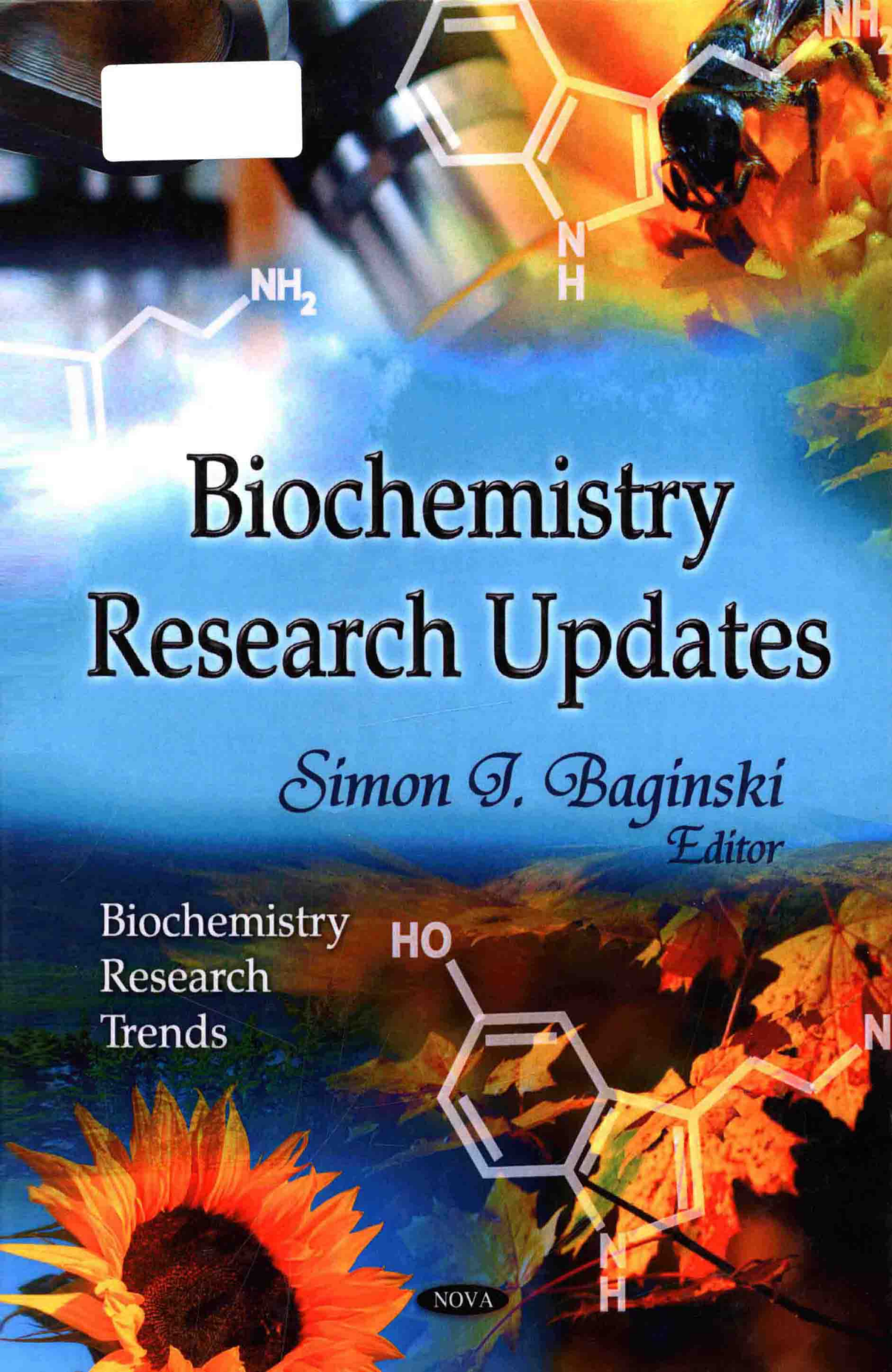
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BIOCHEMISTRY RESEARCH TRENDS

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PREFACE

This book provides a study of biochemistry research trends across a broad spectrum of applications. Topics discussed include the biochemical, physiological and therapeutic aspects of some components of the large class of lipids; streptavidin-biotin binding and immunomodulatory constructs; cardiac opioid peptides and classical opioid receptor systems; angiotensin converting enzyme inhibitors; phylogenomic analyses of the superprotein families of different metabolic systems; aptamers as the new biorecognition element for proteomic biosensing; the interactions of microorganisms with heavy oils and urinary adrenaline measurement in a forest environment setting.

