid and leaf development. Preliminary analyses show that at least 80% of \(^{14}\text{C}\) is directed to cell wall and starch synthesis, up to 7% into proteins, up to 5% into nonpigment lipids and up to 2.5% into pigment synthesis.

The 11th International Plant Lipid Meeting will be held in Paris during 1994; the coordinator will be Paul Mazliak. The two years in between will certainly give the time to experimentally clarify some of the open questions, to raise new ones and to open new avenues toward a better understanding of the functional and regulatory interrelationships between membrane genesis, synthesis and turnover of lipids in plants.