Chapter 1 - This chapter approaches biochemical, physiological and therapeutic aspects of some components of the large class of lipids. The authors begin with a brief history of essential FA and finish presenting the main techniques used in research to analyse the cell content of fat. Lipids are not only the best form of energy storage, cell membrane constituent, or hormones precursors. Lipids and their metabolites participate in intra and intercellular signalling pathways and regulate gene expression. Recent studies on cytotoxicity of FA are discussed. A significant part of the content herein presented is product of the scientific investigation of the own authors and new results, not yet published, are also shown.

Chapter 2 - Several immunomodulatory constructs based on streptavidin-biotin binding have been described that exploit the modularity and flexibility of this system. In general, a streptavidin core serves as the carrier of targeting and effector molecules. Targeting agents, such as whole antibodies or their fragments and natural ligands, deliver antigen to specific receptors on leukocytes. This targeted delivery can both enhance and fine-tune immune responses, which are directed against streptavidin itself or its cargo. Due to its tetrameric structure, streptavidin-based targeting complexes can crosslink cell surface receptors. This property enables such constructs to initiate signaling events in the target cells. Thus, in addition to improving antigen uptake, activation of APCs can be achieved. By using liposome- or nanobead-bound streptavidin, one can both boost the crosslinking effect and target different cell populations, specialized in the uptake of particulate antigens. To generate streptavidin-based constructs, various molecular biological and chemical methods have been applied. In a preferred setup, monobiotinylated components are used, in order to ensure the formation of complexes with controllable stoichiometry. This strategy also allows the combination of distinct targeting and antigenic moieties in the same complex. Here the authors review various approaches of streptavidin-based immunotargeting and their effects on immune responses.

Chapter 3 - Streptavidin is a ~53kDa tetrameric protein purified from the bacterium *Streptomyces avidinii.* It is well known to contain four identical subunits, each containing a single binding site that binds very tightly to the small molecule biotin, also known as vitamin H. This high affinity (K_{assoc}= $10^{14}M^{-1}$) has been applied widely in biotechnology and biosensors. This review will cover the latest trends in the application of streptavidin-biotin system in DNA sensors and immunosensors using electrochemical and optical techniques. Streptavidin is sometimes used in connection with magnetic beads in DNA sensing. By immobilizing the biotinylated DNA probes on streptavidin-conjugated magnetic beads, the target DNA can be easily isolated from a complex matrix using an external magnet. Electrochemical and fluorescence-based immunosensors utilize the biotinylated antibodies with streptavidin-conjugated enzymes (horseradish peroxidase, alkaline phosphatase, etc.) or streptavidin-conjugated fluorescent dyes as the source of analytical signals. Surface plasmon resonance (SPR) and Localized SPR, which provide label-free analysis of dynamic surface events, are valuable optical tools for studying interactions between biomolecules. In immunosensors, SPR is typically applied to detect the formation of a sandwich-type immune complex comprised of a capturing antibody immobilized on a sensor surface, the target biomolecule, and a detecting antibody. The well-studied streptavidin-biotin system serves as an excellent model for the development of LSPR biosensors using metal nanoparticles. This review will also describe the recent fundamental spectroscopic studies that reveal key relationships governing the LSPR spectral location and its sensitivity to the local environment, including changes on the substrate due to binding events. The immobilization of streptavidin on gold nanoparticles is detected with the changes in the peak wavelength and intensity of the LSPR absorption band. Further attachment of the biotinylated ligands on streptavidin-conjugated gold nanoparticles can be detected at high sensitivity and selectivity. The results from these biosensor studies guide the design of new sensing experiments, illustrated through applications of nanomaterials, in which researchers use both the streptavidin-biotin affinity and the advanced transduction techniques to detect molecules of chemical and biological relevance.

Chapter 4 - The endogenous opioidergic system is known to express both receptors and peptides within the cardiovascular system, including within the heart itself. Precursors for all three families of endogenous opioid peptides (EOPs), prodynorphin (PDYN), proenkephalin (PPE) and proopiomelanocortin (POMC) are generated in the heart and alternative processing results in a myriad of mature peptides. Biosynthesis of the opioid peptide precursors, prodynorphin and proenkephalin, occurs predominantly in ventricular cardiomyocytes, not in the atria; whereas, processing of proopiomelanocortin to the cardiac β-endorphin peptide occurs in atrial myocytes. PPE has been shown to be more highly expressed in the adult heart and higher in the ventricles than in the atria, with left-sided preference. Similarly, POMC has been reported to only be detectable in the adult heart and to be preferentially localized to the ventricles compared to the atria. However, PDYN is reported to only be detected in the atria. Similar to the biosynthesis of cardiac peptides from their prohormone precursors, cardiac opioid peptides are spatially distributed; levels of enkephalins are higher in ventricular tissue and β-endorphin peptide is predominantly found in atrial tissue. In atrial tissue, enkephalins are packaged in vesicles, but are found unpackaged in ventricles.

Chapter 5 - Hypertension, a major cause of heart-diseases is caused by malfunction of one or more of the complex set of mechanisms controlling blood pressure. In last four decades specific agents interacting with these regulatory mechanisms have become available.

Introduction in early eighties of Captopril, a rationally designed potent inhibitor of Angiotensin converting enzymes (ACE), heralded a new era in antihypertensive therapy. Since then rapid strides have been made in understanding of the active site and the spatial requirements for ACE inhibitors. This chapter has been aimed to report the existing knowledge and to enable researchers all over the world to exploit further with an objective to develop better therapeutic agents. Bio receptors being chiral, interactions with organic molecules are strongly influenced by the chirality of the substrate. The two enantiomers of a drug are some times known to elicit differential and in some cases even opposite responses, amply heightened by work on ACE inhibitors. The present trend, in drug research, is to design and synthesize molecules with designated chirality. ACE inhibitors represent a class of rationally designed drugs based on the comprehensive knowledge of the enzyme and its binding sites and had led to great revolution in antihypertensive therapy. ACE is a Zn^{++} containing metalloenzyme similar to carboxypeptidases, whose binding sites have been studied properly and a hypothetical model incorporating all the binding sites have been developed. Based on the hypothetical model for ACE binding sites and the role which it plays in living organism, attempts have been made to develop potent inhibitors of ACE with an objective to control blood pressure. Early researches on ACE inhibitors started with snake venom peptides, showing antihypertensive activity. Though these could not be used as drugs due to high toxicity but certainly opened an avenue to look for development of antihypertensive agents on these lines. Further researches coupled with structure activity relationship (SAR) studies led to development of various ACE inhibitors and ultimately in early eighty's first rationally designed drug "Captopril" was introduced in market. Since then various other drugs acting through same mechanism and controlling blood pressure effectively have come out. The chapter entitled "Angiotensin converting enzymes inhibitors: A class of potent antihypertensive agents" is based on literature reports and intended to summarized the available knowledge and to give an update picture of major advances towards the design and synthesis of angiotensin converting enzymes inhibitors and their role in controlling blood pressure and shall undoubtedly be valuable to readers working in the area. This chapter gives information about the designing and developing of ACE inhibitors reported till date with future prospectives to achieve further progress as for as designing and development of even better ACE inhibitors is concerned.

Chapter 6 - Since a metabolic pathway with similar function has similar evolutionary relationship, the operation of metabolic pathways is essential to the survival of organisms, and metabolism related genes usually constitute only 10–20% of the total number of genes, metabolic pathway-based analysis can minimize the effect of the gene number, and thus allow more precise investigation of evolutionary relationships. Herein, the authors have shown that protein superfamilies of archaea were diverged and metabolically converged together within the genomes of this domain as a consequence of long evolutionary and diverged metabolic events. However, a close phylogenetic proximity between archaea and bacteria was observed for sharing metabolic functions by protein family divergence. A close phylogenetic boundary between methanogens and methylotrophs was revealed the reverse methanogenesis hypothesis. Energetic metabolism of methanogens are shared their ancestral behaviors with primitive proteobacteria. A mosaic nature of some functional domains of protein superfamilies was observed between mesophilic methanogens and thermophilic archaea/halophilic archaea, which may be resulted due to selection pressures. Overall results revealed that the phylogenetic analysis of protein superfamilies obtained from archaeal

domain provided a standpoint for the evolutionary history of some key metabolic modules among methanogens. Therefore, molecular evolutionary hypothesis from this finding would provide a new horizon for growth of research in advancement of metabolomics and metabolic engineering in methanogens.

Chapter 7 - Aptamers are single stranded artificial nucleic acid ligands that can be generated against almost any kind of target, such as ions, metabolites aminoacids, drugs, toxins, proteins or whole cells. They are isolated from combinatorial libraries of synthetic nucleic acids by an iterative process of adsorption, recovery and amplification, know as SELEX (Systematic Evolution of Ligands by EXponential enrichment) process. Aptamers, the nucleic acid equivalent to antibodies, are easy to synthesise, is not required the use of animals for its synthesis, for this reason it can be developed again toxins and small molecules that do not produce immune response in animals and can be tuned for affinity in closer to assay conditions permitting recognition out of the physiological state. So, aptamers posses numerous advantages that make them preferred candidates as biorecognition elements. In view of the advantages and simple structure of aptamers, they have been used in a wide range of applications such as therapeutics, diagnosis, chromatography, environmental detection, among other.

Chapter 8 - The objective of this chapter is to demonstrate the basic characteristics of halophilic strain TM-1 and *Bacillus* SP-5 which were isolated from a reservoir of the Shengli oil field in East China and evaluate the effects of strains TM-1 and SP-5 on different heavy oils. Strain TM-1 is a halophilic gram-positive coccus and strain SP-5 is gram-positive rod with spores. The cells of strains TM-1 and SP-5 were grown at temperatures up to 58℃ in the neutral to alkaline pH range. They could grow steadily, and produce various metabolites, have various surface features and use different organic substrates (acetate, D-glucose, fructose, glycerol, maltose, pyruvate, starch, sucrose, and xylose). Laboratory studies have demonstrated that the strains TM-1 and SP-5 affected different heavy oils; converted and degraded various components and changed the physical and chemical properties of heavy oils. The bioconversion of heavy oils leads to an enrichment in lighter hydrocarbons and an overall redistribution of these hydrocarbons. The interactions of microorganisms with heavy oils are variable and depend on the microbial species and the chemical compositions of heavy oils.

Chapter 9 - Humans have enjoyed forest environments for ages because of the quiet atmosphere, beautiful scenery, mild climate, and fresh, clean air. Empirically, forest environments may reduce stress and have relaxing effect on human. Adrenaline is released from the adrenal medulla, and adrenaline levels increase under circumstances of novelty, anticipation, unpredictability, and general emotional arousal. Measurement of free adrenaline in urine provides a reliable measure of the circulating concentration of adrenaline in the bloodstream and thus is a measure of sympathoadrenal medulla activity. To explore the relaxing effect of forest environments on humans, the authors investigated the effect of trips to forest parks on urinary adrenaline and noradrenaline in both male and female subjects. The authors found that three-day/two-night trips to forest parks significantly reduce the concentration of urinary adrenaline and/or noradrenaline. Moreover, a day trip to a forest park also has a similar effect on urinary adrenaline. The Profile of Mood States (POMS) test showed that forest environments significantly increased the score for vigor and decreased the scores for anxiety, depression, anger, confusion, and fatigue suggesting that the subjects were under conditions of lower stress during the forest trips, which support the findings on urinary

adrenaline. Other studies have reported that forest environments reduce the concentration of cortisol in saliva, reduce prefrontal cerebral activity, reduce blood pressure, and stabilize autonomic nervous activity in humans, which also support our findings on urinary adrenaline. These findings indicate that forest environments may reduce stress and have relaxing effect on human by reducing adrenaline level.

Chapter 10 - Here, a microarray-based spectroscopic assay with three readout principles, fluorescence, surface resonance Raman (SERS) and resonance light scattering (RLS), for studying kinase functionality and screening kinase inhibitors has been reported. In this assay, the phosphorylation and inhibition events are marked by biotinylated anti-phosphoserinen antibodies, and gold nanoparticles are attached to the antibodies by standard streptavidin-biotin chemistry, followed by silver deposition for RLS and SERS signal enhancement. The streptavidin conjugated fluorescein is used as fluorescent probe. The utility of this assay to high-throughput screening was demonstrated with the interactions of the α-catalytic subunit of cyclic adenosine 5′-monophosphate (cAMP) dependent protein kinase (PKA) with a commercial inhibitor library, a collection of 80 potential kinase inhibitors, and satisfactory results were obtained. In addition, quantitative determination of binding strength and the inhibiting type (type I) of these inhibitors are also demonstrated by the adenosine 5′-triphosphate (ATP) competing assays.

CONTENTS

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Chapter 1

LIPIDS AND CELL FUNCTION

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ABSTRACT

This chapter approaches biochemical, physiological and therapeutic aspects of some components of the large class of lipids. We begin with a brief history of essential FA and finish presenting the main techniques used in research to analyse the cell content of fat. Lipids are not only the best form of energy storage, cell membrane constituent, or hormones precursors. Lipids and their metabolites participate in intra and intercellular signalling pathways and regulate gene expression. Recent studies on cytotoxicity of FA are discussed. A significant part of the content herein presented is product of the scientific investigation of the own authors and new results, not yet published, are also shown.

1929: THE ESSENTIALITY OF THE FATTY ACIDS

In 1854, Grösmann first described a 20-carbon long saturated fatty acid (FA) derived from peanut (also known as groundnut) oil, which was called arachidic acid, due to the species *Arachis hypogaea*, that in turn was probably named because of the cobweb aspect of its roots. In 1909, Hartley characterized a 20-carbon FA containing four double bonds, isolated from liver lecithin, which was later named arachidonic acid [1, 2]. Up to 1929, FA were regarded exclusively as an efficient form of storing energy. The study of food components at the end of the 1800s and beginning of 1900s revealed various essential substances, particularly vitamins. It was during the study of vitamin E that the first clues for

the discovery of the essential FA appeared. As vitamin E is lipossoluble, diets prepared lacking this vitamin had to be also devoid of lipids. It was then found out that animals that eat lipid-poor diets tend to develop a specific syndrome, different from that known for any vitamin. The results of these studies were published in 1927, by Evans and Burr, in 1929 and 1930 by George and Mildred Burr [3-5]. These researchers not only showed that certain lipids are essential, describing the specific deficiency symptoms, but also pointed out linolenic acid as the main essential FA, which is capable of reverting the syndrome. Among linolenic acid deficiency symptoms, Burr and Burr described *"abnormal scaly condition of skin between the 70th and 90th day"* after specific diet was initiated with 21-day-old rats. Later *"the tail may become inflamed and swollen and the whole tail soon is heavily scaled and ridged. Haemorrhagic spots may arise in the skin throughout the entire length of the tail. The swelling of the tip may gradually be replaced by a true necrosis, resulting in the loss of 1-3 cm of the tail. The hind feet become red and somewhat swollen at times, in some cases with large scales over the dorsal surfaces. The hair on the back of the body become fills with dandruff. There is a tendency to lose the hair, especially about the face, back and throat. Sores often appear on the skin. The skin of the face especially seems to become sore at times and the irritation causes the animal to rub the face continually with his fore feet"* [4, 5]. Other symptoms described include: abnormal kidneys, appearance of blood in urine, cessation of growth, alterations or cessation of ovulation, lowered copulation capacity and infertility in males, high consumption of water, high skin permeability to water (in both directions), elevated respiratory quotient and high metabolic rate [4-6]. When diet lacking fat was given to weanling rats growth ceased in few weeks, a deadline weight was reached in 5 months and animals then died in another 3-4 months. The symptoms could be reverted by the addition of lard, corn oil, linseed oil, butter and coconut oil, although the last three fats were less efficient. The analysis of FA profile led the authors to point out linolenic acid as being essential. Later it was found that the same syndrome appeared on infant fed diluted skim milk plus sugar, which proved that FA can also be essential for humans [7].

Kurzrok e Lieb, who were working with artificial insemination, found that the human uterus could contract or relax upon instillation of semen, made the next breakthrough in the study of lipids. Euf von Euler and Goldblatt independently described during the thirties that prostate extracts caused contractions in uterus and intestine and lowered the blood pressure. Von Euler named the substance responsible for the effect *prostaglandin* (from prostate) having associated it with FA fraction from the prostate. It was in 1958 that the first prostaglandins were crystallized: PGE1 and PGF1, by Sjövall and Bergström. Soon afterwards other six prostaglandins were also purified and characterized. It was also Bergström, in collaboration with van Dorph, who discovered the connection between the essential polyunsaturated FA (PUFA) and prostaglandins, after managing to produce prostaglandin E and F from labelled FA incubated in homogenized sheep glands, in 1964 [8, 9].

Other important discoveries made in the sixties include the inhibitory effect of aspirin and indomethacin on the biosynthesis of prostaglandins, the antisecretory and the protective effect of prostaglandin in the stomach, and the abortion and labor-induction potential of prostaglandins. In a study carried out in 1968, once again with FA deficient mice, it became clear that other FA, not only linoleic acid (9,12-18:2), could abolish symptoms, such as polyenoic 17-carbon FA, gamma-linolenic (6,9,12-18:3) and *bis*-homo gamma-linolenic acid (8,11,14-20:3), thereby expanding the class of the so-called essential FA [6, 8, 9]. Indeed, it

was found that columbinoic acid, which can be converted to leukotrienes, but not to prostaglandins and thromboxanes, can revert the deficiency syndrome, therefore excluding the importance of cyclooxygenase-derived eicosanoids in this pathological condition [10]. The decades of 1970 and 1980 were rich in publications describing other eicosanoids and lipoxins, such as some leukotrienes and prostacyclin. The various metabolic routes related to eicosanoid synthesis and breakdown were elucidated, including both enzymatic and non-enzymatic reactions [8]. Although the discovery of many prostaglandin, thromboxane and leukotriene receptors has happened during the passed decades, these had been found to be exclusively extracellular until recent work characterizing nuclear receptors – the PPAR (peroxissome proliferator activated receptor). With this class of receptors it has become clear that eicosanoids can act both in their original or metabolised forms, upon various different external or internal receptors, signalling both through classic cAMP, GTP, phosphorylation and calcium pathways and directly on gene transcription control. The recent studies on biological effects of FA and eicosanoids derived from them are now enabling researchers to understand the essential nature and the physiological importance of these compounds.

Structure and Biochemistry of Lipids

Lipids are broadly defined as any water-insoluble (lipophilic) or nonpolar compounds of biological origin, such as fats, oils, waxes, cholesterol, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, sphingolipids, glycolipids and terpenoids (eg. retinoids and steroids). Some lipids are linear aliphatic molecules, while others have ring structures. Some are aromatic, while others are not. Some are flexible, while others are rigid. These structural differences are extremely important to define the metabolism and function of the lipids.

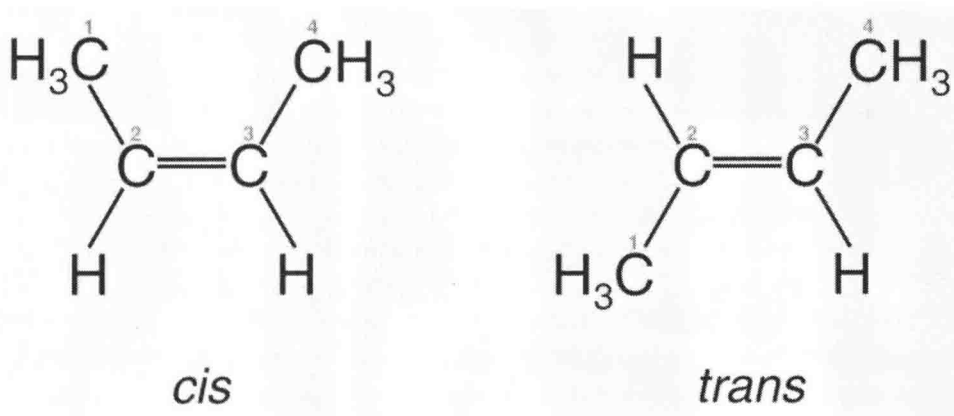

Figure 1. *Cis* and *trans* configuration of double bonds. A *cis* configuration means that adjacent hydrogen atoms are on the same side of the double bond. In *trans* configuration, adjacent hydrogen atoms are on the opposite side of the double bond.

Lipids have many key biological functions, such as structural components of cell membranes, energy storage sources and intermediates in signaling pathways. Lipids originate entirely or in part from two distinct types of biochemical subunits or "building blocks":

ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

Fatty Acids

Fatty acids are long-chain hydrocarbon molecules containing a carboxylic acid moiety at one end that can be represented by the formula RCO_2H. Usually the R group comprises a non-ramified hydrocarbon chain with even number of carbon atoms, that can contain carbon-carbon double bonds or not. The carboxylic acid moiety constitutes the polar portion of the molecule and the R chain is the non-polar portion.

The length of the hydrocarbon chain determines their classification in:

- Short chain FA: formed by 2 or 4 carbon atoms
- Medium chain FA: formed by 6 to 14 carbon atoms
- Long chain FA: formed by more than 14 carbon atoms
- Very long chain FA: formed by 20 and more carbon atoms

The presence of double bonds in the hydrocarbon chain determines their classification in:

- Saturated FA: contain no double bonds in their structure
- Unsaturated FA: contain one (monounsaturated) or more than one (polyunsaturated) double bonds in their structure

The hydrocarbon chain of saturated FA exists mainly as an extended form, once this linear and flexible conformation is the state of minor energy for the molecule. On the other hand, unsaturated FA contain rigid bends in their hydrocarbon chain because double bonds do not spin. The two carbon atoms in the chain that are bound to either side of the double bond can occur in a *cis* or *trans* configuration. A *cis* configuration means that adjacent hydrogen atoms are on the same side of the double bond (Figure 1). The rigidity of the double bond freezes its conformation and, in the case of the *cis* isomer, causes the chain to bend and restricts the conformational freedom of the FA. The more double bonds the chain has in the *cis* configuration, the less flexibility it has. When a chain has many *cis* bonds, it becomes quite curved in its most accessible conformations. The effect of this is that, in restricted environments, such as when FA are part of a phospholipid in a lipid bilayer, or triglycerides in lipid droplets, *cis* bonds limit the ability of FA to be closely packed, and therefore could affect the melting temperature of the membrane or the fat. A *trans* configuration, by contrast, means that the next two hydrogen atoms are bound to opposite sides of the double bond. As a result, they do not cause the chain to bend much, and their shape is similar to straight saturated FA.

Nomenclature

1. Fatty acids can be identified by their trivial names (or common names), which are the most frequent naming system used in the literature. These names do not follow any pattern, but are concise and generally unambiguous. They usually derive from the natural source of the specific FA. For example, palmitic acid was first identified in palm oil and oleic acid was found in olive oil.
2. Systematic names (or IUPAC names) derive from the standard IUPAC Rules for the Nomenclature of Organic Chemistry, published in 1979, along with a recommendation published specifically for lipids in 1977. Counting begins from the carboxylic acid end. The name will have a prefixe refering the number of carbon atoms in the hydrocarbon chain and will always end with the sufix oic, followed by the word acid. This notation is generally more verbose than common nomenclature, but has the advantage of being more technically clear and descriptive.
3. The numeric designations used for FA come from the number of carbon atoms, followed by the number of sites of unsaturation. For example, palmitic acid is a 16 carbon FA with no unsaturation and is designated by 16:0. This notation can be ambiguous, as some different FA can have the same numbers. Consequently, when ambiguity exists this notation is usually paired with either a Δx or n−x term.
4. In Δx (or delta-x) nomenclature, each double bond is indicated by Δx, where the double bond is located on the x^{th} carbon–carbon bond, counting from the carboxylic acid end. Each double bond is preceded by a *cis*- or *trans*- prefix, indicating the conformation of the molecule around the bond. For example, linoleic acid is designated *cis*,*cis*-Δ9,Δ12 18:2.
5. n−x (also ω−x (omega-x)) nomenclature does not provide names for individual compounds, but is a shorthand way to categorize FA by their properties. A double bond is located on the x^{th} carbon–carbon bond, counting from the terminal methyl carbon (designated as n or ω) towards the carbonyl carbon. For example, α-Linolenic acid is classified as a n−3 or ω-3 (omega-3) FA, and so it shares properties with other compounds of this type.

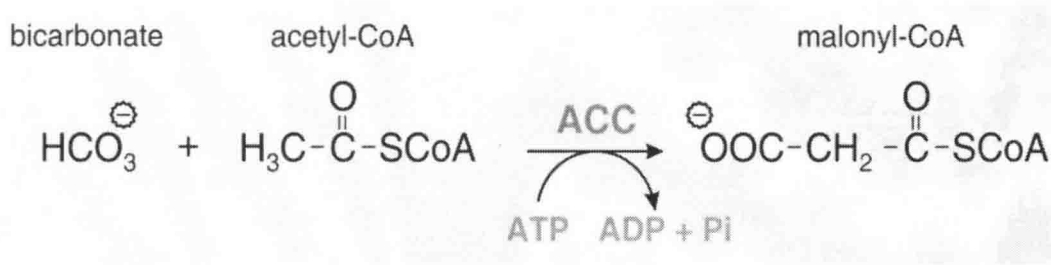

Figure 2. Synthesis of malonyl-CoA. Formation of malonyl-CoA is the first step required for fatty acid synthesis.